Advances in the Understanding of Gluten related Pathology and the Evolution of Gluten-Free Foods

Edited by:

Eduardo Arranz, Fernando Fernández-Bañares,
Cristina M. Rosell, Luis Rodrigo, Amado Salvador Peña

open access

OmniaScience

Monographs

Advances in the Understanding of Gluten related Pathology and the Evolution of Gluten-Free Foods

Edited by:
Eduardo Arranz
Fernando Fernández Bañares
Cristina M. Rosell
Luis Rodrigo
Amado Salvador Peña

Advances in the Understanding of Gluten related Pathology and the Evolution of Gluten-Free Foods

E. Arranz, F. Fernández Bañares, C.M. Rosell, L. Rodrigo and A.S. Peña

www.omniascience.com

DOI: http://dx.doi.org/10.3926/oms.274

ISBN: 978-84-943418-2-3

Cover design: OmniaScience

Cover image: Sticking out. Zoltán Vörös. CC-BY 2.0

Sponsors

Asociación de Celíacos y Sensibles al Gluten

Coeliac Disease and Gluten Sensitivity Association, formerly the Spanish Coeliac Association and later the Madrid Coeliac Association, is a non-profit private organization born around 30 years ago to provide support to families of children diagnosed of what, at that time, was a rare disease presenting with serious gastrointestinal symptoms, malnutrition and failure to thrive.

Today, this Association, with more than 9,000 members, embraces a wide range of patients suffering from a variety of gluten related disorders, mostly represented by coeliac and non-coeliac gluten sensitive people.

Aim

The main goal of this Association is to give support and advice to people diagnosed of coeliac disease and non-coeliac gluten sensitivity, offering:

- Updated information about the disease and the gluten-free diet.
- Training sessions for patients and families.
- Nutritional, medical and psychological advice.
- Cooking courses and other activities for children and adults.
- Different materials, such as a Gluten-free Food Directory, recipe books, list of gluten-free restaurants, hotels and shops, travel guides, etc.

Services

The Association organizes periodically training sessions for health-care professionals and caterers, and dissemination activities for general public to spread the knowledge on coeliac disease in the society and to guarantee an adequate diagnosis and treatment of gluten-related disorders. A permanent contact with the food industry and public authorities, as well as the mass media, is also an important task carried out to support these services.

Training

More than 70% of people with coeliac disease remain undiagnosed, what means they become chronic patients who experience a decrease in their quality of life associated with different gluten-derived health problems occurring over time. This represents extra charges for the public health system that should also be noted.

- **Health-care professionals**: the systemic condition of coeliac disease makes necessary the training not only of primary care doctors, pediatricians and gastroenterologists, but also of other medical specialists involved in the extraintestinal manifestations of the disease. Apart from the training sessions that are carried out in primary care centers and others, it is important to highlight the annual 7-hours accredited course organized by the Association, with 300 health-care professionals attending the sixth edition held in 2014.

- **Catering professionals:** following a gluten-free diet outside home is still a risky matter, so the Association makes a big effort at training caterers from hotels, restaurants and catering companies, and also catering students.

Dissemination

- Conferences for general public.
- Participation in gluten-related events.
- Presence in mass media (press, radio, TV, internet).
- Publication of books, book chapters and other materials.
- Organization of awareness events, such as the Madrid Coeliac Festival, with more than 7,000 people attending the 31st edition in 2014.

Research

Finally, the investigation of gluten-related disorders is a key aspect for this Association, as it is crucial to improve our knowledge of these pathologies to get better diagnostic approaches and healthier and better quality gluten-free products.

- The Association gets updated by reviewing scientific publications periodically and attending the most relevant scientific meetings and events concerning coeliac disease and gluten-free diet around the world. Thus, associated members, health-care professionals, researchers and people in general have a good point of information at this Association.

- To promote research in the field, the Association has been awarding Spanish researchers with up to 24,000 Euro per year since 2003. More than 240,000 Euro have been invested along these years giving support to 13 Spanish research groups.

Asociación de **C**elíacos y **S**ensibles al **G**luten
c/Lanuza 19 bajo – 28028 Madrid
Tel: 91.713.01.47 Fax: 91.725.80.59
www.celiacosmadrid.org

Asociación de Celíacos y Sensibles al Gluten
Comunidad de Madrid

General Preface

As far as we know this book is one of the few combining knowledge of the basic and clinical aspects of gluten-related disorders with the knowledge of the evolution of bread and gluten-free products.

Several articles have fully covered disease entities such as celiac disease, dermatitis herpetiformis, gluten ataxia, gluten allergy and clinical syndromes such as non-celiac gluten sensitive enteropathy. Another article reviewed the complications and diseases associated with the clinical disorders.

In this book, bread refers to a wide concept including a variety of food products with gluten-containing and gluten-free cereals. Further articles refer to the evolution of bread, the different grains and the improvements in raw materials in the preparation of bread, in particular the gluten-free products.

The two above-mentioned different areas of knowledge, are presented in this ambitious volume, with the intention to cover the necessary integration of knowledge between the fields that until recently were wide apart. Both fields are essential for patients, physicians, the food and pharmaceutical industry. If we want to benefit from the recent advances made in different areas of knowledge a common platform is crucial to improve the quality of life of the patients. This book will serve as a first step to build this new platform.

A genetic predisposition is fundamental for the development of celiac disease, dermatitis herpetiformis, gluten allergy and possibly gluten ataxia. Without certain environmental factors, of which the intake of gluten is the main offender these diseases will not become overt and no disease will manifest.

Based on the vision to establish a common platform of knowledge our book has three sections. The first section deals with basic knowledge of disciplines controlling the immune response to the toxic peptides resulting from the incomplete enzymatic digestion of gluten. The second section revises the advances in understanding the clinical spectrum of the disorders. The third section explores the evolution of gluten in particular and bread products most widely consumed in the western world. It also describes the great challenge of the elaboration of high quality gluten-free products but less expensive than the products at present available.

In the preface of section I Eduardo Arranz summarized the topics discussed by a group of experts working in basic areas of clinical investigation. In his preface he draws attention to the chapter of new advances in genetics and genomics in HLA and non-HLA genes. Further, he refers to the immunological mechanisms of intestinal tolerance to dietary proteins present in cereals; to the immunostimulatory and toxic peptides; to the pathogenesis that leads to inflammation; to the modulatory role of intestinal microbiota, which are also described in other chapters. This new knowledge has led to new approaches to develop alternatives for the gluten-free diet. These new possibilities are discussed at the end of section I. With these developments the pharmaceutical industry will probably take an interest in these common disorders.

In the preface of section II, Fernando Fernández-Bañares summarizes the different perspectives of the advances in diagnostics, the most appropriate serological tests and new tools. In one of the chapters he addresses the question whether the intestinal biopsy is still the “gold standard” that until recently has dominated the diagnosis and pathology of celiac disease. Another chapter describes the differences in clinical manifestations and diagnostic criteria among children, adolescents and adults. Other chapters summarize the knowledge on the different clinical entities, the common extraintestinal manifestations, the new syndromes related to gluten and associated disorders that are often encountered in patients suffering from celiac disease and/or dermatitis herpetiformis.

Special attention is given to the chapter on “refractory celiac disease”. This condition has a grave prognostic significance. The chapters to which Fernando Fernández-Bañares draws attention, deal with the follow-up of patients with celiac disease in whom the target of therapy should be a total mucosal recovery. He also refers to the quality of life and to the psychological distress in some patients with celiac disease and those with non-celiac gluten sensitivity. At the end of section II he refers to a comprehensive chapter on medical entities that develop when wheat behaves as an allergen such as Baker’s asthma, food and wheat pollen allergy.

Cristina M. Rosell has written a preface to section III. The evolution of gluten-free foods has been highlighted. She draws attention to chapters on the taxonomy of cereals, the role of domestication and breeding of cereals as well as to recent analytical tools for the detection gluten. These are areas in development that will require new policies and regulation as described in one of the chapters. She also refers to chapters dealing with gluten-free bakery products and pasta, gluten-free autochthonous foodstuffs. These products are still important in Latin America. She also draws attention to the chapter on the developments of gluten-free spirits and drinks. The last chapter of this section emphasizes the marketing and nutrition issues of the quality of gluten-free products.

This book will be interesting to clinical and research scientists in medicine, immunology and pathology, to the professionals in nutritional and health benefits of gluten-free products, to regulatory authorities, food chemists and technologists. We trust it will be of help in the practice of nutritionists, dietitians, industrial bakers, academics involved in undergraduate and post-graduate teaching of gluten related disorders, patients, patient associations as well as to the general public interested in nutrition.

The introductory chapter on epidemiology of celiac disease and gluten related disorders summarizes the latest knowledge and highlights the necessity of systematic studies worldwide in this area. The data available suggest the need to plan further epidemiological studies, in

order to understand the natural history of gluten related disorders and to obtain data to assess the financial burden of these diseases on health systems.

Acknowledgments

We have been very fortunate to work with three editors, leaders in the respective fields of investigation:

Eduardo Arranz, MD, PhD, Full Professor of Immunology at the University of Valladolid, Spain.

Fernando Fernández-Bañares, MD, PhD specialist in Digestive Diseases at the "Servei de Digestiu, Hospital Universitari Mutua Terrassa", Terrassa, Spain. President of SEEC, the Spanish Society of Celiac Disease.

Cristina M. Rosell, PhD from the Food Science Department, Institute of Agrochemistry and Food Technology (IATA-CSIC), Valencia, Spain. She is the current Editor of the Journal of Food Science and Technology.

The three editors have managed to gather a cadre of excellent authors, active and recognized investigators. We are very grateful for sharing their knowledge and expertise.

We also like to acknowledge the contribution of Mrs. Manuela Márquez, Director of the "Asociación de Celíacos y Sensibles al Gluten de la Comunidad de Madrid" and the Board of this Association, for the support and active interest in the progress of this book.

Finally, we like to thank Irene Trullas of OmniaScience for her expertise and organization with respect to the production of this book.

June 2015

Amado Salvador Peña and Luis Rodrigo

Authors' Index

Alvarez Juan B.
Department of Genetics, Escuela Técnica Superior de Ingeniería Agronómica y de Montes.
Campus de Rabanales, Universidad de Córdoba.
Córdoba, Spain.

Arias Rodríguez Laura
Gastroenterology Service. University Hospital of León.
Biomedicine Institute. León University.
León, Spain.

Armentia Alicia
Allergy Unit. Rio Hortega University Hospital.
Valladolid, Spain.

Arranz Sanz Eduardo
Mucosal Immunology Laboratory.
Institute of Biology and Molecular Genetics (IBGM).
University of Valladolid-Spanish National Research Council (CSIC), Consejo Superior de Investigaciones Científicas.
Valladolid, Spain.

Barro Losada Francisco
Institute for Sustainable Agriculture.
Spanish National Research Council (CSIC), Consejo Superior de Investigaciones Científicas.
Córdoba, Spain.

Bernardo David
Gastroenterology Unit. Hospital Universitario de La Princesa
Instituto de Investigación Sanitaria Princesa (ISS-IP)
Centro de Investigación Biomédica en Red de Enfermedades He-páticas y Digestivas (CIBERehd).
Madrid, Spain.
&
Antigen Presentation Research Group. Imperial College London. Northwick Park and St Mark's Campus.
Harrow, United Kingdom.

Bilbao José Ramón
Department of Genetics, Physical Anthropology and Animal Physiology.
Basque Country University (UPV-EHU).
BioCruces Research Institute.
Bizkaia, Spain.

Bustamante María Ángeles
Department of Nutrition & Food Science
University of the Basque Country (UPV-EHU).
Alava, Spain.

Caminero Alberto
Farncombe Family Digestive Health Research Institute, McMaster University.
Hamilton, Canada.

Carrasco Anna
Gastroenterology and Internal Medicine Services, Hospital Universitari Mutua de Terrassa.
University of Barcelona.
Centro de Investigación Biomédica en Red de Enfermedades He-páticas y Digestivas (CIBERehd).
Terrassa, Barcelona, Spain.

Casellas Francesc
Digestive System Research Unit, Hospital Universitari Vall d'Hebron.
Centro de Investigación Biomédica en Red de Enfermedades He-páticas y Digestivas (CIBERehd).
Barcelona, Spain.

Chirdo Fernando G.
Instituto de Estudios Inmunológicos y Fisiopatológicos-IIFP (UNLP-CONICET). Facultad de Ciencias Exactas. Universidad Nacional de La Plata.
La Plata, Argentina.

Escudero-Hernández C.
Mucosal Immunology Laboratory.
Institute of Biology and Molecular Genetics (IBGM).
University of Valladolid-Spanish National Research Council (CSIC), Consejo Superior de Investigaciones Científicas.
Valladolid, Spain.

Esteve Maria
Gastroenterology and Internal Medicine Services, Hospital Universitari Mutua de Terrassa.
University of Barcelona.
Centro de Investigación Biomédica en Red de Enfermedades He-páticas y Digestivas (CIBERehd).
Terrassa, Barcelona, Spain.

Farré Masip Carme
Clinical Biochemistry Department, Hospital Universitari Sant Joan de Déu.
University of Barcelona.
Esplugues de Llobregat, Barcelona, Spain.

Fernández-Bañares Fernando
Gastroenterology and Internal Medicine Services, Hospital Universitari Mutua de Terrassa.
University of Barcelona.
Centro de Investigación Biomédica en Red de Enfermedades He-páticas y Digestivas (CIBERehd).
Terrassa, Barcelona, Spain.

Fernández-Jiménez Nora
Department of Genetics, Physical Anthropology and Animal Physiology.
Basque Country University (UPV-EHU).
BioCruces Research Institute.
Bizkaia, Spain.

García María Alejandra
Universidad Nacional de La Plata (UNLP) – Consejo Nacional de Investigaciones Científicas y Técnicas CONICET-La Plata, Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA).
Argentina.

Garrote José Antonio
Laboratorio de Genética Molecular.
Servicio de Análisis Clínicos.
Hospital Universitario Rio Hortega.
Valladolid, Spain.

Gil-Humanes Javier
Department of Genetics, Cell Biology and Development and Center for Genome Engineering.
University of Minnesota.
Minneapolis, Minnesota, USA.

Giménez María J.
Institute for Sustainable Agriculture.
Spanish National Research Council (CSIC), Consejo Superior de Investigaciones Científicas.
Córdoba, Spain.

Gómez Manuel
Food Technology Area, College of Agricultural Engineering (ETSIIAA)
University of Valladolid.
Palencia, Spain.

Herrera-de guise Claudia
Digestive System Research Unit, Hospital Universitari Vall d'Hebron.
Centro de Investigación Biomédica en Red de Enfermedades He-páticas y Digestivas (CIBERehd).
Barcelona, Spain.

Knight Stella C.
Antigen Presentation Research Group. Imperial College London. Northwick Park and St Mark's Campus.
Harrow, United Kingdom.

Lucendo Alfredo J.
Gastroenterology Department.
Tomelloso General Hospital.
Tomelloso, Ciudad Real. Spain.

Mariné Meritxell
Gastroenterology and Internal Medicine Services, Hospital Universitari Mutua de Terrassa.
University of Barcelona.
Centro de Investigación Biomédica en Red de Enfermedades He-páticas y Digestivas (CIBERehd).
Terrassa, Barcelona, Spain.

Matos S. María Estela
Instituto de Ciencia y Tecnología de Alimentos (ICTA).
Facultad de Ciencias.
Universidad Central de Venezuela.
Caracas, Venezuela.

McCarville Justin L.
Farncombe Family Digestive Health Research Institute, McMaster University.
Hamilton, Canada.

Mearin María Luisa
Unit of Pediatric Gastroenterology. Department of Pediatrics. Leiden University Medical Center (LUMC).
The Netherlands.

Mena Mª Carmen
Proteomics Facility and Gluten Unit.
Spanish National Centre for Biotechnology.
Spanish National Research Council (CSIC), Consejo Superior de Investigaciones Científicas.
Madrid, Spain.

Molina-Infante Javier
Department of Gastroenterology.
Hospital San Pedro de Alcántara.
Cáceres, Spain.

Montoro Huguet Miguel
Department of Medicine.
University of Zaragoza.
Head of Gastroenterology and Hepatolgy Unit.
San Jorge Hospital.
Huesca, Spain.

Olivares Marta
Microbial Ecology, Nutrition and Health.
Institute of Agrochemistry and Food Technology (IATA).
Spanish National Research Council (CSIC), Consejo Superior de Investigaciones Científicas.
Valencia, Spain.

Palazzo Juan P.
Department of Pathology and Laboratory Medicine.
Sidney Kimmel Medical College at Thomas Jefferson University.
Philadelphia, United States.

Peña Amado Salvador
Professor Emeritus, "Vrije Universiteit" University Medical Center (VUmc), Laboratory of Immunogenetics, Department of Medical Microbiology and Infection Control.
Amsterdam, The Netherlands.

Plaza-Izurieta Leticia
Department of Genetics, Physical Anthropology and Animal Physiology.
Basque Country University (UPV-EHU).
BioCruces Research Institute.
Bizkaia, Spain.

Polanco Isabel
Faculty of Medicine, Autonomous University of Madrid.
Head of Gastroenterology and Pediatric Nutrition Service, La Paz. University Children's Hospital.
Madrid, Spain.

Ribes-Koninckx Carmen
Head of the Pediatric Gastrohepatology Unit.
Hospital Universitario y Politécnico La Fe.
Valencia, Spain.

Rodrigo Luis
Professor Emeritus. University of Oviedo.
Oviedo, Spain.

Rosell Cristina M.
Institute of Agrochemistry and Food Technology (IATA).
Spanish National Research Council (CSIC), Consejo Superior de Investigaciones Científicas.
Valencia, Spain.

Santolaria Piedrafita Santos
Gastroenterology and Hepatology Unit.
San Jorge Hospital.
Huesca, Spain.

Santos Javier
Gastroenterology Department.
Rio Hortega University Hospital.
Valladolid, Spain.

Sanz Yolanda
Microbial Ecology, Nutrition and Health.
Institute of Agrochemistry and Food Technology (IATA).
Spanish National Research Council (CSIC), Consejo Superior de Investigaciones Científicas.
Valencia, Spain.

Sciarini Lorena S.
Institute of Food Science and Technology (ICYTAC).
National Scientific and Technical Research Council (CONICET).
National University of Córdoba (UNC).
Cordoba, Argentina.

Simón Edurne
Department of Nutrition & Food Science
University of the Basque Country (UPV-EHU).
Alava, Spain.

Sousa Carolina
Department of Microbiology and Parasitology.
Faculty of Pharmacy. University of Sevilla.
Sevilla, Spain.

Vaquero Ayala Luis
Gastroenterology Service. University Hospital of León.
Biomedicine Institute. León University.
León, Spain.

Verdu Elena F.
Farncombe Family Digestive Health Research Institute, McMaster University; Hamilton.
Canada.

Viña Sonia Zulma
Universidad Nacional de La Plata (UNLP).
Consejo Nacional de Investigaciones Científicas y Técnicas CONICET-La Plata, Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA).
&
Facultad de Ciencias Agrarias y Forestales, Laboratorio de Investigación en Productos Agroindustriales (LIPA) – Curso Bioquímica y Fitoquímica.
Argentina.

Vivas Alegre Santiago
Gastroenterology Service. University Hospital of León.
Biomedicine Institute. León University.
León, Spain.

Index

Section II

Section III

Epidemiology of Celiac Disease and Non-Celiac Gluten-Related Disorders

Amado Salvador Peña[1], Luis Rodrigo[2]

[1] Emeritus Professor, Laboratory of Immunogenetics, Department of Medical Microbiology and Infection Control, "Vrije Universiteit" VU medical center, Amsterdam, the Netherlands.

[2] Emeritus Professor of Medicine, Department of Gastroenterology and Medicine, University of Oviedo, Oviedo, Spain.

pena.as@gmail.com, lrodrigosaez@gmail.com

Doi: http://dx.doi.org/10.3926/oms.248

How to cite this chapter

Peña AS, Rodrigo L. *Epidemiology of Celiac Disease and Non-celiac Gluten-Related Disorders.* In Arranz E, Fernández-Bañares F, Rosell CM, Rodrigo L, Peña AS, editors. *Advances in the Understanding of Gluten Related Pathology and the Evolution of Gluten-Free Foods*. Barcelona, Spain: OmniaScience; 2015. p. 27-73.

Abstract

The epidemiology of celiac disease and Non-Celiac Gluten related disorders is still an open field to be explored. Not many studies have been conducted in well-defined populations. We have reviewed the prevalence reported in studies using different methodology and addressed the findings obtained in old and new areas with the aim to increase awareness of the frequency of these disorders. The data available suggest the need to plan further proper epidemiological studies in order to understand the natural history of the disease and to assess the burden of these diseases on health systems.

Celiac disease has a global distribution. In childhood celiac disease, epidemiological studies have concentrated so far mainly on the determination of the incidence. There is a relative homogeneous prevalence in descendants of the Caucasian race. However, heterogeneity exists in various countries and continents. In some countries studies in blood donors have contributed to raise awareness of celiac disease and are the only information available.

The average prevalence in the United States of celiac disease is very similar to the one observed in Europe. The highest prevalence was found in the Saharawi population and the lowest in Japan. Recent reports have confirmed the occurrence of celiac disease in China and Central America, countries where previously it was considered that gluten-related disorders were nonexistent.

We reviewed the almost non-existent epidemiology of non-celiac gluten related disorders. The worldwide epidemiology of dermatitis herpetiformis suggests stronger heterogeneity than the observed in celiac disease. The incidence of allergy and autoimmune disease in the

U.S.A. and other industrialized nations is increasing. Gluten-related disorders are no exception.

We expect that an improved knowledge of the worldwide distribution of gluten-related disorders will help us to understand the role of different genetic factors and different environmental influences involved in the pathogenesis of these diseases. At a public level the epidemiological studies are necessary to assess the impact on health systems in the different countries.

Keywords

Epidemiology, celiac disease, non-celiac gluten-related disorders, dermatitis herpetiformis, gluten ataxia, prevalence, incidence.

1. Introduction

Celiac disease is a systemic process of autoimmune nature related to the existence of a permanent intolerance to gluten and manifests itself in genetically susceptible individuals. Although it is primarily a disease of the small intestine, it often affects several organs both in and outside the gastrointestinal tract. The clinical features are protean manifestations often without gastrointestinal symptoms, which make the diagnosis as well as the studies of the pathogenesis and its epidemiology more complicated. Studies of epidemiology are important to help to understand the causes of a disease and to quantify the burden of disease.

In childhood celiac disease, epidemiological studies have concentrated so far mainly on the determination of the incidence. Extensive research and literature exist throughout Europe. The determination on the prevalence of celiac disease in different countries involves children and adults.

In relation to the incidence: a demographically homogeneous Danish population study covering a 15-year period[1] found a crude rate of 0.10 by 1000 live births which was the lowest rate described in any epidemiological study per 1000 live births. In the Netherlands, a similar low incidence of 0.18 per 1000 live births[2] was found during the period from 1976 to 1990. In contrast, in other western countries, higher rates of 0.33 to 8 per 1000 live births were found. In Sweden, between 1970 and 1988 the cumulative incidence of celiac disease at 2 years of age per 1000 live born infants increased significantly from 0.31 in the first birth cohort to 2.93 in the last cohort[3]. According to the authors from Goteborg this incidence makes celiac disease one of the most common chronic diseases among Swedish children.

In Sicily, a maximum cumulative incidence rate by birth cohort was reached in 1986, to 1.65 per 1000 live births. When the incidence rate was adjusted for the years of follow-up, the actual standardized rate was 3 cases per 1000 live births[4] and recently in Spain, a prospective, multicenter, nationwide registry of new cases of celiac disease in children <15 years of age conducted from June 1, 2006 to May 31, 2007 an incidence rate of 7.9 cases of

celiac disease per 1000 live births was found. This rate is much higher than the present incidence rates of celiac disease observed in other European countries[5].

In this chapter we focus on the prevalence of celiac disease in adults. We reviewed the prevalence that has been observed in many studies, using different methods of formal epidemiology. Not many studies have been conducted in well-defined populations. We addressed the findings obtained in old and new areas with the aim to increase awareness of the frequency of celiac disease and to draw attention to the need to plan further proper epidemiological studies in order to understand the natural history of the disease.

Celiac disease has a global distribution. There is a relative homogeneous prevalence in descendants of the Caucasian race. Heterogeneity exists in various countries and continents. Several causes may explain the differences observed between countries, even in regions of the same country. A possible explanation is the variability in the knowledge and experience of general practitioners in the diagnosis of the disease due to the multiple forms of clinical presentation. This probably results in a delayed identification of the disease. Also at the specialist level, the awareness to suspect celiac disease varies. Also there are differences in availability of diagnostic tests and a proper interpretation[6]. It is also well-known that there is an increased incidence of subclinical or silent forms of celiac disease. The most frequent extraintestinal markers of subclinical celiac disease are iron-deficiency anemia, dermatitis herpetiformis, osteoporosis and recurrent aphthous stomatitis. The most frequent presentations in silent celiac disease are found in first-degree relatives, in diverse types of thyroid disease and in patients with insulin-dependent diabetes[7].

We expect that an improved knowledge of the worldwide distribution of celiac disease will help us to understand the role of different genetic factors and different environmental influences involved in the pathogenesis of celiac disease. At a public level the determination by epidemiology of celiac disease will help to assess the impact on health systems in the different countries.

2. Heterogeneity and Difficulties in Performing Epidemiological Studies

Most of the epidemiological studies have been carried out through the determination in blood of specific serological markers of celiac disease, like the detection of IgA anti-gliadin (AGA), IgA anti-tissue transglutaminase (tTG) and/or IgA anti-endomysium antibodies (EmA). The most important genetic markers of susceptibility, the HLA class-II antigens: HLA-DQ2 and/or HLA-DQ8 have not been taken into account in general but only in some studies. Full HLA-DQ typing of all patients has been investigated by Hadithi et al.[8]. Some authors have included the histological findings of the duodenal biopsy specimens, based on the presence of villous atrophy and more recent on the increase of epithelial lymphocytes without villous atrophy.

In the last decade, large genome-wide associations studies (GWAS), have identified more than 40 different non-HLA genes associated to celiac disease. However, these genes, identified by single nucleotide polymorphisms (SNPS) inside or near the genes, will only provide a small contribution to the heritability of celiac disease. No screening studies based on other genetic markers that possibly have an influence on the emergence and development of celiac disease, apart from the HLA genes in chromosome 6, have been published[9-11].

In families with celiac disease, the presence of certain SNPS improves the prediction to suffer from celiac disease in first-degree relatives. In particular in the low HLA risk groups[12]. Romanos et al., have suggested using GWAS as a first step to achieve a better diagnosis and prognosis in high-risk families and in population-based screening[13]. In spite of the advances of the GWAS approach this technology still seems to be premature and expensive. Also the genes linked to the high risk SNPS, have not been identified as yet.

Despite the diversity and weaknesses of the epidemiological studies, e.g. the inability to detect Marsh I celiac patients by specific serological tests, it is acknowledged that the worldwide prevalence of celiac disease ranges from 0.5% to 1%. Differences among populations that have low gluten consumption

and/or a limited access to diagnostic tools exhibit lower prevalence. Therefore, in spite of technical failures, lack of orientation and/or the sampling of insufficient biopsies, the "gold standard" for the diagnosis of celiac disease continues to be the small intestinal biopsy[14]. During endoscopy, multiple biopsies in the duodenal bulb and at least 4 in the distal duodenum should be taken. In a multicenter study carried out in children, it was confirmed that in a 2.4% of 665 patients, the lesions were virtually limited to the duodenal bulb[15]. The majority of the studies published so far, do not comply with the protocol suggested by Bonamico et al.[15]. Taking small intestinal biopsies are not feasible for the screening protocol in population studies and without the improvements in the sensibility and specificity of the serological test to diagnose celiac disease, epidemiological studies would not have advance to the state were we are. Serological studies have allowed the possibility of mass screening programs which are useful in identifying patients who can benefit from gluten-free diet and follow-up, because in the general population celiac disease is frequent and clinically relevant, irrespective of histological severity[16]. Nevertheless, since the economic costs of screening and treatment versus the prevention of morbidity have not been calculated, the time for mass screening has not yet been reached[17].

2.1. Prevalence's at the Global Level

Until recently celiac disease was considered to be a disease of the Europeans. It is now endorsed that it affects all races and there is a gradual change in the global distribution; therefore, it is important to quantify the weight of the burden of the disease in each region. The outcome will have implications for the health systems in the different countries.

In Caucasians the average prevalence is estimated to be about 1-2% according to different studies using specific serological tests, evaluated by different methods and markers[18-20].

The presentation forms of celiac disease have changed remarkably. Until some years ago the classical forms predominated. They were clinically characterized by the presence of chronic diarrhea, steatorrhea, malabsorption, and weight-loss. In

the last few decades, the oligosymptomatic and the atypical forms with less or no digestive symptoms have significantly increased. At present we find a notable predominance of extra-intestinal manifestations, such as iron deficiency anemia and osteoporosis. The increase of celiac disease observed in some studies may be due to the success of the case-finding approach and to the accessibility of more sensitive and specific serological tests for diagnosis[21,22].

2.2. Prevalence Studies in Blood Donors

Epidemiologic studies carried out in volunteer blood donors, are not considered representative of what happens in the general population, in part because of the limitation of age selection. In addition anemia, a relatively frequent presentation form of celiac disease, excludes blood donation by healthy volunteers. However, in some countries studies in blood donors have contributed to raise awareness of celiac disease. For example in North India, using tTG antibodies and duodenal biopsy in tTG positive subjects found in 1,610 blood donors of whom 98.2% were males, a prevalence of celiac disease of 1 in 179 donors (0.56%)[23]; In Madrid, Spain in 2,215 apparently healthy blood donors screened with tTG antibodies, they found a prevalence of celiac disease of 1 in 370 or 1 in 222, if Marsh I lesions in duodenal biopsy were included[24].

In many countries the only available information on the prevalence of celiac disease has been obtained from blood donors[25,26].

Many epidemiological studies use the data obtained in blood donors as control of their studies; for example, in Tunisia, Ghozzi et al. have used EmA antibodies to study two hundred and eleven patients suffering from arthritis or arthralgia with no evident cause and two thousand and five hundred blood donors as control group. Five had EmA antibodies positive which represents 2.37% in the patient group and 0.28% in blood donors[27]. In Italy, Carroccio et al. compared the frequency of tTG and EmA in 80 consecutive non-Hodgkin's lymphoma (NHL) patients (median age, 61 years) with 500 blood donors. The frequency in NHL patients was 1.2% versus blood donors 0.4% (p=0.4). Of interest in this study is that in NHL patients the tTG assay often gave discordant results with the EmA assay. They found a high frequency of tTG

false positive tests[28]. Vancikova et al. in the Czech Republic determined the prevalence of celiac disease using a panel of specific antibodies sequentially in 1,312 healthy blood donors and 102 patients with primary osteoporosis, 58 patients with autoimmune diseases and 365 infertile women. They found AGA and/or tTG and EmA positive in 0.45% of healthy blood donors, 0.98% of osteoporotic patients, 2.7% of patients suffering from autoimmune disease and 1.13% of women with infertility[29]. In Eastern Saudi Arabia, Al Attas et al. found in a group of 145 patients with clinical suspicion of celiac disease that the serological (EmA positive) prevalence was 7.6%. Six of these patients had confirmed celiac disease by intestinal biopsy indicating a prevalence of celiac disease of 4%. In 80 patients with autoimmune diseases 2 were EmA-positive (2.5%) whereas none of the 20 patients with inflammatory bowel disease and none of the 100 healthy blood donors were found to be EmA-positive[30]. In Italy, sera from 220 patients with autoimmune thyroiditis, 50 euthyroid subjects with thyroid nodules and 250 blood donors were tested for tTG and EmA antibodies. The prevalence of celiac disease in patients with autoimmune thyroiditis (3.2%) was significantly higher than that found in blood donors (0.4%) (p=0.022, Fisher's exact test). The 50 euthyroid subjects had no antibodies and no signs of celiac disease[31]. Cuoco et al. found among 92 patients with autoimmune thyroid disease that 4 patients had positive AGA and EmA antibodies and celiac disease; among 90 patients with non-autoimmune thyroid disease only 1 patient had celiac disease. In 236 blood donors one subject (0.4%) was AGA and EMA positive and had celiac disease[32].

These studies confirm that the prevalence in blood donors is not representative for the prevalence of celiac disease in a population and the prevalence is inferior to the one found in diseases known to be associated with celiac disease.

2.3. Prevalence in High-Risk Groups

There are various risk groups, which have a greater predisposition to suffer from the disease than the general population. The most common risk groups are first-degree relatives. They show an average prevalence between

10 to 20%[33]. The family members who carry the HLA-DQ2 and/or HLA-DQ8 antigen and the siblings exhibit the highest risk to suffer from celiac disease. Some authors have found a higher prevalence in brothers, than among the rest of family members[34-36]. Hansen et al found a high prevalence of celiac disease 10.4% (95% C.I. 4.6-16.2%) in young Danish type-1 diabetics[37]. A study in young people under the age of 20 in Sweden, suffering from type-1 diabetes mellitus, found a low prevalence of 0.7% in symptomatic children. However, at 5 year's follow-up after diagnosis, the prevalence increased to 10%[38].

Studies of prevalence of celiac disease have been carried out in high risk groups as shown in Table 1, in first-degree relatives, in individuals with Down´s syndrome and in type-1 insulin-dependent juvenile diabetes (See Table 1). A study in 35 patients with Turner syndrome found a prevalence of celiac disease of 8.1 (3 patients with villous atrophy, or 10.8 (if 4 antiendomysium antibody-positive are considering as suffering from celiac disease. This prevalence is quite high and Bonamico and coworkers have suggested that the association of these two disorders could not be coincidental[39].

Table 1. Risk Groups for the Development of Celiac Disease.

Risk groups for celiac disease (Ref 33 and 40-69)
• First- and Second-Degree Family Members[33]
• Chronic Iron Deficiency Anemia and Refractory Anemia[40,41]
• Osteoporosis, Osteopenia and Osteomalacia[42,43]
• Diabetes Mellitus type-1 (mainly in Children and Adolescents)[44-46]
• Endocrinopathies of Autoimmune Origin, especially Thyroid Diseases[47-49]
• Autoimmune Hepatitis and Primary Biliary Cirrhosis[50]
• Skin Diseases, Dermatitis Herpetiformis, psoriasis[51-53]
• Chromosomal Abnormalities such as Down syndrome[54], Turner syndrome[55], and Williams Syndrome[56,57]
• Neurological disorders, Gluten Ataxia, Epilepsies, Occipital Calcifications, Polyneuropathies[58-61]
• Recurrent Polyarthritis and Poly-Arthralgias[53,62]
• Recurrent Headaches of Migraine-type[63]
• IgA Nephropathy[64-67]
• Repeated Miscarriages, Menstrual Disorders, Infertility[68,69]

2.4. Prevalence in Europe

The prevalence in Europe is slightly higher in the countries of Northern Europe than in the Mediterranean basin. It appears that the differences in prevalence have diminished in recent years[70]. The Scandinavian countries, the United Kingdom and Ireland, have shown a prevalence ranging from 1 to 2.5%[71-74].

A study conducted in Holland among blood donors found a low prevalence of 0.3% in 1,999[75]. A larger study in the Netherlands including 50,760 individuals who had previously participated in two large population-based studies on health status in relation to lifestyle factors, were screened by identification of self-reported adherence to a gluten-free diet and subsequent confirmation of the diagnosis of celiac disease found a prevalence of coeliac disease 0.016% (95% confidence interval 0.008-0.031). In a random sample of 1,440 of all participating subjects were screened by serological tests and by the typing of human lymphocyte antigens. A prevalence of 0.35% (95% C.I. 0.15-0.81) was found. The prevalence of adult-recognized celiac disease in the Netherlands is one of the lowest in Europe, while the prevalence of unrecognized celiac disease is comparable with other Southern European countries, which suggests that celiac disease is underdiagnosed in the Netherlands[76].

A study performed in adolescents in Switzerland showed a prevalence of 0.75%[77].

The mean prevalence of celiac disease in European countries is within the medium range at global level. Although celiac disease was traditionally regarded as a disease with predominance in children, in the last decades the majority of cases are diagnosed in adults[78].

There are longitudinal studies conducted in Finland which confirm the increase in the prevalence of celiac disease, over the past few decades. In a large cohort of 8,000 participants selected from the general population, the average prevalence from 1978 to 1980 was 1%, rising to 2% in the period from 2000 to 2001[79].

A centralized international mass screening of 29,212 participants in Finland, Germany, and Italy by means of a tTG antibody test and when the tTG test was positive or showed border-line results then an EmA test was performed. This large study found a mean prevalence of celiac disease of 2.4% (2.0-2.8) in Finland, 0.3% (0.1-0.4) in Germany, 0.7% (0.4-1.0) in Italy. Sixty-eight percent of antibody-positive individuals showed small-bowel mucosal changes typical for celiac disease (Marsh II/III) lesions[80].

The epidemiological differences between neighboring countries may be due to differences in the socio-economic levels of the different populations, as well as to environmental health measures. The prevalence of tTG in celiac disease is lower in Russian Karelia than in Finland, in spite of the similar frequency of HLA risk haplotypes in both regions. It has been hypothesized that this may be associated with a protective environment characterized by inferior prosperity and standards of hygiene in Karelia[81]. Unfortunately the availability of the diagnostic tools and knowledge of the disease in primary care centers are very different from one country to the other.

2.5. Prevalence in United States

The average prevalence in the United States of celiac disease is very similar to the one observed in Europe. In the last years, a greater awareness of celiac disease and a more active search through information campaigns and the dissemination of knowledge by patient´s associations and internet active groups have contributed to a higher prevalence. Patients with symptomatic celiac disease in the USA showed prevalence of 1.7% in 2003[82].

Another study compared the results obtained with samples collected between the years 1948-54, to two cohorts of samples collected between 1995-2003 and 2006-2008. The authors found a notable increase of up to four times higher in the last periods[83].

In a retrospective comparative study during a 15-year period of follow-up conducted in healthy volunteers found a prevalence of 1 in 501 subjects in

1974 versus 1 in 219 subjects was found[84]. (Table 2 shows a summary of studies performed in U.S.A.).

These epidemiological changes in time of celiac disease in the U.S.A. are seen in many other countries. Not only in the well-known geographical areas where celiac disease is present, such as northern Europe, but also in regions where celiac disease was unknown, such as in Central America and Asian countries. This aspect is described later. To a large extent, the increase in the prevalence of celiac disease is due to the changes in dietary habits in the last few decades. There has been a considerable increase in the consumption of foods containing gluten.

Table 2. Prevalence in United States.

Characteristics	Year (Ref)	Studied population	Prevalence in (%)
Global	2003[82]	13,145 at-risk and non-at-risk	4.54-0.75
Nationwide	2012[83]	7,798 adolescents and adults	0.71
Cohort study	2010[84]	4,351 adults	0.19-0.45

2.6. Prevalence in Africa

In the countries of North Africa, Morocco, Algeria, Tunisia, Libya and Egypt, a high prevalence of celiac disease has been reported. The highest prevalence was found in the Saharawi population of Arab-Berber origin. The prevalence varies between 0.3 to 5.6%. There is a strong association with the haplotype HLA-DR3-DQ2 in the general population and a high consumption of cereal-based foods with gluten with less intake of vegetables and fruits[85].

There is little information on the prevalence of celiac disease in the countries of Sub-Saharan Africa. Some individual studies such as one conducted in Djibouti in the Horn of Africa region clearly confirms that

celiac disease does exist in these regions. The clinical presentation is similar to the observed in the rest of the world. Its diagnosis is more difficult due to the limited knowledge, low index of suspicion of the disease, as well as for the limited facilities to carry out a diagnosis[86]. In Africa and in general in the tropical countries the major causes of iron deficiency anemia are an increased Hookworm infestation, *Schistosoma mansoni* particularly in Egyptian patients and *Trichuris trichiura*. In the continent of Africa, the etiology of anemia in children besides iron deficiency includes malaria, bacterial or viral infections, folate deficiency and sickle-cell disease[87] (Table 3 illustrates as far as we know the only information available on celiac disease in Africa.

Table 3. Prevalence in Africa.

Country	Year (Ref)	Studied population	Prevalence in (%)
Sahara	2010[85]	975 Children and Adults	5.6
Inter-tropical (Horn of Africa)	2008[86]	Children and Adolescents During 3 years period 8 celiac disease patients diagnosed	Unspecified

2.7. Prevalence's in Middle East

Celiac disease is a frequent cause of chronic diarrhea, mainly in children and in patients with type-1 diabetes mellitus in diverse countries of the Middle East, such as Iran, Iraq and Kuwait[88].

The prevalence of celiac disease in adult blood donors in Iran[89] and Israel[26] is 0.6% for both countries; in Syria and Turkey the prevalence is 1.6%[90]. In Anatolia a similar result of 1% was found[91]. An Iranian study in children with chronic diarrhea, found a prevalence of 6.5%[92] and the prevalence in healthy children from Turkey was 1 in 115 (0.86%) based on serology. However the

prevalence of biopsy proven celiac disease was 1 in 158 (0.63%)[93]. Table 4 shows studies on prevalence in the middle east.

Table 4. Prevalence in Middle East.

Country	Year (Ref)	Studied population	Prevalence in (%)
Iran (South)	2013[88]	83 T1DM children	4.80
Iran (Tehran)	2003[89]	2,000 blood donors	0.60
Iran	2005[92]	825 children with chronic diarrhea	6.50
Iran(north and south)	2006[94]	2799 individuals	0.96
Israel	2002[26]	1571 blood donors	0.63
Turkey	2004[95]	2000 blood donors	1.30
Turkey (Anatolia)	2005[91]	906 adults	0.99
Turkey	2005[93]	1263 healthy children	0.86

T1DM = Type-1 Diabetes Mellitus

2.8. Prevalence in Asia

Celiac disease is still uncommon in Asia. Only several cases have been reported. The World Gastroenterology Organization and the Asian Pacific Association of Gastroenterology commissioned a working party to address the key issues in the emergence of celiac disease in Asia[96]. The working party suggested performing studies on the prevalence of celiac disease increase the awareness among physicians and patients as well as increase the recognition of atypical manifestations of the disease. Several problems were identified and represent challenges to be overcome. The working party found variability in performance of serological tests, a lack of population-specific cut-off values for

tests positive or negative, a need to educate dietitians for proper counseling and supervision of patients and improve the gluten-free infrastructure in food supply. To establish celiac patient's advocacy organizations was also emphasized.

2.9. Prevalence in India and Pakistan

In India celiac disease was recently described by an Indian Task Force as being "submerged in an ocean of malnutrition"[97]. Its frequency in India, seems to be higher in the Northern part of the country, creating the so-called "celiac belt". This finding is at least partially explained by the wheat-rice shift from the North to the South[98,99]. The "All India Institute of Medical Sciences" in New Delhi, has prospectively studied adolescent and adult patients presenting with nutritional anemia by tTG antibodies. Positive patients underwent an upper gastrointestinal endoscopy and duodenal biopsy. Ninety-six patients with a median duration of anemia of 11 months (range 1 to 144 months) were screened. 10 patients with nutritional anemia (iron deficiency 9, vitamin B12 deficiency 1) were diagnosed to have celiac disease[41].

There is limited data on the epidemiology in India. Possibly because of the presence of generalized malnutrition and epidemics of chronic diarrhea as well as the difficulty to make a diagnosis of celiac disease[100,101].

In the Delhi area with a large population sample of 2,879 participants, the prevalence of celiac disease was 1.04% (1 in 96)[102]. In a questionnaire-based survey of 4,347 schoolchildren (3–17 years) from Ludhiana, a city in Northern part of Punjab, India the prevalence was 1 in 310[103].

Based on these studies, it is estimated that 5 to 8 million individuals can be expected to have celiac disease in India, yet so far only a few thousand cases appear to have been diagnosed. There is a clear need for further epidemiological studies, in order to determine the regional differences in prevalence.

No epidemiological studies have been reported in Pakistan some studies however have reported patients with celiac disease and explained the difficulties such as in India, in making the diagnosis[104,105].

2.10. Prevalence of Celiac Disease in China

In China the major causative factor—gluten consumption (particularly in the Northern part of the country)—and risk HLA genotypes (HLADQ2 and -DQ8) are present, although with a lower prevalence than in Western countries[106-108]. It appears that there is a clear predominance in its distribution in the North. The knowledge on celiac disease in China has started in recent years, though no formal epidemiological studies have been performed yet[108,109]. In a recent series of 118 children with chronic diarrhea, admitted in pediatric hospitals in four major Chinese cities (Shanghai, Wuhan, Jinan, and Chengdu) the diagnosis of celiac disease was made in 14 patients (11.9%)[110]. The reports are of great importance since they confirmed the occurrence of celiac disease in China, a country where previously was considered to be nonexistent.

Table 5. Prevalence in Asia.

Country	Year (Ref)	Studied population	Prevalence in (%)
India Punjabis (City of Leicester UK)	1993[111]	20 celiac adults	2.7-3.8
India(north)	2011[102]	10,488 adults	1.04
India(Punjab)	2006[103]	4,347 children	0.32
China	2011[110]	199 children with Chronic diarrhea	11.9
Japan	2014[112]	172 IBD adults Positive tTG and DGP No HLA high risk	0

IBD = Inflammatory Bowel Disease; DGP = Deamidated Gliadin Peptides

2.11. Prevalence of celiac disease in Japan and the South East Asian Islands

In Japan, in a recent study, the prevalence of celiac serological markers was 18% in a series of 172 patients with inflammatory bowel disease, compared with the 1.6% in 190 healthy individuals recruited in the general population. However, no duodenal biopsies were performed and no information on genetic markers of susceptibility were available[112].

There are no data on the prevalence of celiac disease in the South East Asian countries, including Malaysia, Korea, Taiwan, the Philippines and the smaller islands of the Pacific. It is assumed that there is a low incidence, due to the low consumption of products containing wheat flour, along with a low frequency of HLA-DQ2 and HLA-DQ8 in the general population. There is a limited availability to study celiac disease by specific serological markers in these countries.

The average prevalence of the risk haplotype HLA-DQ2 is low in Japan and in Southeast Asia. It is present only in 5-10% in the general population and the mean prevalence of the HLA-DQ8 in Asia is less than 5%[113]. The ingestion of wheat-based products is low, but has increased in the past few years.

2.12. Prevalence of Celiac Disease in New Zealand

New Zealand forms part of an island continent whose inhabitants have a large proportion of predecessors of white race, with Anglo-Saxon predominance and genetic haplotypes of susceptibility for celiac disease as well as a high cereal consumption of wheat.

In a comprehensive study conducted in Western Australia, a prevalence of 0.4% of celiac disease was found in this population[113].

A study to determine the prevalence of celiac disease and of gluten avoidance in New Zealand children, found that 1% of these had celiac disease, but 5% reported gluten avoidance. The predictors of gluten avoidance in children without properly diagnosed celiac disease suggest important regional

differences in community belief or medical practice regarding implementation of a gluten-free diet[114].

2.13. Prevalence of Celiac Disease in Australia

In a retrospective analysis performed in an Australian community of stored serum samples taken in 1994-1995 from 3,011 subjects, assays for IgA-tTG and IgG-tTG antibodies were performed. Positive or equivocal samples were retested with a different commercial tTG assay. The prevalence of tTG antibodies in this population is 1.56%; the prevalence of celiac disease is 0.56%. According to these authors, the value of a single positive result of a tTG assay in screening for celiac disease in the community is poor and an assessment with different assays may decrease the need for gastroscopy and distal duodenal biopsy[115].

In an Australian rural community a prevalence of 12 of 3,011 found (1 in 251) was based on positive EmA antibodies and duodenal biopsy compatible with celiac disease[116].

Table 6. Prevalence in Australia and New Zealand.

Country	Year (Ref)	Studied population	Prevalence in (%)
New Zealand	2000[113]	1,064 adults	1.2
Australia	2001[116]	3,011 adults	0.4
New Zealand	2002[114]	916 children	1.0
Australia	2009[115]	3,011 adults	0.6

2.14. Prevalence of Celiac Disease in Mexico

Information regarding celiac disease in Mexico is limited; however, on the basis of the prevalence of tTGA in a large group of healthy blood donors a high prevalence of tTGA positivity 27 of 1,009 (2.6%) was found. This

suggests that in the adult Mexican Mestizo population the presence of celiac disease is high[117]. A recent update[118] using the weighted prevalence for double-positive serology IgA tTG and IgA EmA the prevalence was 0.59% (95% CI, 0.27 – 1.29). A high prevalence of 5.9% biopsy-proven celiac disease was found in Mexican Mestizo patients with type-1 diabetes mellitus[119]. Interestingly, in a study of prevalence in United States in 7,798 persons aged 6 years and older who participated in the National Health and Nutrition Examination Survey 2009-2010, they found a prevalence of 0.71% (95% confidence interval (CI), 0.58-0.86%) i.e. 1 in 141. This study also reported celiac disease to be rare among minority groups, including Hispanics. The prevalence was 0.03%, or 1 of 2,519 and in Mexican Americans (0%)[83]. This discrepancy illustrates the possible effect of environmental factors in determining the prevalence of celiac disease in people with the same genetic background.

2.15. Prevalence of Celiac Disease in El Salvador and Costa Rica

There are no epidemiological studies published in Central America. The first study using the modified Marsh classification and the full HLA-DQ typing in El Salvador, has been recently published. Of the 32 cases, 23 were celiac disease risk genotype carriers[120]. Similar results have been reported from Costa Rica, in 35 patients[121].

2.16. Prevalence of Celiac Disease in Brazil

A study carried out in Brazil, which included a total of 214 symptomatic children, aged between 12 and 36 months, were studied by serological screening and subsequent confirmation by jejunal biopsy in the positive cases. Five cases (2.3%) were found[122]. The prevalence of celiac disease in a group of first-degree relatives of Brazilian celiac patients, between March 2001 and November 2004, in two centers in Brasilia was studied. They found among the 188 first-degree relatives a prevalence of 4.8%[123].

In another study in adults carried out in Brazil, conducted in an urban area on 2,086 blood donors reported a prevalence of 1.4%. This prevalence is lower than the previous study but it is similar to the prevalence found in European countries or in North America[124].

2.17. Prevalence of Celiac Disease in Argentina

Similar studies in hospitals have been carried out in several cities in Argentina including a multicenter study of the prevalence in a pediatric population in 5 urban districts of Argentina. A total of 2,219 patients, ages between 3 and 16 years were analyzed. A prevalence of celiac disease 1.26% was found. 33% of the cases were symptomatic[125].

In the adult population in Argentina, Gómez et al., found a prevalence of 1.16%, in a study of 2,000 individuals, chosen in the general population[126].

Table 7. Prevalence in Mexico, Central and South America.

Country	Year (Ref)	Studied population	Prevalence in (%)
Mexico	2006[117]	1,009 adults tTG screening	2.6
El Salvador	2014[120]	32 adults	Undefined
Costa Rica	2014[121]	35 adults	Undefined
Brazil(Brasilia)	2010[122]	214 children	2.3
Brazil(Brasilia)	2008[123]	188 First-degree relatives	4.8
Brazil(Curitiba)	2,086[124]	Adults blood donors	0.23
Argentina	2012[125]	2,219 children	1.26
Argentina(La Plata)	2001[126]	2,000 adults	0.59

2.18. Conclusions

The true prevalence of celiac disease is still impossible to ascertain. A multidisciplinary approach to make the diagnosis is necessary. The collaboration between clinicians, immunologists, geneticists, and pathologists is essential to integrate clinical, serological, genetic, histological criteria as well as the response to the gluten-free diet. Many patients have atypical symptoms or none at all. Many patients have minimal lesions without villous atrophy such as those patients with Marsh 1 lesion. These patients require a differential diagnosis that is beyond the current epidemiological studies.

The most challenging observation to understand the epidemiology of the disease is the observation demonstrating that there is a clear difference in prevalence between children and adults.

In a large study of 4,230 subjects in Terrassa, Barcelona, Spain found population-based celiac disease prevalence of 1:250. The prevalence of celiac disease in childhood was five times higher than in adults. The authors correctly have pointed out that whether this difference is due to environmental factors in childhood or due to latent celiac disease in adulthood. This remains to be demonstrated in prospective longitudinal studies[127]. The outcome will have consequences to understand the epidemiology and natural history of the disease.

Major changes that are bound to alter the epidemiology of celiac disease and gluten related disorders are taking place such as the rice cultivation in several regions in China where wheat cultivation now predominates. Maize from the Mexican highlands has been the dominant food in Mexico and Central America, the potato developed in the Peruvian Andes and the Quinoa in Bolivia, is now complemented with gluten containing diets.

Several environmental changes point to an increase of celiac disease in these regions. The eradication of intestinal parasites which contributes to a change in the intestinal immune response, from TH2 to TH1, changes in the intestinal microbiota probably in individuals living in urban areas, changes in dietary habits due to the influence of "fast foods" and changes in traditional

diets as described above as well as the widespread use of antibiotics will in some way or other alter the epidemiology of celiac disease[106].

The review presented in this chapter indicates the worldwide importance of celiac disease and the gluten related pathologies and should help to clarify the need to implement measures in National Health systems, to cope with this expanding disease. As Greco et al have estimated:-"*In the near future, the burden of celiac disease will increase tremendously. Few Mediterranean countries are able to face this expanding epidemic*"[128].

3. Epidemiology of Non-celiac Gluten-Related Disorders

3.1. Epidemiology of Dermatitis Herpetiformis

A study in the UK at the Clinical Practice Research Datalink of the University of Nottingham, has quantified the incidence and prevalence of celiac disease and dermatitis herpetiformis between 1990 and 2011. A total of 9,087 incident cases of celiac disease and 809 incident cases of dermatitis herpetiformis were identified. Although dermatitis herpetiformis has been called celiac disease of the skin[129] to underline the common genetic background and the relation to gluten, the expression of either or both of these diseases is different. In Nottingham the incidence rate of celiac disease increased from 5.2 per 100,000 (95% CI, 3.8-6.8) to 19.1 per 100,000 person-years (95% CI, 17.8-20.5; IRR, 3.6; 95% CI, 2.7-4.8) while the incidence of dermatitis herpetiformis has decreased over the same time period from 1.8 per 100,000 to 0.8 per 100,000 person-years (average annual IRR, 0.96; 95% CI, 0.94-0.97)[72]. Although the prevalence of nutritional deficiencies, autoimmune diseases, and lymphoma occurred at a similar rate in patients with dermatitis herpetiformis as in patients with celiac disease without dermatitis herpetiformis, a recent study has confirmed that the prevalence of villous atrophy is significantly higher in the patients who presented with celiac disease than in those who presented with dermatitis herpetiformis only (61.8vs.12.5%;p = 0.005)[51].

At least two studies in the USA and in Finland show an expected prevalence of dermatitis herpetiformis in families significantly higher than previously calculated. This is possibly due to shared genetic factors and environment factors[130,131]. In Tampere and Helsinki, an analysis of 105 families with dermatitis herpetiformis showed that 13.6% of parents, 18.7% of siblings and 14.0% of children were affected. The authors suggested that this segregation pattern fits well to a dominant mode of Mendelian inheritance. However they added that gender may also be important because the first-degree relatives affected with dermatitis herpetiformis were more often females[131]. In Brazil two pairs of monozygotic twins have been studied after a gluten-free diet for 16 to 21 years. They were concordant for celiac disease. However, dermatitis herpetiformis was present in three patients belonging to the two pairs of twins, demonstrating partial concordance of dermatitis herpetiformis in monozygotic twins[132].

These observations fit with a multifactorial and polygenic disease pathogenesis similar to other autoimmune diseases.

At the Tampere University Hospital, the causes of death during 1971-2010 were studied in 476 consecutive patients with dermatitis herpetiformis diagnosed from 1970 onwards. 97.7% of the patients adhered to a gluten-free diet. All-cause and cerebrovascular disease mortality was significantly reduced. The standardized mortality rate due to lymphoproliferative malignancies was significantly increased (6.86) only in the first 5 years of follow-up[133].

The worldwide epidemiology of dermatitis herpetiformis shows a great heterogeneity. In Asia dermatitis herpetiformis is very rare. Twenty two cases have been described from China[134] and 35 cases in Japan[135]. Very few cases have been reported from Iran[136], Singapore[137], and Maylasia[138].

In southern Sweden, there were 96 cases in a defined population of 425,000 inhabitants. The incidence of dermatitis herpetiformis was 1.05-1.13/100,000 inhabitants/year and the prevalence was approximately 20 to 25 per 100,000 inhabitants.

In Utah in the U.S.A. with a main population of European descendants have higher incidence prevalence than in Asia. The prevalence of dermatitis herpetiformis in Utah in 1987 was 11.2 per 100,000. The mean incidence for the years 1978 through 1987 was 0.98 per 100,000 per year. The mean age at onset of symptoms for male patients was 40.1 years, and the one for female patients was 36.2 years. The male-female ratio was 1.44:1[139].

In Buenos Aires, Argentina 18 patients with dermatitis herpetiformis were found to have increased intestinal permeability even in patients with no evidence of histologic damage in biopsy specimens. They found that patients with linear IgA dermatosis appear to be a distinct population without gluten sensitivity[140]. Since the majority of patients with celiac disease and dermatitis herpetiformis have European ancestors it would be interesting to perform proper epidemiological studies in Argentina to study the environmental triggers.

The highest incidence and prevalence of dermatitis herpetiformis has been reported in Finland but there is some evidence that contrary to celiac disease, dermatitis herpetiformis is diminishing. The prevalence of dermatitis herpetiformis was 75.3 per 100,000 which is eight times lower than the prevalence of celiac disease in the Tampere area. The annual incidence of dermatitis herpetiformis for the entire 40 year period was 3.5 per 100,000, and in the three 10-year periods 5.2, 2.9 and 2.7 per 100,000, respectively[141].

The worldwide epidemiology of dermatitis herpetiformis suggests stronger heterogeneity than the observed in celiac disease.

3.2. Epidemiology of Gluten Ataxia

As pointed out by Hadjivassiliou gluten ataxia is one of the most common immune-mediated cerebellar ataxias and one of the few ataxias that are potentially treatable[142].

From a total of two hundred and twenty-four patients with various causes of ataxia in North Trent England, 132 were diagnosed as sporadic idiopathic ataxia patients. In The Institute of Neurology in London England 44 patients

were diagnosed with sporadic ataxia. From these groups of patients, 41% and 32% respectively had AGA antibodies and were confirmed to have gluten ataxia[143]. Familial and isolated cases of gluten ataxia have been described in Spain[144] and in Japan[145].

Further studies in neurological centers in other countries are necessary, because the experimental evidence seems to be incomplete. There is sufficient evidence to support immune-mediated basal ganglia dysfunction as an emerging clinical concept. The central nervous system dysfunction may be caused by a deviant immune response triggered by exogenous antigens such as gluten or streptococcal infection[146].

3.3. Epidemiology of Non-celiac Gluten Sensitivity

The incidence of allergy and autoimmune disease in the U.S.A. and other industrialized nations is increasing. Gluten-related disorders are no exception[147]. Many researchers particularly in the U.S.A. claim that non-celiac gluten sensitivity is the most common syndrome of gluten intolerance[148]. We have previously summarized the current thinking on non-celiac gluten sensitivity as follows[149]. This issue may have been the one with the greatest impact during the last decades, especially on the internet, in patients' associations and in the food industry. There is a lack of systematic studies which could improve the understanding and definition of this syndrome for the patients and assess the impact on public health services. We fully agree with the view expressed by Corazza and his group, who emphasize the lack of a clear definition of non-celiac gluten sensitivity. This hindrance is fundamentally related to the cause of this proteiform disease whose symptoms are presumably caused by different mechanisms[150].

It is therefore not surprising that Spence of Glasgow, Scotland wrote an article: - "*Do you think non-celiac gluten sensitivity exists*?" He describes the results of a recent poll undertaken by the general practitioners' journal in England, the British Medical Journal. 66% of the 941 who were asked and have had access to a higher education, responded that they believe it does exist, despite a lack of scientific evidence. "*Besides, about 20% of the*

American population purchase gluten-free products and, by 2017, it is estimated that this market will be worth about 6.6 million dollars"[151].

Recently Aziz et al. have determined the population prevalence of self-reported gluten sensitivity and referral characteristics to secondary care in Sheffield, UK. This study on a population-based questionnaire screened for gluten sensitivity, related symptoms and exclusion of celiac disease found that the self-reported prevalence for non-celiac gluten sensitivity was 13% (female 79%, mean age 39.5 years). These individuals had an increased prevalence of complying with the Rome III criteria for irritable bowel syndrome, in comparison with those without gluten sensitivity. The majority of patients with non-celiac gluten sensitivity have clinical and immunological differences to celiac disease[152].

3.4. Epidemiology of Gluten Allergy

According to clinical presentations and allergy testing, there are three types of food allergy: IgE mediated, mixed (IgE/Non-IgE), and non-IgE mediated (cellular, delayed type hypersensitivity). Among the most common of these allergies in children is wheat allergy. The prevalence of this kind of allergy in infancy is increasing and may affect up to 15-20% of infants. According to Ho et al. the alarming rate of increase calls for a public health approach in the prevention and treatment of food allergy in children[153]. The epidemiology of food allergy in general is outside the scope of this chapter. Wheat is one of the most common allergy caused by food. A recent study has reported that the prevalence in Japanese adults was found to be 0.21% by using a combination of questionnaire-based examination, skin-prick test and serum omega-5 gliadin-specific IgE test[154].

The prevalence of food allergy was investigated among patients reporting to The Institute of Child Health and Mediland Diagnostics in Kolkata, India. Among the 5,161 patients tested, wheat (22%) was the predominant allergen[155]. A large recent review has found that the overall prevalence of food allergy in Asia is fairly comparable to the West, although this kind of types of allergies differ in order of relevance in the consumption of type of food. Wheat allergy, though uncommon in most Asian countries, is the most

common cause of anaphylaxis in Japan and Korea and is increasing in Thailand[156].

See also the chapter with detail description of recent advances of gluten allergy.

3.5. Burden of Disease

As stated in the introduction and recently underscored:-"understanding epidemiology is crucial for hypothesizing about causes and quantifying the burden of disease"[72]. It is well known that patients with celiac disease have a greater burden of disease than the general population because of osteoporosis, autoimmune diseases, and malignancies (See also chapter by Lucendo et al.).

The statement made 10 years ago by Green et al. is valid today:-"There is a need for screening studies of patients with conditions associated with celiac disease to determine whether the large numbers of people with undiagnosed celiac disease currently are seeking health care"[157]. Currently, there is a need to quantify the increase in wheat allergy, as part of the increase in allergic conditions. Also it is necessary to quantify the relevance of other gluten related disorders for the awakening of the officers of national health systems to assess the total burden of these diseases and to be prepared for the application of adequate funds.

Greco and coworkers have called attention to the burden of celiac disease in the Mediterranean countries. They have calculated that in the next 10 years, the Mediterranean area will have about half a billion inhabitants, of which 120 million will be children. The projected number of celiac disease diagnoses in 2020 is 5 million cases (1 million celiac children), with a relative increase of 11% compared to 2010. Based on the 2010 rate, there will be about 550,000 symptomatic adults and about 240,000 sick children: 85% of the symptomatic patients will suffer from gastrointestinal complaints, 40% are likely to have anemia, 30% will likely have osteopenia, 20% of children will have short stature, and 10% will have abnormal liver enzymes[128]. The economic impact as discussed earlier with reference to non-celiac gluten sensitivity is already having major consequences, particularly in the U.S.A. In cases of non-celiac gluten sensitivity

the priority is in finding adequate criteria and tests to confirm the diagnosis and clearly separate the different entities which are included in this diagnosis.

References

1. Weile B, Krasilnikoff PA. *Low incidence rates by birth of symptomatic coeliac disease in a Danish population of children.* Acta Paediatr. 1992; 81: 394-8.
   http://dx.doi.org/10.1111/j.1651-2227.1992.tb12256.x
   PMid:1498504
2. George EK, Mearin ML, van der Velde EA, et al. *Low incidence of childhood celiac disease in The Netherlands.* Pediatr Res. 1995; 37: 213-8.
   http://dx.doi.org/10.1203/00006450-199502000-00015
   PMid:7731760
3. Ascher H, Krantz I, Kristiansson B. *Increasing incidence of coeliac disease in Sweden.* Arch Dis Child. 1991; 66: 608-11.
   http://dx.doi.org/10.1136/adc.66.5.608
   PMid:2039251 PMCid:PMC1792945
4. Magazzu G, Bottaro G, Cataldo F, et al. *Increasing incidence of childhood celiac disease in Sicily: results of a multicenter study.* Acta Paediatr. 1994; 83: 1065-9.
   http://dx.doi.org/10.1111/j.1651-2227.1994.tb12987.x
   PMid:7841706
5. Cilleruelo ML, Roman-Riechmann E, Sanchez-Valverde F, et al. *Spanish national registry of celiac disease: incidence and clinical presentation.* J Pediatr Gastroenterol Nutr. 2014; 59: 522-6.
   http://dx.doi.org/10.1097/MPG.0000000000000446
   PMid:24886992
6. Bai D, Brar P, Holleran S, et al. *Effect of gender on the manifestations of celiac disease: evidence for greater malabsorption in men.* Scand J Gastroenterol. 2005; 40: 183-7.
   http://dx.doi.org/10.1080/00365520510011498
   PMid:15764149
7. Tursi A, Giorgetti G, Brandimarte G, et al. *Prevalence and clinical presentation of subclinical/silent celiac disease in adults: an analysis on a 12-year observation.* Hepato-gastroenterology. 2001; 48: 462-4.
   PMid:11379333
8. Hadithi M, von Blomberg B, Crusius J, et al. *Accuracy of serologic tests and HLA-DQ typing for diagnosing celiac disease.* Ann Intern Med. 2007; 147: 294-302.
   http://dx.doi.org/10.7326/0003-4819-147-5-200709040-00003
   PMid:17785484

9. Karell K, Louka AS, Moodie SJ, et al. *HLA types in celiac disease patients not carrying the DQA1*05-DQB1*02 (DQ2) heterodimer: results from the European Genetics Cluster on Celiac Disease.* Hum Immunol. 2003; 64: 469-77.
http://dx.doi.org/10.1016/S0198-8859(03)00027-2

10. Megiorni F, Mora B, Bonamico M, et al. *HLA-DQ and risk gradient for celiac disease.* Hum Immunol. 2009; 70: 55-9.
http://dx.doi.org/10.1016/j.humimm.2008.10.018
PMid:19027045

11. Sollid LM. *Coeliac disease: dissecting a complex inflammatory disorder.* Nat Rev Immunol. 2002; 2: 647-55.
http://dx.doi.org/10.1038/nri885
PMid:12209133

12. Izzo V, Pinelli M, Tinto N, et al. *Improving the estimation of celiac disease sibling risk by non-HLA genes.* PLoS One. 2011; 6: e26920.
http://dx.doi.org/10.1371/journal.pone.0026920
PMid:22087237 PMCid:PMC3210127

13. Romanos J, van Diemen CC, Nolte IM, et al. *Analysis of HLA and non-HLA alleles can identify individuals at high risk for celiac disease.* Gastroenterology. 2009; 137: 834-40, 840 e1-3.

14. Villanacci V, Ceppa P, Tavani E, et al. *Coeliac disease: the histology report.* Dig Liver Dis. 2011; 43 Suppl 4: S385-95.
http://dx.doi.org/10.1016/S1590-8658(11)60594-X

15. Bonamico M, Thanasi E, Mariani P, et al. *Duodenal bulb biopsies in celiac disease: a multicenter study.* J Pediatr Gastroenterol Nutr. 2008; 47: 618-22.
http://dx.doi.org/10.1097/MPG.0b013e3181677d6e
PMid:18979585

16. Marine M, Fernandez-Banares F, Alsina M, et al. *Impact of mass screening for gluten-sensitive enteropathy in working population.* World J Gastroenterol. 2009; 15: 1331-8.
http://dx.doi.org/10.3748/wjg.15.1331
PMid:19294762 PMCid:PMC2658830

17. Mearin ML, Ivarsson A, Dickey W. *Coeliac disease: is it time for mass screening?* Best Pract Res Clin Gastroenterol. 2005; 19: 441-52.
http://dx.doi.org/10.1016/j.bpg.2005.02.004
PMid:15925848

18. Rostom A, Murray JA, Kagnoff MF. *American Gastroenterological Association (AGA) Institute technical review on the diagnosis and management of celiac disease.* Gastroenterology. 2006; 131: 1981-2002.
http://dx.doi.org/10.1053/j.gastro.2006.10.004
PMid:17087937

19. Green PH, Cellier C. *Celiac disease.* N Engl J Med. 2007; 357: 1731-43.
http://dx.doi.org/10.1056/NEJMra071600
PMid:17960014

20. Catassi C, Yachha SK. *The global village of celiac disease.* In: Fasano A, Troncone R, Branski D, eds. Frontiers in celiac disease. Volume 23-31. Basel: Switzerland Karger, 2008.
http://dx.doi.org/10.1159/000128610

21. Murray JA, Van Dyke C, Plevak MF, et al. *Trends in the identification and clinical features of celiac disease in a North American community, 1950-2001.* Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2003; 1: 19-27.
http://dx.doi.org/10.1053/jcgh.2003.50004
PMid:15017513

22. McGowan KE, Castiglione DA, Butzner JD. *The changing face of childhood celiac disease in north america: impact of serological testing.* Pediatrics. 2009; 124: 1572-8.
http://dx.doi.org/10.1542/peds.2008-2373
PMid:19948628

23. Kochhar R, Sachdev S, Kochhar R, et al. *Prevalence of coeliac disease in healthy blood donors: a study from north India.* Dig Liver Dis. 2012; 44: 530-2.
http://dx.doi.org/10.1016/j.dld.2012.01.004
PMid:22497903

24. Garcia Novo MD, Garfia C, Acuña Quiros MD, et al. *Prevalence of celiac disease in apparently healthy blood donors in the autonomous community of Madrid.* Rev Esp Enferm Dig. 2007; 99: 337-42.
PMid:17883297

25. Melo SB, Fernandes MI, Peres LC, et al. *Prevalence and demographic characteristics of celiac disease among blood donors in Ribeirao Preto, State of Sao Paulo, Brazil.* Dig Dis Sci. 2006; 51: 1020-1025.
http://dx.doi.org/10.1007/s10620-006-9340-9
PMid:16758312

26. Shamir R, Lerner A, Shinar E, et al. *The use of a single serological marker underestimates the prevalence of celiac disease in Israel: a study of blood donors.* Am J Gastroenterol. 2002; 97: 2589-94.
http://dx.doi.org/10.1111/j.1572-0241.2002.06028.x
PMid:12385444

27. Ghozzi M, Sakly W, Mankai A, et al. *Screening for celiac disease, by endomysial antibodies, in patients with unexplained articular manifestations.* Rheumatol Int. 2014; 34: 637-42.

http://dx.doi.org/10.1007/s00296-013-2906-x
PMid:24292850

28. Carroccio A, Iannitto E, Di PL, et al. *Screening for celiac disease in non-Hodgkin's lymphoma patients: a serum anti-transglutaminase-based approach.* Dig Dis Sci. 2003; 48: 1530-1536.

http://dx.doi.org/10.1023/A:1024811707311
PMid:12924648

29. Vancikova Z, Chlumecky V, Sokol D, et al. *The serologic screening for celiac disease in the general population (blood donors) and in some high-risk groups of adults (patients with autoimmune diseases, osteoporosis and infertility) in the Czech republic.* Folia Microbiol (Praha). 2002; 47: 753-8.

http://dx.doi.org/10.1007/BF02818684

30. Al Attas RA. *How common is celiac disease in Eastern Saudi Arabia?* Ann Saudi Med. 2002; 22: 315-9.

PMid:17146251

31. Volta U, Ravaglia G, Granito A, et al. *Coeliac disease in patients with autoimmune thyroiditis.* Digestion. 2001; 64: 61-5.

http://dx.doi.org/10.1159/000048840
PMid:11549838

32. Cuoco L, Certo M, Jorizzo RA, et al. *Prevalence and early diagnosis of coeliac disease in autoimmune thyroid disorders.* Ital J Gastroenterol Hepatol. 1999; 31: 283-7.

PMid:10425571

33. Freeman HJ. *Risk factors in familial forms of celiac disease.* World J Gastroenterol. 2010; 16: 1828-31.

http://dx.doi.org/10.3748/wjg.v16.i15.1828
PMid:20397258 PMCid:PMC2856821

34. Farre C, Humbert P, Vilar P, et al. *Serological markers and HLA-DQ2 haplotype among first-degree relatives of celiac patients. Catalonian Coeliac Disease Study Group.* Dig Dis Sci. 1999; 44: 2344-9.

http://dx.doi.org/10.1023/A:1026685527228
PMid:10573385

35. Maki M, Mustalahti K, Kokkonen J, et al. *Prevalence of Celiac disease among children in Finland.* N Engl J Med. 2003; 348: 2517-24.

http://dx.doi.org/10.1056/NEJMoa021687
PMid:12815137

36. Book L, Zone JJ, Neuhausen SL. *Prevalence of celiac disease among relatives of sib pairs with celiac disease in U.S. families.* Am J Gastroenterol. 2003; 98: 377-81.

http://dx.doi.org/10.1111/j.1572-0241.2003.07238.x
PMid:12591058

37. Hansen D, Bennedbaek FN, Hansen LK, et al. *High prevalence of coeliac disease in Danish children with type I diabetes mellitus.* Acta Paediatr. 2001; 90: 1238-43.

http://dx.doi.org/10.1111/j.1651-2227.2001.tb01568.x
PMid:11808892

38. Larsson K, Carlsson A, Cederwall E, et al. *Annual screening detects celiac disease in children with type 1 diabetes.* Pediatr Diabetes 2008; 9: 354-9.

http://dx.doi.org/10.1111/j.1399-5448.2008.00367.x
PMid:18774995

39. Bonamico M, Pasquino AM, Mariani P, et al. *Prevalence and clinical picture of celiac disease in Turner syndrome.* J Clin Endocrinol Metab. 2002; 87: 5495-8.

http://dx.doi.org/10.1210/jc.2002-020855
PMid:12466343

40. Smukalla S, Lebwohl B, Mears JG, et al. *How often do hematologists consider celiac disease in iron-deficiency anemia? Results of a national survey.* Clin Adv Hematol Oncol. 2014; 12: 100-5.

PMid:24892255

41. Kavimandan A, Sharma M, Verma AK, et al. *Prevalence of celiac disease in nutritional anemia at a tertiary care center.* Indian J Gastroenterol. 2014; 33: 114-8.

http://dx.doi.org/10.1007/s12664-013-0366-6
PMid:23996798

42. Hjelle AM, Apalset E, Mielnik P, et al. *Celiac disease and risk of fracture in adults-a review.* Osteoporosis international: a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA. 2014; 25: 1667-76.

http://dx.doi.org/10.1007/s00198-014-2683-8
PMid:24691647

43. Stobaugh DJ, Deepak P, Ehrenpreis ED. *Increased risk of osteoporosis-related fractures in patients with irritable bowel syndrome.* Osteoporosis international: a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA. 2013; 24: 1169-75.

http://dx.doi.org/10.1007/s00198-012-2141-4
PMid:22993020

44. Troncone R, Discepolo V. *Celiac disease and autoimmunity.* J Pediatr Gastroenterol Nutr. 2014; 59 Suppl 1: S9-S11.

http://dx.doi.org/10.1097/01.mpg.0000450394.30780.ea
PMid:24979198

45. Liu E, Lee HS, Aronsson CA, et al. *Risk of pediatric celiac disease according to HLA haplotype and country.* N Engl J Med. 2014; 371: 42-9.

http://dx.doi.org/10.1056/NEJMoa1313977
PMid:24988556 PMCid:PMC4163840

46. Virta LJ, Kolho KL. *The risk of contracting pediatric inflammatory bowel disease in children with celiac disease, epilepsy, juvenile arthritis and type 1 diabetes-a nationwide study.* J Crohns Colitis. 2013; 7: 53-7.

http://dx.doi.org/10.1016/j.crohns.2012.02.021
PMid:22445838

47. Mehrdad M, Mansour-Ghanaei F, Mohammadi F, et al. *Frequency of celiac disease in patients with hypothyroidism.* J Thyroid Res. 2012; 2012: 201538.

http://dx.doi.org/10.1155/2012/201538
PMid:22545223 PMCid:PMC3321550

48. Megiorni F, Pizzuti A. *HLA-DQA1 and HLA-DQB1 in Celiac disease predisposition: practical implications of the HLA molecular typing.* J Biomed Sci. 2012; 19: 88.

http://dx.doi.org/10.1186/1423-0127-19-88
PMid:23050549 PMCid:PMC3482388

49. Ludvigsson JF, Kampe O, Lebwohl B, et al. *Primary Hyperparathyroidism and Celiac Disease: A Population-Based Cohort Study.* J Clin Endocrinol Metab. 2012; 97: 897-904.

http://dx.doi.org/10.1210/jc.2011-2639
PMid:22238405 PMCid:PMC3319223

50. Efe C, Wahlin S, Ozaslan E, et al. *Autoimmune hepatitis/primary biliary cirrhosis overlap syndrome and associated extrahepatic autoimmune diseases.* Eur J Gastroenterol Hepatol. 2012; 24: 531-4.

http://dx.doi.org/10.1097/MEG.0b013e328350f95b
PMid:22465972

51. Krishnareddy S, Lewis SK, Green PH. *Dermatitis herpetiformis: clinical presentations are independent of manifestations of celiac disease.* Am J Clin Dermatol. 2014; 15: 51-6.

http://dx.doi.org/10.1007/s40257-013-0051-7
PMid:24293087

52. Plotnikova N, Miller JL. *Dermatitis herpetiformis.* Skin therapy letter. 2013; 18: 1-3.

PMid:23674144

53. Iqbal T, Zaidi MA, Wells GA, et al. *Celiac disease arthropathy and autoimmunity study.* J Gastroenterol Hepatol. 2013; 28: 99-105.

http://dx.doi.org/10.1111/j.1440-1746.2012.07272.x
PMid:22988822

54. Malt EA, Dahl RC, Haugsand TM, et al. *Health and disease in adults with Down syndrome.* Tidsskr Nor Laegeforen. 2013; 133: 290-4.

http://dx.doi.org/10.4045/tidsskr.12.0390
PMid:23381164

55. Grossi A, Palma A, Zanni G, et al. *Multiorgan autoimmunity in a Turner syndrome patient with partial monosomy 2q and trisomy 10p.* Gene. 2013; 515: 439-43.

http://dx.doi.org/10.1016/j.gene.2012.12.007
PMid:23262341

56. Giannotti A, Tiberio G, Castro M, et al. *Coeliac disease in Williams syndrome.* J Med Genet. 2001; 38: 767-8.

http://dx.doi.org/10.1136/jmg.38.11.767
PMid:11694549 PMCid:PMC1734770

57. Dirks MH. *Practical considerations for the identification and follow-up of children with celiac disease.* Paediatr Child Health. 2004; 9: 714-8.

PMid:19688081 PMCid:PMC2724146

58. Ruuskanen A, Kaukinen K, Collin P, et al. *Gliadin antibodies in older population and neurological and psychiatric disorders.* Acta Neurol Scand. 2013; 127: 19-25.

http://dx.doi.org/10.1111/j.1600-0404.2012.01668.x
PMid:22494246

59. Bukulmez A, Dalgic B, Gunduz B, et al. *The evaluation of hearing loss in children with celiac disease.* Int J Pediatr Otorhinolaryngol. 2013; 77: 175-9.

http://dx.doi.org/10.1016/j.ijporl.2012.10.012
PMid:23137854

60. Hadjivassiliou M, Aeschlimann P, Strigun A, et al. *Autoantibodies in gluten ataxia recognize a novel neuronal transglutaminase.* Ann Neurol. 2008; 64: 332-43.

http://dx.doi.org/10.1002/ana.21450
PMid:18825674

61. Crosato F, Senter S. *Cerebral occipital calcifications in celiac disease.* Neuropediatrics. 1992; 23: 214-7.

http://dx.doi.org/10.1055/s-2008-1071345
PMid:1407390

62. De Maddi F, Pellegrini F, Raffaele CG, et al. *Celiac disease and juvenile idiopathic arthritis: a still enigmatic crossover.* Scand J Gastroenterol. 2013; 48: 511-2.

http://dx.doi.org/10.3109/00365521.2013.772232
PMid:23448357

63. Zelnik N, Pacht A, Obeid R, et al. *Range of neurologic disorders in patients with celiac disease.* Pediatrics. 2004; 113: 1672-6.

http://dx.doi.org/10.1542/peds.113.6.1672
PMid:15173490

64. Welander A, Sundelin B, Fored M, et al. *Increased Risk of IgA Nephropathy Among Individuals With Celiac Disease.* J Clin Gastroenterol. 2013; 47: 678-83.

http://dx.doi.org/10.1097/MCG.0b013e318284792e
PMid:23442839

65. Smerud HK, Fellstrom B, Hallgren R, et al. *Gluten sensitivity in patients with IgA nephropathy.* Nephrol Dial Transplant. 2009; 24: 2476-81.

http://dx.doi.org/10.1093/ndt/gfp133
PMid:19332868

66. Prasad D, Khara HS, Gupta M, et al. *Celiac disease associated membranous nephropathy - a rare cause or coincidence? A case report.* Cases J. 2009; 2: 7018.

http://dx.doi.org/10.1186/1757-1626-0002-0000007018
PMid:19829897 PMCid:PMC2740114

67. Collin P, Syrjanen J, Partanen J, et al. *Celiac disease and HLA DQ in patients with IgA nephropathy.* Am J Gastroenterol. 2002; 97: 2572-6.

http://dx.doi.org/10.1111/j.1572-0241.2002.06025.x
PMid:12385441

68. Tursi A, Giorgetti G, Brandimarte G, et al. *Effect of gluten-free diet on pregnancy outcome in celiac disease patients with recurrent miscarriages.* Dig Dis Sci. 2008; 53: 2925-8.

http://dx.doi.org/10.1007/s10620-008-0242-x
PMid:18368491

69. Martinelli P, Troncone R, Paparo F, et al. *Coeliac disease and unfavourable outcome of pregnancy.* Gut. 2000; 46: 332-5.

http://dx.doi.org/10.1136/gut.46.3.332
PMid:10673293 PMCid:PMC1727862

70. Volta U, Bellentani S, Bianchi FB, et al. *High prevalence of celiac disease in Italian general population.* Dig Dis Sci. 2001; 46: 1500-5.

http://dx.doi.org/10.1023/A:1010648122797
PMid:11478502

71. Sjoberg K, Eriksson S. *Regional differences in coeliac disease prevalence in Scandinavia?* Scand J Gastroenterol. 1999; 34: 41-5.

http://dx.doi.org/10.1080/00365529950172817
PMid:10048731

72. West J, Fleming KM, Tata LJ, et al. *Incidence and prevalence of celiac disease and dermatitis herpetiformis in the UK over two decades: population-based study.* The American journal of gastroenterology. 2014; 109: 757-68.

http://dx.doi.org/10.1038/ajg.2014.55
PMid:24667576 PMCid:PMC4012300

73. Biagi F, Trotta L, Alfano C, et al. *Prevalence and natural history of potential celiac disease in adult patients.* Scand J Gastroenterol. 2013; 48: 537-42.

http://dx.doi.org/10.3109/00365521.2013.777470
PMid:23506211

74. Dickey W, Hughes D. *Prevalence of celiac disease and its endoscopic markers among patients having routine upper gastrointestinal endoscopy.* Am J Gastroenterol. 1999; 94: 2182-6.

http://dx.doi.org/10.1111/j.1572-0241.1999.01348.x
PMid:10445547

75. Rostami K, Mulder C, Werre J, et al. *High prevalence of celiac disease in apparently healthy blood donors suggests a high prevalence of undiagnosed celiac disease in the Dutch population.* Scand J Gastroenterol. 1999; 34: 276-9.

http://dx.doi.org/10.1080/00365529950173681
PMid:10232872

76. Schweizer JJ, von Blomberg BM, Bueno-de Mesquita HB, et al. *Coeliac disease in The Netherlands.* Scand J Gastroenterol. 2004; 39: 359-64.

http://dx.doi.org/10.1080/00365520310008503
PMid:15125468

77. Rutz R, Ritzler E, Fierz W, et al. *Prevalence of asymptomatic celiac disease in adolescents of eastern Switzerland.* Swiss Med Wkly. 2002; 132: 43-7.

PMid:11953905

78. Cataldo F, Pitarresi N, Accomando S, et al. *Epidemiological and clinical features in immigrant children with coeliac disease: an Italian multicentre study.* Dig Liver Dis. 2004; 36: 722-9.

http://dx.doi.org/10.1016/j.dld.2004.03.021
PMid:15571002

79. Lohi S, Mustalahti K, Kaukinen K, et al. *Increasing prevalence of coeliac disease over time.* Aliment Pharmacol Ther. 2007; 26: 1217-25.

http://dx.doi.org/10.1111/j.1365-2036.2007.03502.x
PMid:17944736

80. Mustalahti K, Catassi C, Reunanen A, et al. *The prevalence of celiac disease in Europe: results of a centralized, international mass screening project.* Ann Med. 2010; 42: 587-95.

http://dx.doi.org/10.3109/07853890.2010.505931
PMid:21070098

81. Kondrashova A, Mustalahti K, Kaukinen K, et al. *Lower economic status and inferior hygienic environment may protect against celiac disease.* Ann Med. 2008; 40: 223-31.

http://dx.doi.org/10.1080/07853890701678689
PMid:18382888

82. Fasano A, Berti I, Gerarduzzi T, et al. *Prevalence of celiac disease in at-risk and not-at-risk groups in the United States: a large multicenter study.* Arch Intern Med. 2003; 163: 286-92.

http://dx.doi.org/10.1001/archinte.163.3.286
PMid:12578508

83. Rubio-Tapia A, Ludvigsson JF, Brantner TL, et al. *The prevalence of celiac disease in the United States.* Am J Gastroenterol. 2012; 107: 1538-44.

http://dx.doi.org/10.1038/ajg.2012.219
PMid:22850429

84. Catassi C, Kryszak D, Bhatti B, et al. *Natural history of celiac disease autoimmunity in a USA cohort followed since 1974.* Ann Med. 2010; 42: 530-8.

http://dx.doi.org/10.3109/07853890.2010.514285
PMid:20868314

85. Teresi S, Crapisi M, Vallejo MD, et al. *Celiac disease seropositivity in Saharawi children: a follow-up and family study.* J Pediatr Gastroenterol Nutr. 2010; 50: 506-9.

http://dx.doi.org/10.1097/MPG.0b013e3181bab30c

86. Coton T, Grassin F, Maslin J, et al. *Celiac disease: special features in Africa. Description of 8 cases in Djibouti (horn of Africa).* Med Trop (Mars). 2008; 68: 144-8.

87. Fleming AF. *Iron deficiency in the tropics.* Clin Haematol. 1982; 11: 365-88.

PMid:7042157

88. Honar N, Karamizadeh Z, Saki F. *Prevalence of celiac disease in patients with type 1 diabetes mellitus in the south of Iran.* Turk J Gastroenterol. 2013; 24: 122-6.

PMid:23934458

89. Shahbazkhani B, Malekzadeh R, Sotoudeh M, et al. *High prevalence of coeliac disease in apparently healthy Iranian blood donors.* Eur J Gastroenterol Hepatol. 2003; 15: 475-8.

http://dx.doi.org/10.1097/00042737-200305000-00003
PMid:12702902

90. Elsurer R, Tatar G, Simsek H, et al. *Celiac disease in the Turkish population.* Dig Dis Sci. 2005; 50: 136-142.

http://dx.doi.org/10.1007/s10620-005-1291-z
PMid:15712651

91. Gursoy S, Guven K, Simsek T, et al. *The prevalence of unrecognized adult celiac disease in Central Anatolia.* J Clin Gastroenterol. 2005; 39: 508-11.

http://dx.doi.org/10.1097/01.mcg.0000165664.87153.e1
PMid:15942437

92. Imanzadeh F, Sayyari AA, Yaghoobi M, et al. *Celiac disease in children with diarrhea is more frequent than previously suspected.* J Pediatr Gastroenterol Nutr. 2005; 40: 309-11.

http://dx.doi.org/10.1097/01.MPG.0000154012.10420.08
PMid:15735484

93. Ertekin V, Selimoglu MA, Kardas F, et al. *Prevalence of celiac disease in Turkish children.* J Clin Gastroenterol. 2005; 39: 689-91.

http://dx.doi.org/10.1097/01.mcg.0000174026.26838.56
PMid:16082278

94. Akbari MR, Mohammadkhani A, Fakheri H, et al. *Screening of the adult population in Iran for coeliac disease: comparison of the tissue-transglutaminase antibody and anti-endomysial antibody tests.* Eur J Gastroenterol Hepatol. 2006; 18: 1181-6.

http://dx.doi.org/10.1097/01.meg.0000224477.51428.32
PMid:17033439

95. Tatar G, Elsurer R, Simsek H, et al. *Screening of tissue transglutaminase antibody in healthy blood donors for celiac disease screening in the Turkish population.* Dig Dis Sci. 2004; 49: 1479-84.

http://dx.doi.org/10.1023/B:DDAS.0000042250.59327.91
PMid:15481323

96. Makharia GK, Mulder CJ, Goh KL, et al. *Issues associated with the emergence of coeliac disease in the Asia-Pacific region: a working party report of the World Gastroenterology Organization and the Asian Pacific Association of Gastroenterology.* J Gastroenterol Hepatol. 2014; 29: 666-77.

http://dx.doi.org/10.1111/jgh.12514
PMid:24783246

97. Gupta R, Reddy DN, Makharia GK, et al. *Indian task force for celiac disease: current status.* World J Gastroenterol. 2009; 15: 6028-33.

http://dx.doi.org/10.3748/wjg.15.6028
PMid:20027674 PMCid:PMC2797658

98. Yachha SK, Poddar U. *Celiac disease in India.* Indian J Gastroenterol. 2007; 26: 230-7.

PMid:18227574

99. Catassi C, Anderson RP, Hill ID, et al. *World perspective on celiac disease.* J Pediatr Gastroenterol Nutr. 2012; 55: 494-9.

http://dx.doi.org/10.1097/MPG.0b013e318272adf4
PMid:22983371

100. Agrawal S, Srivastava SK, Borkar M, et al. *Genetic affinities of north and northeastern populations of India: inference from HLA-based study.* Tissue Antigens. 2008; 72: 120-30.

http://dx.doi.org/10.1111/j.1399-0039.2008.01083.x
PMid:18721272

101. Shanmugalakshmi S, Balakrishnan K, Manoharan K, et al. *HLA-DRB1*, -DQB1* in Piramalai Kallars and Yadhavas, two Dravidian-speaking castes of Tamil Nadu, South India.* Tissue Antigens. 2003; 61: 451-64.

http://dx.doi.org/10.1034/j.1399-0039.2003.00061.x
PMid:12823769

102. Makharia GK, Verma AK, Amarchand R, et al. *Prevalence of celiac disease in the northern part of India: a community based study.* J Gastroenterol Hepatol. 2011; 26: 894-900.

http://dx.doi.org/10.1111/j.1440-1746.2010.06606.x
PMid:21182543

103. Sood A, Midha V, Sood N, et al. *Prevalence of celiac disease among school children in Punjab, North India.* J Gastroenterol Hepatol. 2006; 21: 1622-5.

http://dx.doi.org/10.1111/j.1440-1746.2006.04281.x
PMid:16928227

104. Masood N, Ali Shaikh I. *Clinical presentations and biochemical profile in adult celiac disease patients in Hyderabad: Pakistan.* Pak J Med Sci. 2014; 30: 287-90.

PMid:24772128 PMCid:PMC3998995

105. Rashid M, Khan AG. *Celiac disease in Pakistan: challenges and opportunities.* J Ayub Med Coll Abbottabad. 2009; 21: 1-2.

PMid:20929000

106. Perez Villavicencio A, Beirute Lucke C, Peña AS. *Perspectives to take into account when studying celiac disease in China and Central America.* In: Rodrigo L, Peña AS, eds. Celiac disease and non-celiac gluten sensitivity. Barcelona, Spain: OmniaScience, 2014: 61-64.

http://dx.doi.org/10.3926/oms.237

107. Yuan J, Gao J, Li X, et al. *The tip of the "celiac iceberg" in China: a systematic review and meta-analysis.* PLoS One. 2013; 8: e81151.

http://dx.doi.org/10.1371/journal.pone.0081151
PMid:24324669 PMCid:PMC3852028

108. Wu J, Xia B, von Blomberg BM, et al. *Coeliac disease in China, a field waiting for exploration.* Rev Esp Enferm Dig. 2010; 102: 472-7.

http://dx.doi.org/10.4321/S1130-01082010000800003
PMid:20670067

109. Wu J, Xia B, von Blomberg BM, et al. *Coeliac disease: emerging in China?* Gut. 2010; 59: 418-9.

http://dx.doi.org/10.1136/gut.2009.197863
PMid:20207652

110. Wang XQ, Liu W, Xu CD, et al. *Celiac disease in children with diarrhea in 4 cities in China.* J Pediatr Gastroenterol Nutr. 2011; 53: 368-70.

PMid:21701402

111. Sher KS, Fraser RC, Wicks AC, et al. *High risk of coeliac disease in Punjabis. Epidemiological study in the south Asian and European populations of Leicestershire.* Digestion. 1993; 54: 178-82.

http://dx.doi.org/10.1159/000201035
PMid:8359561

112. Watanabe C, Komoto S, Hokari R, et al. *Prevalence of serum celiac antibody in patients with IBD in Japan.* J Gastroenterol. 2014; 49: 825-34.

http://dx.doi.org/10.1007/s00535-013-0838-6
PMid:23754511 PMCid:PMC4019828

113. Cook HB, Burt MJ, Collett JA, et al. *Adult coeliac disease: prevalence and clinical significance.* Journal of gastroenterology and hepatology. 2000; 15: 1032-6.

http://dx.doi.org/10.1046/j.1440-1746.2000.02290.x
PMid:11059933

114. Tanpowpong P, Ingham TR, Lampshire PK, et al. *Coeliac disease and gluten avoidance in New Zealand children.* Arch Dis Child. 2012; 97: 12-26.

http://dx.doi.org/10.1136/archdischild-2011-300248
PMid:22075107

115. Chin MW, Mallon DF, Cullen DJ, et al. *Screening for coeliac disease using anti-tissue transglutaminase antibody assays, and prevalence of the disease in an Australian community.* Med J Aust. 2009; 190: 429-32.

PMid:19374615

116. Hovell CJ, Collett JA, Vautier G, et al. *High prevalence of coeliac disease in a population-based study from Western Australia: a case for screening?* Med J Aust. 2001; 175: 247-50.

PMid:11587254

117. Remes-Troche JM, Ramirez-Iglesias MT, Rubio-Tapia A, et al. *Celiac disease could be a frequent disease in Mexico: prevalence of tissue transglutaminase antibody in healthy blood donors.* J Clin Gastroenterol. 2006; 40: 697-700.

http://dx.doi.org/10.1097/00004836-200609000-00007
PMid:16940881

118. Remes-Troche JM, Nunez-Alvares C, Uscanga-Dominguez LF. *Celiac disease in Mexican population: an update.* Am J Gastroenterol. 2013; 108: 283-4.

http://dx.doi.org/10.1038/ajg.2012.408
PMid:23381082

119. Remes-Troche JM, Rios-Vaca A, Ramirez-Iglesias MT, et al. *High prevalence of celiac disease in Mexican Mestizo adults with type 1 diabetes mellitus.* J Clin Gastroenterol. 2008; 42: 460-5.

http://dx.doi.org/10.1097/MCG.0b013e318046ea86
PMid:18344893

120. Cromeyer M, Gutierrez RA, Zaldivar K, et al. *Celiac disease in El Salvador.* In: Rodrigo L, Peña AS, eds. Celiac disease and non-celiac gluten sensitivity. Barcelona, Spain: OmniaScience, 2014: 75-88.

http://dx.doi.org/10.3926/oms.231

121. Brenes-Pino F, Brenes A. *Small Intestine Biopsy and its Interpretation: Preliminary Results in Costa Rica.* In: Rodrigo L, Peña AS, eds. Celiac disease and non-celiac gluten sensitivity. Barcelona, Spain: OmniaScience, 2014: 203-218.

http://dx.doi.org/10.3926/oms.229

122. Modelli IC, Gandolfi L, Almeida RC, et al. *Serological screening for celiac disease in symptomatic 12 to 36 month-old children.* Arq Gastroenterol. 2010; 47: 61-5.

http://dx.doi.org/10.1590/S0004-28032010000100011
PMid:20520977

123. Almeida PL, Gandolfi L, Modelli IC, et al. *Prevalence of celiac disease among first degree relatives of Brazilian celiac patients.* Arq Gastroenterol. 2008; 45: 69-72.

http://dx.doi.org/10.1590/S0004-28032008000100013
PMid:18425232

124. Pereira MA, Ortiz-Agostinho CL, Nishitokukado I, et al. *Prevalence of celiac disease in an urban area of Brazil with predominantly European ancestry.* World J Gastroenterol. 2006; 12: 6546-50.

PMid:17072989 PMCid:PMC4100646

125. Mora M, Litwin N, Toca Mdel C, et al. *Prevalence of celiac disease: multicentric trial among pediatric population from five urban districts in Argentina.* Arch Argent Pediatr. 2012; 110: 490-6.

http://dx.doi.org/10.5546/aap.2012.eng.490

126. Gomez JC, Selvaggio GS, Viola M, et al. *Prevalence of celiac disease in Argentina: screening of an adult population in the La Plata area.* The American journal of gastroenterology. 2001; 96: 2700-4.

http://dx.doi.org/10.1111/j.1572-0241.2001.04124.x
PMid:11569698

127. Marine M, Farre C, Alsina M, et al. *The prevalence of coeliac disease is significantly higher in children compared with adults.* Aliment Pharmacol Ther. 2011; 33: 477-86.

http://dx.doi.org/10.1111/j.1365-2036.2010.04543.x
PMid:21166832

128. Greco L, Timpone L, Abkari A, et al. *Burden of celiac disease in the Mediterranean area.* World J Gastroenterol. 2011; 17: 4971-8.

http://dx.doi.org/10.3748/wjg.v17.i45.4971
PMid:22174546 PMCid:PMC3236588

129. Reunala T. *Dermatitis herpetiformis: coeliac disease of the skin.* Annals of medicine. 1998; 30: 416-8.

http://dx.doi.org/10.3109/07853899809002482
PMid:9814827

130. Meyer LJ, Zone JJ. *Familial incidence of dermatitis herpetiformis.* Journal of the American Academy of Dermatology. 1987; 17: 643-7.

http://dx.doi.org/10.1016/S0190-9622(87)70250-3

131. Reunala T. *Incidence of familial dermatitis herpetiformis.* The British journal of dermatology. 1996; 134: 394-8.

http://dx.doi.org/10.1111/j.1365-2133.1996.tb16220.x
PMid:8731659

132. da Silva Kotze LM, Nisihara R, Kotze LR, et al. *Celiac disease and dermatitis herpetiformis in Brazilian twins: a long-term follow-up and screening of their relatives.* Journal of pediatric endocrinology and metabolism. 2013; 26: 71-5.

http://dx.doi.org/10.1515/jpem-2012-0282
PMid:23329745

133. Hervonen K, Alakoski A, Salmi TT, et al. *Reduced mortality in dermatitis herpetiformis: a population-based study of 476 patients.* The British journal of dermatology. 2012; 167: 1331-7.

http://dx.doi.org/10.1111/j.1365-2133.2012.11105.x
PMid:22708883

134. Zhang F, Yang B, Lin Y, et al. *Dermatitis herpetiformis in China: a report of 22 cases.* Journal of the European Academy of Dermatology and Venereology : JEADV. 2012; 26: 903-7.

http://dx.doi.org/10.1111/j.1468-3083.2011.04204.x
PMid:22032403

135. Shibahara M, Nanko H, Shimizu M, et al. *Dermatitis herpetiformis in Japan: an update.* Dermatology. 2002; 204: 37-42.

http://dx.doi.org/10.1159/000051808
PMid:11834848

136. Daneshpazhooh M, Chams-Davatchi C, Payandemehr P, et al. *Spectrum of autoimmune bullous diseases in Iran: a 10-year review.* International journal of dermatology. 2012; 51: 35-41.

http://dx.doi.org/10.1111/j.1365-4632.2011.04946.x
PMid:22182376

137. Wong SN, Chua SH. *Spectrum of subepidermal immunobullous disorders seen at the National Skin Centre, Singapore: a 2-year review.* The British journal of dermatology. 2002; 147: 476-80.

http://dx.doi.org/10.1046/j.1365-2133.2002.04919.x

138. Adam BA. *Bullous diseases in Malaysia: epidemiology and natural history.* International journal of dermatology. 1992; 31: 42-5.

http://dx.doi.org/10.1111/j.1365-4362.1992.tb03519.x
PMid:1737688

139. Smith JB, Tulloch JE, Meyer LJ, et al. *The incidence and prevalence of dermatitis herpetiformis in Utah.* Archives of dermatology. 1992; 128: 1608-10.

http://dx.doi.org/10.1001/archderm.1992.04530010046006
PMid:1456754

140. Smecuol E, Sugai E, Niveloni S, et al. *Permeability, zonulin production, and enteropathy in dermatitis herpetiformis.* Clinical gastroenterology and hepatology: the official clinical practice journal of the American Gastroenterological Association. 2005; 3: 335-41.

http://dx.doi.org/10.1016/S1542-3565(04)00778-5

141. Salmi TT, Hervonen K, Kautiainen H, et al. *Prevalence and incidence of dermatitis herpetiformis: a 40-year prospective study from Finland.* The British Journal of Dermatology. 2011; 165: 354-9.

http://dx.doi.org/10.1111/j.1365-2133.2011.10385.x
PMid:21517799

142. Hadjivassiliou M. *Immune-mediated acquired ataxias.* Handb Clin Neurol. 2012; 103: 189-99.

http://dx.doi.org/10.1016/B978-0-444-51892-7.00011-5
PMid:21827889

143. Hadjivassiliou M, Grunewald R, Sharrack B, et al. *Gluten ataxia in perspective: epidemiology, genetic susceptibility and clinical characteristics.* Brain. 2003; 126: 685-91.

http://dx.doi.org/10.1093/brain/awg050
PMid:12566288

144. Hernandez-Lahoz C, Rodrigo-Saez L, Vega-Villar J, et al. *Familial gluten ataxia.* Mov Disord. 2014; 29: 308-10.

http://dx.doi.org/10.1002/mds.25783
PMid:24375771

145. Nanri K, Mitoma H, Ihara M, et al. *Gluten ataxia in Japan.* Cerebellum. 2014; 13: 623-7.

http://dx.doi.org/10.1007/s12311-014-0582-3
PMid:24997752

146. Wills A, Dale R, Giovannoni G. *Gluten Ataxia and Post-Streptococcal Central Nervous System Syndromes: Emerging Immune-mediated Disorders of the Central Nervous System?* Curr Treat Options Neurol. 2005; 7: 183-189.

http://dx.doi.org/10.1007/s11940-005-0011-5
PMid:15814071

147. Leonard MM, Vasagar B. *US perspective on gluten-related diseases.* Clin Exp Gastroenterol. 2014; 7: 25-37.

PMid:24493932 PMCid:PMC3908912

148. Czaja-Bulsa G. *Non coeliac gluten sensitivity - A new disease with gluten intolerance.* Clin Nutr. 2014.

PMid:25245857

149. Peña AS, Rodrigo L. *Celiac disease and non-celiac gluten sensitivity.* In: Rodrigo L, Peña AS, eds. Celiac disease and Non-Celiac Gluten Sensitivity. Volume. Barcelona: OmniaScience, 2014: 25-43.

http://dx.doi.org/10.3926/oms.223

150. Di Sabatino A, Corazza GR. *Nonceliac gluten sensitivity: sense or sensibility?* Ann Intern Med. 2012; 156: 309-11.

http://dx.doi.org/10.7326/0003-4819-156-4-201202210-00010
PMid:22351716

151. Spence D. *Bad medicine: food intolerance.* BMJ. 2013; 346: f529.

http://dx.doi.org/10.1136/bmj.f529
PMid:23364064

152. Aziz I, Lewis NR, Hadjivassiliou M, et al. *A UK study assessing the population prevalence of self-reported gluten sensitivity and referral characteristics to secondary care.* Eur J Gastroenterol Hepatol. 2014; 26: 33-9.

http://dx.doi.org/10.1097/01.meg.0000435546.87251.f7
PMid:24216570

153. Ho MH, Wong WH, Chang C. *Clinical spectrum of food allergies: a comprehensive review.* Clin Rev Allergy Immunol. 2014; 46: 225-40.

http://dx.doi.org/10.1007/s12016-012-8339-6
PMid:23229594

154. Morita E, Chinuki Y, Takahashi H, et al. *Prevalence of wheat allergy in Japanese adults.* Allergol Int. 2012; 61: 101-5.

http://dx.doi.org/10.2332/allergolint.11-OA-0345
PMid:22377522

155. Dey D, Ghosh N, Pandey N, et al. *A hospital-based survey on food allergy in the population of Kolkata, India.* Int Arch Allergy Immunol. 2014; 164: 218-21.

http://dx.doi.org/10.1159/000365629
PMid:25138428

156. Lee AJ, Thalayasingam M, Lee BW. *Food allergy in Asia: how does it compare?* Asia Pac Allergy. 2013; 3: 3-14.

http://dx.doi.org/10.5415/apallergy.2013.3.1.3
PMid:23403837 PMCid:PMC3563019

157. Green PH. *The many faces of celiac disease: clinical presentation of celiac disease in the adult population.* Gastroenterology. 2005; 128: S74-8.

http://dx.doi.org/10.1053/j.gastro.2005.02.016
PMid:15825130

SECTION I: GENETICS, GENOMICS, IMMUNOLOGY AND APPLICATION TO THERAPY

Preface Section I

Eduardo Arranz MD, PhD

Chapters 1 to 6

Go to TOC

The scope of **Section I** is to review the current knowledge on environmental, genetic and immunological factors involved in celiac disease, as well as to describe alternative therapies, which are at different stages of development. Gluten is a complex mixture of storage proteins with a low nutritional value, but unique functional properties for the elaboration of a wide variety of food products. Gliadins and glutenins from wheat and their counterparts in barley and rye, also called prolamins, are partially digested in the human intestine and, as a result, different immunogenic peptides are generated with the ability to stimulate an immune response in individual with genetic susceptibility.

Chapter 1 describes genetic factors known to have a central role in the susceptibility to celiac disease, though the mode of inheritance is still unknown. The contribution of environmental and genetic factors has been estimated in studies on the prevalence of celiac disease in affected families and, especially, by comparing twin pairs. The genetic component of celiac disease is higher than the estimated contribution for other immunological complex diseases. The genetic risk is mainly based on the presence of certain Human Leucocyte Antigen (HLA) alleles, though their contribution to the heredity is modest, and other non-HLA susceptibility loci may contribute with of many small effects.

Chapter 2 describes the unique properties of the lymphoid tissue associated to the gastrointestinal tract for the maintenance of the immune homeostasis while dealing with an antigen rich environment. Here, the default response is oral tolerance, which controls the immune response against food antigens and the commensal flora. However, there are situations where the mechanisms of immune tolerance are not developed and/or maintained, leading to the activation of immune responses against gluten proteins (celiac disease), or the commensal

flora (Crohn's disease). The main role of dendritic cells in controlling the mechanisms of immune homeostasis in the gastrointestinal tract is also discussed.

Chapter 3 provides relevant information on cereal proteins which are toxic: gliadins and glutenins from wheat, and other prolamins from barley and rye. Adherence to a gluten-free diet is the actual treatment of celiac disease and, to this end, certified gluten-free products are mandatory. Immunochemical techniques for gluten analysis are based on polyclonal and monoclonal antibodies raised against prolamins. Luminal digestion generates different immune-modulatory and toxic peptides which are responsible for an exacerbated immune response in the intestinal mucosa of celiac disease patients, with a central role for the adaptive immunity and gluten-reactive T lymphocytes, though the innate immunity may be also involved, as it has been shown that some gliadin peptides may induce structural changes in the intestine as well as inflammatory reactions.

Chapter 4 discusses the most widely-accepted model of the pathogenesis of celiac disease which focuses on the stimulation of gluten-reactive CD4+ T cells by TG2-deamidated gluten peptides presented by HLA-DQ2/DQ8 molecules, and the production of inflammatory cytokines. Other gliadin peptides may have a direct effect on the epithelium, with interleukin (IL)-15 as the main mediator, and manifested by the expression of stress molecules and the activation of CD8+ intra-epithelial T-cell cytotoxic function. An abnormal immune response to gliadin peptides may lead leads to the development of intestinal lesions with intraepithelial lymphocytosis, epithelial destruction, mucosal re-modeling, and the production of auto-antibodies to tissue transglutaminase.

Chapter 5 reviews the reported association between celiac disease and changes in the composition of intestinal microbiota, which is not completely restored after a gluten-free diet, and may be associated with the HLA-DQ genotype, as shown in healthy infants at family risk of celiac disease. The gut microbiota composition may have a role in the pathogenesis of celiac disease, and its proteolytic activity may be responsible for the generation of immunogenic and toxic peptides, and microbiota is known to have the ability to regulate the epithelial barrier function. Further studies are necessary to confirm these effects and to learn how the administration of specific bacterial strains may modulate the immune homeostasis at the gastrointestinal level and to reducing the risk of celiac disease.

Chapter 6. To date, the only accepted therapy for celiac disease is a lifetime gluten-free diet, which is safe and effective in most patients, though some of its limitations and the growing understanding of celiac disease pathogenesis have led to the development of alternatives. These new therapies include: a) Gluten detoxification strategies in foods; b) Luminal therapies aiming to neutralize gluten peptides in intestine by enzymes, probiotics and gluten binders; c) Intestinal barrier enhancing therapies to inhibit the passage of peptides to the lamina propria; c) Immune targeted therapies, among them, those targeting T cells or inflammatory mediators, and vaccine therapy; and d) Experimental therapies using compounds or biological strategies in discovery phase, for example, the Elafin molecule studied by the authors in an animal model.

Chapter 1

Genetics of Celiac Disease. HLA and Non-HLA Genes

Leticia Plaza-Izurieta, Nora Fernandez-Jimenez, Jose Ramon Bilbao

Dpt. of Genetics, Physical Anthropology and Animal Physiology, BioCruces Health Research Institute, University of the Basque Country-UPV/EHU, Leioa, Basque Country, Spain.

Immunogenetics.let@gmail.com, joseramon.bilbao@ehu.es

Doi: http://dx.doi.org/10.3926/oms.249

How to cite this chapter

Plaza-Izurieta L, Fernandez-Jimenez N, Bilbao JR. *Genetics of Celiac Disease. HLA and Non-HLA Genes.* In Arranz E, Fernández-Bañares F, Rosell CM, Rodrigo L, Peña AS, editors. *Advances in the Understanding of Gluten Related Pathology and the Evolution of Gluten-Free Foods*. Barcelona, Spain: OmniaScience; 2015. p. 79-104.

Abstract

Although the mode of inheritance of celiac disease is still unknown, it has been known for a long time that Genetics participates in the susceptibility to the disease. Studies on the prevalence of CD in affected families, and especially those comparing twin pairs, have been very useful to estimate the proportion in which environmental and genetic factors contribute to the development of this complex disorder. According to these studies, Genetics is a fundamental player both in the triggering and in the latter development of CD.

In general, it is well accepted that the proportion of monozygotic or identical twins concordant for CD is around 75-86%, while in the case of dizygotic twins, this proportion is reduced to 16-20%. This difference between mono- and dizygotic twins has allowed scientists to estimate the genetic component of CD, which is higher than what has been calculated for other immunological complex diseases, such as type 1 diabetes (T1D) (around 30% concordance in monozygotic and 6% in dizygotic twins)[1]. Moreover, concordance rates between sib pairs and dizygotic twins are almost the same, indicating that the environmental component has a minimum contribution to the risk of developing CD. In summary, accumulated evidence suggests that CD has a very strong genetic component and it has been calculated that the heritability of this disease (proportion of the risk of suffering from CD attributable to genetic factors, compared to environmental determinants) is around 87%[2]. The largest portion of the genetic risk to develop CD comes from the presence of certain Human Leucocyte Antigen (HLA) alleles. However, even if the role of these HLA molecules is essential in the pathogenesis of the disease, their contribution to the heredity is

modest, and thus, it has been hypothesized on the existence of many small effect, non-HLA susceptibility *loci*.

Keywords

Celiac disease, autoimmune disease, immune-mediated disease, HLA, linkage studies, genome-wide association studies (GWAS), gene expression, pathway analysis.

---

1. HLA Region and Celiac Disease

1.2. HLA Region

HLA is the name for the Mayor Histocompatibility Complex (MHC) in humans; it is a super *locus* located on the chromosomal region 6p21 and contains a large number of genes related to the immune response. HLA genes encode antigen presenting proteins that are expressed in most human cells and are essential for the ability of the organism to distinguish between self and foreign molecules.

HLA genes are involved in many inflammatory and autoimmune disorders and also contribute to the susceptibility to develop infectious diseases such as AIDS or malaria. However, due to the high genetic complexity of the region, most of the particular genetic factors and pathogenic mechanisms underlying the susceptibility to each of these disorders remain unknown. In fact, the HLA region presents the highest genic density of the entire genome and a very strong gene expression seems to be favored[3].

1.2. Contribution to the Genetic Risk and Susceptibility Genes

As previously mentioned, the HLA region is the most important susceptibility *locus* in CD and explains around 40% of the genetic component of the disease. The first evidence supporting the association between HLA and CD was published in 1973 and was detected using serological methods[4]. Due to the strong linkage disequilibrium present in the area, initial studies identified HLA-A1, HLA-B8 and HLA-DR3 as the etiological variants in the region, but subsequent molecular studies have revealed that the factors directly implicated are the HLA class II genes encoding both HLA-DQ2 and -DQ8 molecules (Figure 1). The strongest association has been found with HLA-DQ2, and 90% of celiac patients present at least one copy of the HLA-DQ2.5 heterodimer (formed by the combination of the products of DQA1*05 and DQB1*02 alleles, that encode the α and β chains of the

heterodimer, respectively). On the other hand, 20-30% of the non-celiac population also presents this HLA-DQ2 variant, making it clear that, even though it is very important, it is not sufficient to develop the disease. Most of the patients who do not carry the HLA-DQ2 genotype are HLA-DQ8 carriers and so have at least one copy of the haplotype containing DQA1*03:01 and DQB1*03:02 alleles[5]. A very small portion of the patients are negative for both DQ2 and DQ8, but it has been observed that in these few cases, individuals present at least one of the two alleles encoding the DQ2 molecule (DQA1*05 or DQB1*02)[6,7].

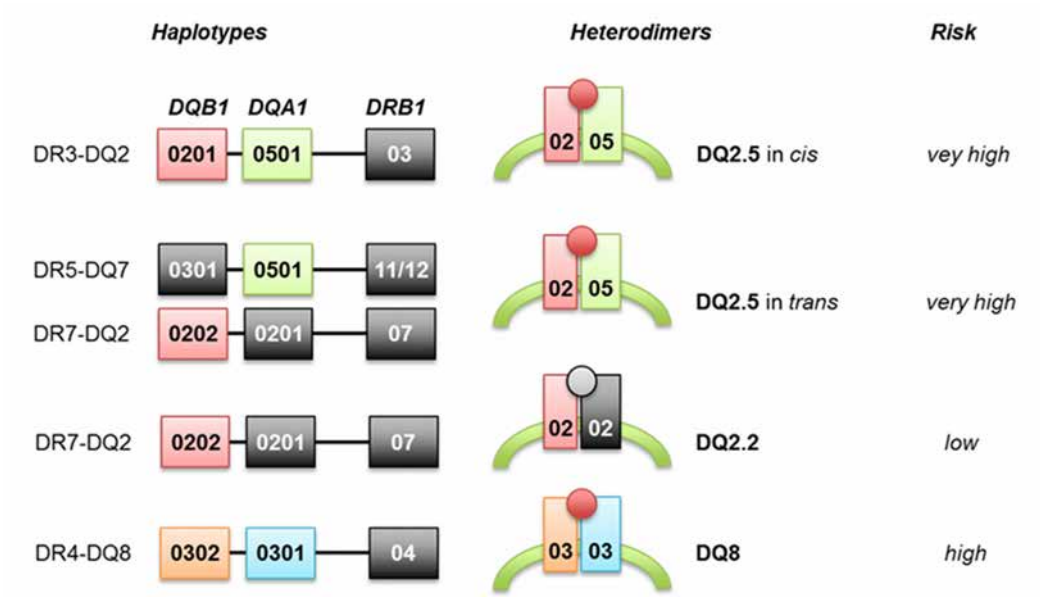


*Figure 1. Association of the HLA locus with CD. HLA-DQ2 molecule is the major factor conferring risk to CD. Most celiac patients express the heterodimer HLA-DQ2.5, encoded by the alleles HLA-DQA1*05 (α chain) and HLA-DQB1*02 (β chain), that can be present in cis in the DR3-DQ2 haplotype or in trans, in the heterozygotes DR5-DQ7 and DR7-DQ2.2. The HLA-DQ2.2 dimer, a variant of HLA-DQ2 encoded by the alleles HLA-DQA1*02:01 and HLA-DQB1*02:02, confer a low risk to develop the disease. Most of the patients that are negative for DQ2 express HLA-DQ8, encoded by the DR4-DQ8 haplotype[8].*

HLA-DQ2 and -DQ8 variants are in linkage disequilibrium with DR3 and DR4, respectively. Thus, we often refer to these risk variants as DR3-DQ2 and DR4-DQ8 haplotypes[9]. In several haplotypes, as is the case of DR3-DQ2, the two alleles of the HLA-DQ2.5 heterodimer (DQA1*05:01 and DQB1*02:01) are located in the same chromosome and therefore, encoded in *cis*. In the heterozygous individuals carrying DR5-DQ7 and DR7-DQ2 haplotypes, the two molecules taking part in the risk heterodimer are encoded in *trans*, because they are located in different chromosomes. The differences between these two types of HLA-DQ2.5 rely on a single amino acid of the DQα chain (DQA1*05:01 *vs.* DQA1*05:05) and another residue of the membrane region of the DQβ chain (DQB1*02:01 versus DQB1*02:02), but they seem not to have any functional consequences and are associated with a similar risk effect. However, the risk conferred by another HLA-DQ2 variant, the HLA-DQ2.2 dimer, is very low[1,10].

There is also a relationship between the degree of susceptibility to CD and the number of DQ2.5 heterodimers. Homozygous individuals with two DR3-DQ2 haplotypes as well as heterozygous patients presenting DR3-DQ2/DR7-DQ2 express the highest levels of DQ2.5 heterodimers and thus, confer the maximum genetic risk to develop CD[11-13]. In this sense, it has to be mentioned that patients with refractory CD (those not responding to GFD) present a higher degree of homozygosity for DR3-DQ2 (44-62%) than other celiac patients (20-24%). A similar dose-dependent effect has also been suggested for DQ8 molecules.

Apart from the genes encoding DQ molecules, the HLA region also contains many other genes that participate to the immune response and that could contribute to the susceptibility to CD. Several studies have postulated that polymorphisms in genes such as *MICA*, *MICB* or *TNF* could contribute to the genetic risk to develop this disorder. Nonetheless, most of these works have not paid enough attention to the strong linkage disequilibrium among genes and results are not conclusive. Deep-sequencing and exhaustive mapping of the region will help to determine whether it contains susceptibility factors other than HLA-DQ. Although HLA genes importantly contribute to the

genetic susceptibility, the concordance of the disease in siblings identical for HLA genotype approaches only 30%, so that we can conclude that HLA genes are important but not sufficient to develop CD[10].

1.3. Role of HLA in the Pathogenesis of CD

The strong association of the HLA class II genes with CD is directly linked to the fundamental role of CD4+ T lymphocytes in the pathogenesis of the disease. In fact, CD4+ T cells that are able to recognize gluten-derived peptides are present in the intestinal mucosa of celiac patients, but not in the case of healthy, non-celiac individuals. When genetically susceptible individuals are exposed to certain gluten-derived epitopes, they are presented by the HLA-DQ2/HLA-DQ8 molecules on the surface of antigen presenting cells (APC), stimulating the proliferation of gluten-specific CD4+ T cells[14].

An important landmark in the molecular basis underlying the association between HLA and CD was the discovery that the binding capacity between the HLA-DQ2 and/or -DQ8 and the gliadin peptides increases substantially when the latter have been enzymatically modified by the enzyme tissue transglutaminase type 2, or TG2. The enzyme catalyzes a reaction that provokes the increase of negative charges in the gluten-derived peptides, favoring their binding to certain HLA molecules (DQ2 and DQ8) and thus, triggering the presentation of these gluten peptides to CD4+ T cells.

Given the importance of HLA molecules in the activation of auto-reactive gluten-specific T cells, it is expected that any modification in their coding sequence will provoke alterations in different steps of this process. In this way, polymorphisms in the sequence encoding the antigen binding sites could affect affinity, favoring or hampering the recognition of the gluten-derived peptides[15]. On the other hand, several polymorphisms located in regulatory sites can repress or enhance the expression of the HLA molecules, reducing or augmenting the immune response to gluten.

2. Search for Genetic Susceptibility Genes in CD

Given the fact that HLA alone can only explain around 40% of the genetic component of CD, large efforts have been done to localize and identify non-HLA susceptibility genes that could clarify the complex genetics of this disorder. Two have been the major strategies used with this aim: on the one hand, linkage studies in affected families, and on the other hand, association studies based on population screening. More recently, CD has also been studied using Genome Wide Association Studies (GWAS), in which thousands of Single Nucleotide Polymorphisms (SNP) have been genotyped and analyzed. These studies have allowed us to identify several associated *loci*, but functional studies will be needed to confirm the implication of the proposed candidate genes.

2.1. Linkage Studies

Linkage studies in families have allowed the identification of chromosomal regions which are repeatedly and consistently inherited by the affected members of a family through several generations. Thus, regions potentially relevant to the development of the disease can be selected and fenced in. Genes localized in these regions are considered positional candidates, due to the fact that it is their position in the genome that is conferring them the candidate identity. In the case of CD, apart from the HLA region (or CELIAC1) which obviously is the most consistently replicated signal and the one showing the strongest linkage disequilibrium, three regions containing positional candidates such as a number of interleukins, the SPINK family, *CD28*, *CTLA4*, *ICOS* and *MYO9B* have been described in the different linkage studies (Figure 2). However, even though consistently replicated in several studies, the certain causes of association with CD have not been identified for these linkage regions.

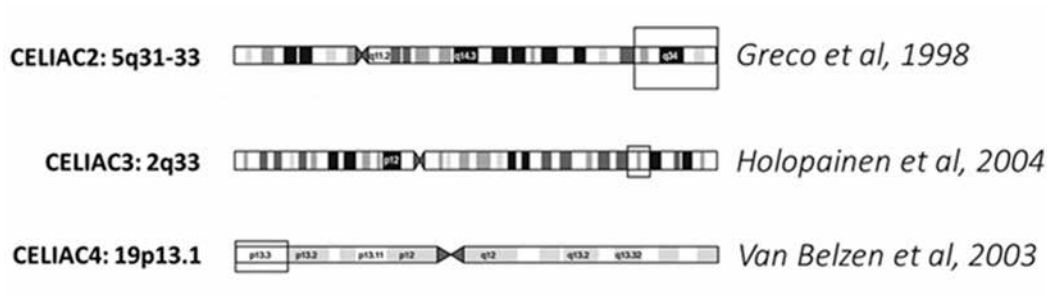


Figure 2. Linkage regions replicated in different families affected by CD[16-18].

2.2. Functional Candidate Genes

The candidate gene approach for genetic association studies focuses on associations between genetic variation within pre-specified genes of interest and phenotypes or disease states. This is in contrast to GWA studies, which scan the entire genome for common genetic variation. Candidate genes are most often selected for study based on *a priori* knowledge of the gene's biological functional impact on the trait or disease in question. This approach has been commonly used in complex disease studies, and also in CD.

Most of the candidate gene studies to date has focused on immune response, since it is generally accepted that CD is a T cell mediated disease, in which gliadin-derived peptides, either in native form or deamidated by transglutaminase, activate *lamina propria* infiltrating T lymphocytes, leading to both Th1 and Th17 inflammatory responses of the adaptive immune system[19]. Thus, both the most Th1 response-characteristic cytokine INFγ (encoded by *INFG*)[20] and *IL23R* the receptor of the best known interleukin in the Th17 cascade[21-23] have been studied among many others, with not many strong association evidences as conclusion.

During the last decade, however, a growing interest has focused on the possible implication of the innate immune response, based on the fact that gliadin peptides are also able to trigger a non-T-cell-dependent response that could establish the proinflammatory environment necessary for subsequent

T-cell activation and tissue destruction[24]. Different innate immune genes and gene families have been proposed as putative susceptibility candidates to CD such as the inflammatory mediators *IL1A*, *IL1B*, *IL1RN*, *IL18*, *RANTES* and *MCP1*[25], the Killer Immunoglobulin-like receptor (KIR) family[26], the Toll-like receptor (TLR) family[27,28] and the stress molecules *MICA* and *MICB*[29] but although a general activation of the innate immune system is well known to occur in CD, none of the proposed candidates have shown a strong association with the disease.

Finally, functional players involved in the remodeling of the intestinal epithelia and in the maintenance of the extracellular matrix have also been proposed as putative susceptibility genes, but again, no association has been confirmed for any of them.

2.3. Genome-wide association and follow-up studies in CD

Millions of SNPs have been identified thanks to the Human Genome sequencing projects. Some of those SNPs, called tag SNPs, have been used as genetic markers in GWAS and allow the identification of thousands of susceptibility variants for many complex diseases. The two GWAS performed in CD, together with several follow-up studies, revealed a total of 26 non-HLA associated regions[30-32]. The most recent large-scale project performed to identify variants associated with CD and other autoimmune diseases is the Immunochip Project, in which a denser genotyping of 186 GWAS *loci* associated with 12 immune-related diseases identified 13 additional regions associated with CD[33].

Hence, there is a total of 39 non-HLA regions associated with CD, containing 57 independent association signals. Nineteen of those regions pinpoint to a single candidate gene, but only 3 associated SNPs are linked to protein-altering variants located in exonic regions, although some potentially causative genes have been proposed due to the existence of signals near the 5' or 3' regulatory regions(Figure 3).

Even though most SNPs localize to nonprotein coding intergenic and intronic regions, CD associated variants seem to be located in expression

quantitative trait *loci* or eQTLs, genomic *loci* that regulate expression levels of mRNAs or proteins. When eQTLs map to a genomic location close to the regulated gene they are referred to as *cis*-eQTLs; in contrast, when the eQTL maps far from the gene (even on different chromosome), it is referred to as *trans*-eQTL. After a meta-analysis of a genome-wide eQTL dataset of 1,469 human whole blood samples, supposed to reflect primary leukocyte gene expression, 38 genome-wide CD associated non-HLA *loci* were assessed for *cis* expression-genotype correlation[32]. Twenty significant eQTLs were identified, more than expected by chance, indicating that CD associated regions are greatly enriched for eQTLs. These data may indicate that some risk variants could have an influence in CD susceptibility by altering gene expression, however, there are many evidences indicating that *cis*-eQTLs differ between different tissues and can even have completely opposite effects.

Thereby, it is important to perform functional analysis of the proposed candidate genes in the disease tissue. The eight association peaks from the first CD GWAS were replicated in a Spanish population in 2011, identifying four genes (*IL12A*, *LPP*, *SCHIP1* and *SH2B3*) whose expression in the intestinal mucosa varied according to disease status and the genotype of the associated variant[34]. These results suggest that these genes may be constitutively altered in celiac patients, probably before the onset of observable symptoms of the disease, and therefore could have a primary role in its pathogenesis.

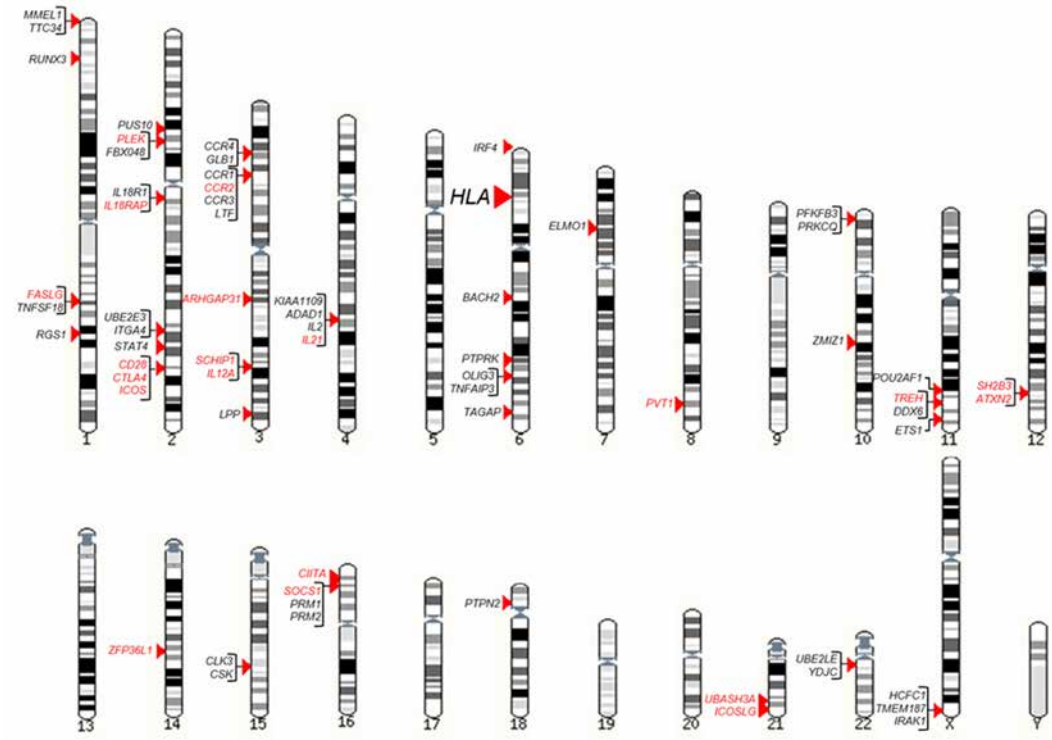


Figure 3. Celiac disease associated regions and proposed candidate genes. Genes highlighted in red showed differential expression in functional analysis.

A second work took a step forward and identified two genes (PTPRK and THEMIS), located in the same associated region, which were co-expressed both in active disease and in response to *in vitro* stimulation by gliadin of intestinal biopsies of celiac patients with inactive disease who have adhered to the gluten free diet for at least two years[35]. Therefore, it seems that associated variants in this region affect the expression of different genes, but not constitutively from the time of birth of the future celiac patient, but only in the presence of a toxic stimulus that triggers an immune response. The implications of this finding are of great importance because they highlight the existence of common regulatory mechanisms for different genes in the DNA

sequence that only have an effect in the presence of a disease-provoking immunogenic stimulus.

In order to elucidate the substantial fraction of heritability that remains unexplained in most complex diseases, a novel hypothesis has recently been postulated. It has been called the "rare-variant synthetic genome-wide-association hypothesis" and it is based on the assumption that unobserved rare causal variants lead to association detected at common tag variants. However, a recent work in which sequencing and genotyping for coding exons of 25 GWAS risk genes were performed in 41,911 UK residents of white European origin (24,892 subjects with six autoimmune disease phenotypes and 17,019 controls) has revealed that rare coding-region variants at known *loci* have a negligible role in common autoimmune disease susceptibility, including CD[36].

A different approach was taken to fine map the *LPP locus* in the search for possible functional variants. This strategy revealed 6 SNPs that overlap regulatory sites, with rs4686484 having a possible effect on LPP gene expression in patients[37].

Almost all associated regions contain genes with an immunological function, many of which act in the same biological pathways. T-cell development in the thymus, a pathway previously not explored in CD pathogenesis, is one of those pathways. A study carried out by Amundsen et al. aimed to explore the regulatory potential of the CD-associated SNPs by eQTL analysis in thymic tissue[38]. They found 43 nominally significant ($p<0.05$) eQTLs within 24 CD-associated chromosomal regions, corresponding to 27 expression-altering SNPs and 40 probes that represent 39 unique genes. When compared across different tissues, they found that 14 eQTLs could represent potentially novel thymus-specific eQTLs. This implies that CD risk polymorphisms could affect gene regulation in the thymus

Given the diversity of cell types and specialization of functions within the immune system, Xinli Hu et al. studied genetic and cellular traits of CD4+ effector memory T (CD4+ TEM) cells, which are particularly important in the onset of CD[39]. They purified CD4+ T cells form a cohort of healthy

individuals and assayed genome-wide SNPs, abundance of CD4+ TEM cells in blood, proliferation upon T cell receptor stimulation, and 215 gene transcripts both in resting and stimulated states. They found that expression levels of 46 genes were regulated by nearby SNPs, including disease-associated SNPs. Many of these eQTLs had not been previously observed in studies of more heterogeneous peripheral blood cells, however they were not able to demonstrate that disease alleles confer risk by modulating these traits in this particular cell type.

The last work published in this field tried to scrutinize the functional implication of 45 candidate genes that were not studied in previous works[40] (Figure 3). The expression of those genes was analyzed in the disease tissue of celiac patients at diagnosis and after treatment, and compared to non-celiac controls. Moreover, the SNP genotype effect in gene expression was also investigated and coexpression analyses were performed. Several genes showed differential expression among disease groups, most of them related to immune response. Multiple *trans*- but only 4 *cis*- eQTLs were found, and surprisingly the genotype effect seems to be stimulus dependent as it differs among groups. Coexpression levels vary from higher to lower levels in active patients at diagnosis, treated patients and non-celiac controls respectively. A subset of 18 genes tightly correlated in both groups of patients but not in controls was identified. Interestingly, this subset of genes was influenced by the genotype of 3 SNPs. These results strongly suggest that the effects of disease-associated SNPs go far beyond the oversimplistic idea of transcriptional control at a nearby *locus*.

In conclusion, recent studies stress the need of developing functional studies and the importance of avoiding arbitrary selection of susceptibility candidate genes. Additionally, they reveal the huge work that remains to be done in order to identify the elements underlying the complex regulatory system of the genome, while opening the door to future studies, in which the scientific community will need to exhaustively analyze both different classes of variation (such as structural variants of the genome or epigenetic features) and the vast non-coding genome, in order to shed light on the complex

genetics of common disorders and to be able to understand the effect of the disease-associated variants found by the numerous GWA studies.

3. Novel Approaches to Unravel the Genetics of CD

A unique Copy Number Variation (CNV) study has been performed in CD. In this work, *TLR2*, *TLR4*, and the β-defensin cluster (*DEFB4*, *DEFB103* and *DEFB104*) were analyzed by gene-specific, real-time PCR in 376 CD patients and 376 controls[41]. TLR genes did not show CNV, and all samples presented with two copies. β-defensin clusters varied between 2 and 9 copies per genome, and when grouped into bins, high copy numbers (>4) were underrepresented among patients, suggesting that increased copy numbers could protect from CD, possibly by impeding bacterial infiltration more efficiently and preserving gut epithelial integrity.

On the other hand, genome-wide expression analysis have consistently been used to draw maps of the most common functional alterations in different complex diseases. Sometimes, these approaches have also been used to identify associated variants that could explain different pathological situations. In the case of CD, Castellanos-Rubio et al. designed in 2008 a strategy that combined gene expression profiling of intestinal biopsy specimens, linkage region information, and different bioinformatics tools for the selection of potentially regulatory single-nucleotide polymorphisms[42]. Among other results, they found evidence of association with several SNPs and identified *SERPINE2* in 2q33, and *PBX3* or *PPP6C* in 9q34 as potential role players in the development of the disease.

Following the results from the ENCODE project, it is now known that a substantial fraction of genetic variants contributing to complex traits in humans are involved in gene regulation[43]. Most phenotype-associated variants discovered in GWA studies are far away from protein coding regions, and even appear in gene deserts[44]. This distribution is similar to that shown by most of the *cis* regulatory modules such as promoters and enhancers, and it is expected that many variants associated with complex traits may affect gene

expression. Furthermore, virtually any noncoding sequence in the human genome could potentially be a regulatory element[45] and even act far away from its genomic location and globally alter whole pathways and signaling routes. Thus, oversimplistic and arbitrary selection of nearby and single candidate genes should be avoided and Systems Biology approaches should be implemented to find the relations and common regulatory mechanisms conjugating the genes that interact to generate the celiac phenotype.

In this sense, in a genome-wide expression microarray carried out several years ago, some signaling routes were found to be altered in CD, such as the Jak-Stat, NFκB, MAPK or TGFB pathways[46]. Some of the genes participating in these routes have been studied to determine whether they contain CD-associated variants. One of these genes is *STAT1*, whose expression is altered in the disease. However, no associated SNPs have been found[47]. *NFκB1* has also been studied but, although it is constitutively active in the intestinal mucosa of CD patients, it does not seem to contain any genetic alteration that could explain its overexpression. It has been suggested that the pathogenic effects assigned to this transcription factor (TF) could be caused by a regulatory defect and that variants or alterations in genes upstream NFκB could trigger the enhanced transcriptional activity observed in CD. It has been speculated that two of the genes identified in a follow-up study after the GWAS (*REL* and *TNFAIP3*) could underlie the deregulation of this biological route[48]. A regulatory SNP in the *UBD* gene that is involved in the activation of NFκB has been associated to CD in Spanish population. This gene is overexpressed in active disease and the allelic distribution of the associated polymorphism presents a significant correlation with expression levels[49].

In this context, a recent study that tried to normalize the altered expression of the NFκB pathway *in vitro* using a MALT1 paracaspase inhibitor discovered a strong coexpression among genes of the route in healthy gut mucosa, while intestinal biopsies from active CD patients presented a completely deregulated pathway (Figure 4)[50]. This disruption of coexpression persisted in treated, inactive patients, especially after acute gliadin

stimulation *in vitro*, and could be reverted to a regulated pattern similar to the one seen in controls through MALT1 inhibition. These results strongly suggest that unknown regulatory mechanisms behind the tight coexpression of the NFκB pathway observed in non-inflamed gut mucosa could be the ones affected by putative genetic or epigenetic alterations rather than single genes taking part in the activating cascade.

Figure 4. Gene pair coexpression matrixes for the different disease statuses. Each small square represents the p value for the correlation of the expression level in a specific gene pair. White, light gray, dark gray and black indicate Pearson's correlation p values >0.05, <0.05, <0.01 and <0.001, respectively[50].

Other recent pathway analysis includes the study of genes whose expression was previously shown to be altered in celiac disease and that shared "angiogenesis" GO terms[51]. A regulatory polymorphism mapping to *TNFSF13* was shown to be associated with CD, and several antiangiogenic genes such as *TGM2* and *PML* were found to be upregulated, while some proangiogenic genes were notably downregulated. Another study has confirmed the involvement of tight junction genes related to permeability, polarity, and cell proliferation in the epithelial destruction observed in CD[52]. Coexpression patterns of several genes of the tight junction pathway support the idea of a common regulatory mechanism that seems to be altered in active CD. In general, GFD normalization confirms the reversibility of the process, except for the constitutive downregulation of *PPP2R3A*, suggestive of a genetic implication.

4. Preliminary steps on the epigenetics of CD

For the moment, only a few attempts have been performed to unravel the epigenetic landscape of CD. However, taking into account that genetic and epigenetic variation, together with environmental factors that shape expression and methylation patterns are known to underlie the vast complexity of common disorders, is probable that epigenetic studies will increase in the following years.

As far as we know, the first miRNA expression analysis in CD was performed in 2011 by Capuano et al. In this study, they tested the expression of a large set of miRNA molecules and found out that nearly the 20% was differentially expressed when celiac patients were compared to control individuals[53]. Moreover, they discovered that high miR-449a levels targeted and reduced *NOTCH1* signaling and suggested that NOTCH pathway could be constitutively altered in the celiac small intestine due to the overexpression of this miRNA, and therefore, could drive the increased proliferation and the decreased differentiation of intestinal cells towards the secretory goblet cell lineage.

On the other hand, DNA methylation has also been studied in the context of CD. It is known that methylation of cytosines, usually at CpG dinucleotides, is involved in epigenetic regulation of gene expression. Promoter methylation is typically associated with repression, whereas genic methylation correlates with transcriptional activity. It has been recently found that 96% of CpGs exhibit differential methylation in at least one cell type or tissue assayed and that levels of DNA methylation correlate with chromatin accessibility[43]. Additionally, chronic inflammation have been linked to several epigenetic alterations. Thus, methylation level was measured in several NFκB-related genes in celiac active and inactive mucosa and compared to control, non-inflamed tissue[50]. Surprisingly, partially reversible, subtle but still significant methylation differences were found in active celiac biopsies and disease samples showed significant correlations among the methylation levels

of different genes (co-methylations). These relationships seemed to somehow disrupt the coexpression patterns observed in health among those same genes.

The ENCODE project has also been able to find CpGs with allele-specific methylation consistent with genomic imprinting, and determined that these *loci* exhibit aberrant methylation in cancer cell lines. Very recently, Hutchinson and collaborators hypothesized that the phenomenon of allele-specific methylation may underlie the phenotypic effects of multiple variants identified by genome-wide association studies, so that they evaluated this in an initial screen at up to 380,678 sites within the genome[54]. They showed that many of the *cis*-regulated allele-specific methylation variants are also eQTLs in peripheral blood mononuclear cells and monocytes and/or in high linkage-disequilibrium with variants associated to complex disease. Finally, they found out that, among others, the CD-associated SNP rs2762051, was associated to one of such methylation variants, opening the door to a novel way to relate the epigenetic, non-coding variation to the GWAS-derived results.

References

1. Sollid LM, Thorsby E. *HLA susceptibility genes in celiac disease: genetic mapping and role in pathogenesis.* Gastroenterology. 1993; 105: 910-22. Erratum in: Gastroenterology. 1994; 106: 1133.

   PMid:8359659
2. Greco L, Romino R, Coto I, Di Cosmo N, Percopo S, Maglio M et al. *The first large population based twin study of coeliac disease.* Gut. 2002; 50: 624-8.

   http://dx.doi.org/10.1136/gut.50.5.624
   PMid:11950806 PMCid:PMC1773191
3. Horton R, Wilming L, Rand, V, Lovering RC, Bruford EA, Khodiyar VK et al. *Gene map of the extended human MHC.* Nat Rev Genet. 2004; 5: 889-99.

   http://dx.doi.org/10.1038/nrg1489
   PMid:15573121
4. Ludwig H, Polymenidis Z, Granditsch G, Wick G. *Association of HL-A1 and HL-A8 with childhood celiac disease.* Z Immunitatsforsch Exp Klin Immunol. 1973; 146: 158-67.

   PMid:4282973
5. Mäki M, Collin P. *Coeliac disease.* Lancet. 1997; 349: 1755-9.

   http://dx.doi.org/10.1016/S0140-6736(96)70237-4
6. Karell K, Louka AS, Moodie SJ, Ascher H, Clot H, Clot F et al. *HLA types in celiac disease patients not carrying the DQA1*05-DQB1*02 (DQ2) heterodimer: results from the European Genetics Cluster on Celiac Disease.* Hum Immunol. 2003; 64: 469-77.

   http://dx.doi.org/10.1016/S0198-8859(03)00027-2
7. Spurkland A, Sollid LM, Polanco I, Vartdal F, Thorsby E. *HLA-DR and –DQ genotypes of celiac disease patients serologically typed to be non-DR3 or non-DR5/7.* Hum Immunol. 1992; 35: 188-92.

   http://dx.doi.org/10.1016/0198-8859(92)90104-U
8. Abadie V, Sollid LM, Barreiro LB, Jabri B. *Integration of genetic and immunological insights into a model of celiac disease pathogenesis.* Ann Rev Immunol. 2011; 29: 493-525.

   http://dx.doi.org/10.1146/annurev-immunol-040210-092915
   PMid:21219178
9. Sollid LM, Markussen G, Ek J, Gjerde H, Vartdal F, Thorsby E. *Evidence for a primary association of celiac disease to a particular HLA-DQ alpha/beta heterodimer.* J Exp Med. 1989; 169: 345-50.

   http://dx.doi.org/10.1084/jem.169.1.345
   PMid:2909659

10. Sollid LM. *Coeliac disease: dissecting a complex inflammatory disorder.* Nat Rev Immunol. 2002; 2: 647-55.
http://dx.doi.org/10.1038/nri885
PMid:12209133

11. van Belzen MJ, Koeleman BP, Crusius JB, Meijer JW, Bardoel AF, Pearson PL et al. *Defining the contribution of the HLA region to cis DQ2-positive coeliac disease patients.* Genes Immun. 2004; 5: 215-20.
http://dx.doi.org/10.1038/sj.gene.6364061
PMid:15014431

12. Ploski R, Ek J, Thorsby E, Sollid LM. *On the HLA-DQ(alpha 1*0501, beta 1*0201)-associated susceptibility in celiac disease: a possible gene dosage effect of DQB1*0201.* Tissue Antigens. 1993; 41: 173-7.
http://dx.doi.org/10.1111/j.1399-0039.1993.tb01998.x
PMid:8362409

13. Lundin KE, Scott H, Hansen T, Paulsen G, Halstensen TS, Fausa O et al. *Gliadin-specific, HLA-DQ(alpha 1*0501,beta 1*0201) restricted T cells isolated from the small intestinal mucosa of celiac disease patients.* J Exp Med. 1993; 178: 187-96.
http://dx.doi.org/10.1084/jem.178.1.187
PMid:8315377

14. Schuppan D, Junker Y, Barisani D. *Celiac disease: From pathogenesis to novel therapies.* Gastroenterology. 2009; 137: 1912-33.
http://dx.doi.org/10.1053/j.gastro.2009.09.008
PMid:19766641

15. Hovhannisyan Z, Weiss A, Martin A, Wiesner M, Tollefsen S, Yoshida K et al. *The role of HLA-DQ8 beta57 polymorphism in the anti-gluten T-cell response in coeliac disease.* Nature. 2008; 456: 534-8.
http://dx.doi.org/10.1038/nature07524
PMid:19037317 PMCid:PMC3784325

16. Greco L, Corazza G, Babron MC, Clot F, Fulchignoni-Lataud MC, Percopo S et al. *Genome search in celiac disease.* Am J Hum Genet. 1998; 62: 669-75.
http://dx.doi.org/10.1086/301754
PMid:9497251 PMCid:PMC1376948

17. Holopainen P, Naluai AT, Moodie S et al. *Candidate gene region 2q33 in European families with coeliac disease.* Tissue Antigens. 2004; 63: 212-22.
http://dx.doi.org/10.1111/j.1399-0039.2004.00189.x
PMid:14989710

18. van Belzen MJ, Meijer JW, Sandkuijl LA et al. *A major non-HLA locus in celiac disease maps to chromosome 19.* Gastroenterology. 2003; 125: 1032-41.
http://dx.doi.org/10.1016/S0016-5085(03)01205-8

19. Castellanos-Rubio A, Santin I, Irastorza I, Castano L, Carlos Vitoria J, Ramon Bilbao J. *TH17 (and TH1) signatures of intestinal biopsies of CD patients in response to gliadin.* Autoimmunity. 2009; 42: 69-73.

http://dx.doi.org/10.1080/08916930802350789
PMid:19127457

20. Wapenaar MC, van Belzen MJ, Fransen JH, Sarasqueta AF, Houwen RH, Meijer JW et al. *The interferon gamma gene in celiac disease: augmented expression correlates with tissue damage but no evidence for genetic susceptibility.* J Autoimmun. 2004; 23: 183-90.

http://dx.doi.org/10.1016/j.jaut.2004.05.004
PMid:15324937

21. Weersma RK, Zhernakova A, Nolte IM et al. *ATG16L1 and IL23R are associated with inflammatory bowel diseases but not with celiac disease in the Netherlands.* Am J Gastroenterol. 2008; 103: 621-7.

http://dx.doi.org/10.1111/j.1572-0241.2007.01660.x
PMid:18047540

22. Einarsdottir E, Koskinen LL, Dukes E, Kainu K, Suomela S, Lappalainen M et al. *IL23R in the Swedish, Finnish, Hungarian and Italian populations: association with IBD and psoriasis, and linkage to celiac disease.* BMC Med Genet. 2009; 10: 8.

http://dx.doi.org/10.1186/1471-2350-10-8
PMid:19175939 PMCid:PMC2642807

23. Medrano LM, García-Magariños M, Dema B, Espino L, Maluenda C et al. *Th17-related genes and celiac disease susceptibility.* PLoS One. 2012; 7: e31244.

http://dx.doi.org/10.1371/journal.pone.0031244
PMid:22359581 PMCid:PMC3281077

24. Maiuri L, Ciacci C, Ricciardelli I, Vacca L, Raia V, Auricchio S et al. *Association between innate response to gliadin and activation of pathogenic T cells in coeliac disease.* Lancet. 2003; 362: 30-7.

http://dx.doi.org/10.1016/S0140-6736(03)13803-2

25. Rueda B, Zhernakova A, López-Nevot MA, Martín J, Koeleman BPC. *Association study of functional genetic variants of innate immunity related genes in celiac disease.* BMC Med Genet. 2005; 6: 29.

http://dx.doi.org/10.1186/1471-2350-6-29
PMid:16078996 PMCid:PMC1190178

26. Santin I, Castellanos-Rubio A, Perez de Nanclares G. *Association of KIR2DL5B gene with celiac disease supports the susceptibility locus on 19q13.4.* Genes Immun. 2007; 8: 171-6.

http://dx.doi.org/10.1038/sj.gene.6364367
PMid:17215859

27. Dezsofi A, Szebeni B, Hermann CS, Kapitany A, Veres G, Sipka S et al. Frequencies of genetic polymorphisms of TLR4 and CD14 and of HLA-DQ genotypes in children with celiac disease, type 1 diabetes mellitus, or both. J. Pediatr Gastroenterol Nutr. 2008; 47: 283-7.

http://dx.doi.org/10.1097/MPG.0b013e31816de885
PMid:18728522

28. Santin I, Castellanos-Rubio A, Hualde I, Castaño L, Vitoria JC, Bilbao JR. *Toll-like receptor 4 (TLR4) gene polymorphisms in celiac disease.* Tissue Antigens. 2007: 70: 495-8.

http://dx.doi.org/10.1111/j.1399-0039.2007.00945.x
PMid:17927684

29. Martín-Pagola A, Perez-Nanclares G, Ortiz L et al. *MICA response to gliadin in intestinal mucosa from celiac patients.* Immunogenetics. 2004; 56: 549-54.

http://dx.doi.org/10.1007/s00251-004-0724-8
PMid:15490153

30. Van Heel DA, Franke L, Hunt KA, Gwilliam R, Zhernakova A, Inouye M et al. *A genome-wide association study for celiac disease identifies risk variants in the region harboring IL2 and IL21.* Nat Genet. 2007; 39: 827-9.

http://dx.doi.org/10.1038/ng2058
PMid:17558408 PMCid:PMC2274985

31. Hunt KA, Zhernakova A, Turner G, Heap GAR, Franke L, Bruinenberg M et al. *Newly identified genetic risk variants for celiac disease related to the immune response.* Nat Genet. 2008; 40: 395-402.

http://dx.doi.org/10.1038/ng.102
PMid:18311140 PMCid:PMC2673512

32. Dubois PCA, Trynka G, Franke L, Hunt KA, Romanos J, Curtotti A et al. *Multiple common variants for celiac disease influencing immune gene expression.* Nat Genet. 2010; 42: 295-302.

http://dx.doi.org/10.1038/ng.543
PMid:20190752 PMCid:PMC2847618

33. Trynka G, Hunt KA, Bockett NA, Romanos J, Mistry V, Szperl A et al. *Dense genotyping identifies and localizes multiple common and rare variant association signals in celiac disease.* Nat Genet. 2011; 43: 1193-201.

http://dx.doi.org/10.1038/ng.998
PMid:22057235 PMCid:PMC3242065

34. Plaza-Izurieta L, Castellanos-Rubio A, Irastorza I, Fernandez-Jimenez N, Gutierrez G, CEGEC, Bilbao JR. *Revisiting genome wide association studies (GWAS) in coeliac disease: replication study in Spanish population and expression analysis of candidate genes.* J Med Genet. 2011; 48: 493-6.

http://dx.doi.org/10.1136/jmg.2011.089714
PMid:21490378

35. Bondar C, Plaza-Izurieta L, Fernandez-Jimenez N, Irastorza I, Withoff S, Wijmenga C et al. *THEMIS and PTPRK in celiac intestinal mucosa: coexpression in disease and after in vitro gliadin challenge*. Eur J Hum Genet. 2014; 22: 358-62.

36. Hunt KA, Mistry V, Bockett NA, Ahmad T, Ban M, Barker JN et al. *Negligible impact of rare autoimmune-locus coding-region variants on missing heritability*. Nature. 2013; 498-232-5.

37. Almeida RC, Ricaño-Ponce I, Kumar V, Deelen P, Szperl A, Trynka G et al. *Fine mapping of the celiac disease-associated LPP locus reveals a potential functional variant.* Hum Mol Genet. 2014; 2481-9.

http://dx.doi.org/10.1093/hmg/ddt619
PMid:24334606 PMCid:PMC3976328

38. Amundsen SS, Viken MK, Sollid LM, Lie BA. *Coeliac disease-associated polymorphisms influence thymic gene expression.* Genes Immun. 2014; 15:355-60.

http://dx.doi.org/10.1038/gene.2014.26
PMid:24871462

39. Hu X, Kim H, Raj T, Brennan PJ, Trynka G, Teslovich N et al. *Regulation of gene expression in autoimmune disease loci and the genetic basis of proliferation in CD4+ effector memory T cells.* PloS Genet. 2014; 10(6), e1004404.

http://dx.doi.org/10.1371/journal.pgen.1004404
PMid:24968232 PMCid:PMC4072514

40. Plaza-Izurieta L, Fernandez-Jimenez N, Irastorza I, Jauregi-Miguel A, Romero-Garmendia I et al. *Expression analysis in intestinal mucosa reveals complex relations among genes under the association peaks in celiac disease*. Eur J Hum Genet. 2014.

http://dx.doi.org/10.1038/ejhg.2014.244
PMCid:PMC3925264

41. Fernandez-Jimenez N, Castellanos-Rubio A, Plaza-Izurieta L, Gutierrez G, Castaño L, Vitoria JC, Bilbao JR. *Analysis of beta-defensin and Toll-like receptor gene copy number variation in celiac disease.* Hum Immunol. 2010; 71: 833-6.

http://dx.doi.org/10.1016/j.humimm.2010.05.012
PMid:20483368

42. Castellanos-Rubio A, Martin-Pagola A, Santín I, Hualde I, Aransay AM, Castaño L, Vitoria JC, Bilbao JR. *Combined functional and positional gene information for the identification of susceptibility variants in celiac disease.* Gastroenterology. 2008; 134: 738-46.

http://dx.doi.org/10.1053/j.gastro.2007.11.041
PMid:18241860

43. ENCODE *Project Consortium et al. An integrated encyclopedia of DNA elements in the human genome.* Nature. 2012; 489: 57-74.

http://dx.doi.org/10.1038/nature11247
PMid:22955616 PMCid:PMC3439153

44. Hardison RC. *Genome-wide epigenetic data facilitate understanding of disease susceptibility association studies.* J Biol Chem. 2012; 287: 30932-40.

http://dx.doi.org/10.1074/jbc.R112.352427
PMid:22952232 PMCid:PMC3438926

45. Hindorff LA, Sethupathy P, Junkins HA, Ramos EM, Mehta JP, Collins FS et al. *Potential etiologic and functional implications of genome-wide association loci for human diseases and traits.* Proc Natl Acad Sci USA. 2009; 106: 9362-7.

http://dx.doi.org/10.1073/pnas.0903103106
PMid:19474294 PMCid:PMC2687147

46. Castellanos-Rubio A, Santin I, Martín-Pagola A, Irastorza I, Castaño L, Vitoria JC et al. *Long-term and acute effects of gliadin on small intestine of patients on potentially pathogenic networks in celiac disease.* Autoimmunity. 2010; 43: 131-9.

http://dx.doi.org/10.109/08916930903225229
PMid:19814655

47. Diosdado B, Monsuur AJ, Mearin ML et al. *The downstream modulator of interferon-gamma, STAT1 is not genetically associated to the Dutch coeliac disease population.* Eur J Hum Genet. 2006; 14: 1120-4.

http://dx.do.org/10.1038/sj.ejhg.5201667
PMid:16773129

48. Trynka G, Zhernakova A, Romanos J, Franke L, Hunt KA, Turner G, Bruinenberg M et al. *Coeliac disease-associated risk variants in TNFAIP3 and REL implicate altered NF-kappaB signalling.* Gut. 2009; 58: 1078-83.

http://dx.oi.org/10.1136/gut.2008.169052
PMid:19240061

49. Castellanos-Rubio A et al. *A regulatory single nucleotide polymorphism in the ubiquitin D gene associated with celiac disease.* Hum Immunol. 2010; 71: 96-9.

http://dx.doi.org/10.1016/j.humimm.2009.09.359
PMid:19808075

50. Fernandez-Jimenez N, Castellanos-Rubio A, Plaza-Izurieta L et al. *Coregulation and modulation of NFκB-related genes in celiac disease: uncovered aspects of gut mucosal inflammation.* Hum Mol Genet. 2014; 23: 1298-310.

http://dx.oi.org/10.1093/hmg/ddt520
PMid:24163129 PMCid:PMC3919015

51. Castellanos-Rubio A, Caja S, Irastorza I et al. *Angiogenesis-related gene expression analysis in celiac disease.* Autoimmunity. 2012; 45: 264-70.

http:/dx.doi.org/10.3109/08916934.2011.637531
PMid:22136669

52. Jauregi-Miguel A, Fernandez-Jimenez N, Irastorza I, Plaza-Izurieta L, Vitoria JC, Bilbao JR. *Alteration of tight junction gene expression in celiac disease.* J Pediatr Gastroenterol Nutr. 2014; 58: 762-7.

http://dx.doi.org/10.1097/mpg.0000000000000338

53. Capuano M, Iaffaldano L, Tinto N, Montanaro D, Capobianco V et al. *MicroRNA-449a overexpression, reduced NOTCH1 signals and scarce goblet cells characterize the small intestine of celiac patients.* PLoS One. 2011; 6: e29094.

http://dx.doi.org/10.1371/journal.pone.0029094
PMid:22194996 PMCid:PMC3240641

54. Hutchinson JN, Raj T, Fagerness J, Stahl E et al. *Allele-specific methylation occurs at genetic variants associated with complex disease.* PLoS One. 2014; 9: e98464.

http://dx.doi.org/10.1371/journal.pone.0098464
PMid:24911414 PMCid:PMC4049588

Chapter 2

Mechanisms of Intestinal Tolerance to Dietary Proteins

David Bernardo[1,2], Stella C. Knight[2]

[1] Gastroenterology Unit. Hospital Universitario de La Princesa and Instituto de Investigación Sanitaria Princesa (IIS-IP), Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBEREHD) Madrid, Spain.

[2] Antigen Presentation Research Group, Imperial College London, Northwick Park and St. Mark's Campus, Mark's Hospital, Harrow, UK.

d.bernardo.ordiz@gmail.com, s.knight@imperial.ac.uk

Doi: http://dx.doi.org/10.3926/oms.250

How to cite this chapter

Bernardo D, Knight SC. *Mechanisms of Intestinal Tolerance to Dietary Proteins*. In Arranz E, Fernández-Bañares F, Rosell CM, Rodrigo L, Peña AS, editors. *Advances in the Understanding of Gluten Related Pathology and the Evolution of Gluten-Free Foods*. Barcelona, Spain: OmniaScience; 2015. p. 105-139.

Abstract

Oral tolerance is defined as the lack of a systemic immune response against antigens previously administered through the gastrointestinal tract. Therefore, in an antigen rich environment such as the intestine, the oral tolerance avoids the development of immune responses against food antigens and the commensal microbiota maintaining immune homeostasis in health. Nevertheless, in some circumstances the immune system fails to develop and/or maintain immune tolerance, triggering an abnormal immune response against the commensals, which occurs in inflammatory bowel diseases and/or against food antigens as evident in celiac disease. In this chapter, we will discuss the unique properties of the immune system in the gastrointestinal tract and study how dendritic cells, the most potent antigen presenting cells, control mechanisms of immune homeostasis in the intestine.

Keywords

Dendritic cells, tolerance, intestine, immunity.

1. Characteristics of the Gastrointestinal Mucosa

The mucosa of the gastrointestinal tract (GIT) is the longest in the human body comprising $100m^2$ (200 times bigger than the skin surface). It consists of a monolayer of epithelial cells specialized in the absorption of water and nutrients and also provides a physical barrier with the external environment.

The intestinal epithelial cells (IEC) constitute the frontier between the external antigen-rich environment [in its lower or distal compartments the GIT carries a total of 10^{12} bacteria per gram of human tissue[1]] and the immune system in the lamina propria (LP) underneath, which comprises the connective tissue between the apical epithelial layer and the inner muscularis mucosae. Barrier function of the IEC is elicited by an array of tight-junctions between the IEC blocking the passage of substances from the lumen. In addition to the epithelial barrier, some IEC like the Goblet cells secrete mucins which constitute the mucus layer on the apical membrane of the IEC. This mucus layer carries a high concentration of anti-microbial defensins, neutrophils and secreted IgA helping to maintain immune homeostasis in the GIT[2,3].

Although IEC are not immune cells, their role in GIT homeostasis and disease cannot be disregarded since some pathologies display increased epithelial gut permeability due to defective or "leaky" tight-junctions. The leakage of food and microbiota antigens through the IEC occurs in some forms of inflammatory bowel disease (IBD) like in Crohn's disease; mucosal exposure to luminal antigens probably provides the basis for sensitivity to food antigens in Crohn's disease, responses to which can then be elicited only through challenge via gut mucosa but not through skin challenge[4]. Patients with celiac disease (CD) have increased epithelial gut permeability too, allowing passage of luminal content antigens including gluten to the LP. The composition of the mucus layer is also altered in CD patients[5,6] as well as the microbiota composition[6-11]. Nevertheless, it remains elusive whether such altered properties of the IEC compartment and the microbiota are cause or consequence of the disease.

2. The Immune System in the Gastrointestinal Tract

Dendritic cells (DC) and macrophages (Mφ) are the main antigen presenting cells (APC) in the GIT and changes in their numbers, phenotype and function have been reported in GIT diseases including CD[12-15]. Nevertheless, DC and Mφ have different functions. DC, the most potent APC, are unique in their capacity to migrate to the lymph nodes to perform antigen presentation and indeed are the only cells which can present antigens to stimulate naïve T-cells[16]. DC, therefore, control the mechanisms of immunity/tolerance in the GIT, maintaining immune tolerance against harmless antigens (mainly derived from the diet and the commensals) whilst also maintaining the capacity to trigger active immune responses, against invading pathogens[17]. Mφ, on the contrary, do not migrate to the lymph nodes and fail to perform antigen presentation to naïve T-cells. However, Mφ provide a first line of phagocytic defence against invading antigens[18] and also modulate effector T-cell responses in the tissues[19,20]. They also help to maintain intestinal tolerance by reducing local inflammation[21] and contributing to epithelial cell renewal[22]. Differential functions at induction and effector sites influence the outcome of the immune responses in the GIT allowing the establishment of regulatory mechanisms required to maintain the properties of the mucosal immune system[23]. Different compartments of the immune system in the GIT can be classified, according to their function and location, into i) sampling; ii) induction; and iii) effector areas.

2.1. Sampling Areas

The sampling areas of the GIT immune system are those areas where antigens are sampled by the DC[24] (Figure 1).

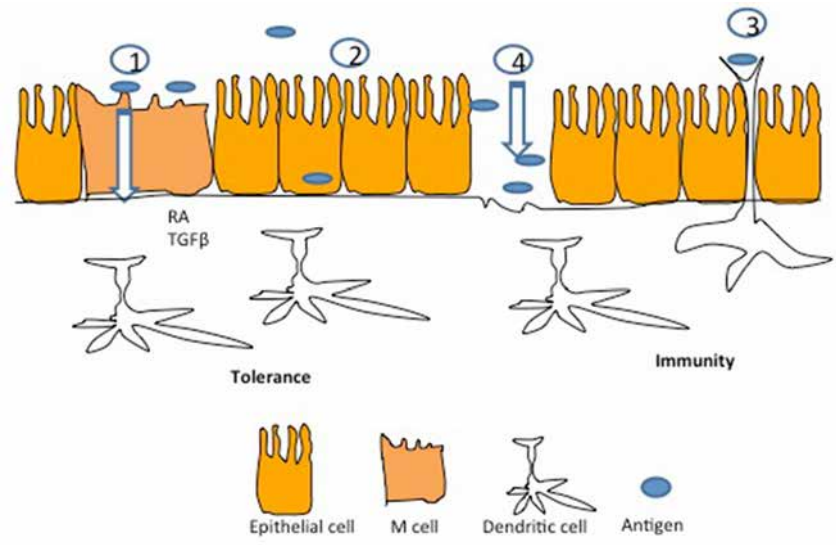


Figure 1. Dendritic cell antigen sampling. DC can sample antigens via (1) M cells at Peyer's Patches, (2) intestinal epithelial cell derived tolerosomes, (3) following direct uptake after sending their veils or dendrites between the epithelial cells or (4) after breakdown of the epithelial integrity. While the first two mechanisms promote immune tolerance, the last two are related with development of active immune responses.

2.1.1. Antigen Transfer Via M Cells at the Peyer's Patches

Peyer's Patches (PP) are lympho-epithelial organs mainly located in the small bowel submucosa. On their apical and external surface PP are covered by a subset of specialized IEC called Microfold or M cells. Such M cells are specialized for direct transfer of particulate antigens from the GIT lumen into tissue beneath the dome of the PP, a compartment rich in DC which will sample the antigens.

2.1.2. Indirect Sampling Via Enterocytes

In contrast to the underdome compartment of the PP, where DC are enriched, DC and other APC such as Mφ are also spread throughout the whole lamina propria of the GIT where they constitute a cell network in intimate contact with the basal membrane of the IEC. In order to maintain the GIT epithelial integrity, IEC can sample the luminal content and secrete antigens onto the basolateral membrane through release of vesicles into the LP where they will be taken up by DC. Such vesicles have been defined as "tolerosomes" as they promote development of tolerogenic responses via LP-DC[25,26]. Nevertheless, DC can also get indirect access to luminal antigens following phagocytosis of apoptotic IEC although in that case they would promote active immune responses against the foreign antigens[24].

2.1.3. Direct Uptake by DC

LP-DC expressing CX3CR1 can extend their veils, or dendrites, between the IEC while establishing tight-junctions in order to maintain the integrity of the epithelial barrier[25] and hence gaining direct access to luminal antigens. Nevertheless, recent evidence has redefined such CX3CR1$^+$ cells as a subset of tissue-resident tolerogenic Mφ[20,28].

2.1.4. Direct Access Following Epithelial Breakdown

When the epithelial integrity is compromised, due to an increase in transepithelial permeability and/or IEC apoptosis (as induced in CD by IL-15 as discussed in other chapters), then the luminal content will have direct access to LP-DC which will trigger an active immune response against the invading pathogens or, in disease, to food or microbiota antigens[24]. Increased epithelial permeability has been associated with several GIT diseases including CD.

2.2. Induction Areas

Following antigen update by the DC, induction areas are those compartments where DC present antigen to naive T-cells. In the GIT, induction areas are comprised of organized lymphoid tissues (including the PP as previously described, the appendix and some lymph nodes) and the mesenteric lymph nodes draining the gut. During antigen presentation DC will not only generate antigen-specific T-cells but will also control their differentiation into pro-inflammatory and/or tolerogenic T-cells

2.3. Effector Areas

Following T-cell priming, antigen-specific effector lymphocytes will migrate back to the GIT to elicit their function at the effector areas in the epithelial compartment and/or the LP.

2.3.1. Intraepihelial Lymphocytes

Intraepitheilal lymphocytes (IEL) constitute a heterogeneous pool of T-cells on the basal membrane of the epithelial and intercalating with the enterocytes. In contrast to immune cells in the LP and non-mucosal immune tissues, IEL constitute a unique mix of lymphocytes. In resting conditions, in healthy controls, human IEL constitute around 20-40 cells per 100 enterocytes in the ileum where they are more frequent. They are characterized by the expression of the CD103 integrin, and most of them (70-90%) have a cytotoxic $CD3^{+}CD4^{-}CD8^{+}$ profile with a classical TCR$\alpha\beta$. Although non-classical TCR$\gamma\delta$ lymphocytes are not very common in other compartments, they represent up to 30% of the total IEL in the GIT being the tissue where they are mainly found. Finally, the IEL compartment comprises a third $CD45^{+}CD3^{-}CD7^{+}$ NK-like cells with cytotoxic capacity[29,30].

2.3.2. Lamina Propria

The LP contains an array of immune cells in addition to fibroblast, smooth muscle cells, lymph and blood vessels. Indeed, although it is not an organized lymphoid tissue, LP of the GIT contains the largest number of immune cells (mainly effector B and T-cells but also DC and Mφ) in the human body.

2.3.2.1. B-cells and IgA

Different B-cell subsets produce different types of immunoglobulins (Ig). IgM/IgG are involved in systemic antibody responses and IgE mediates allergic reactions but the major component of antibody responses in the GIT is IgA. Therefore, IgA is the main Ig in mucosal compartments and the human body secretes over 3g/day. Ig-A promotes a non-aggressive exclusion of pathogens, limiting their access to the IEC, and accumulates in the mucus layer which is also rich in other immune molecules like defensins and bacteriocines, enhancing all together its immune protective function forming the first immune barrier of the GIT[3,31].

2.3.2.2. T-cells

Following antigen presentation, DC determine the outcome (pro-inflammatory/tolerogenic) of the responding antigen-specific T-cells. In both cases, T-cells will migrate from the lymph nodes to the LP where, as the effector site, they will elicit their function (either pro-inflammatory or regulatory).

The role of the *pro-inflammatory lymphocytes* in the GIT has been clearly stated in several intestinal pathologies including CD. Production of pro-inflammatory cytokines by the T-cells compromises the integrity of the epithelial barrier and is also related to structural modifications of the extracellular matrix[32,33]. Production of pro-inflammatory cytokines promote a positive auto- and paracrine feedback for production of chemokines and other pro-inflammatory cytokines which exacerbate the immune response and the

tissue injury. Generation of gluten-specific pro-inflammatory T-cells following antigen presentation by DC is the ultimate cause of CD pathogenesis.

Regulatory T-cells, are $CD4^+$ lymphocytes characterized by the expression of high levels of CD25 in which activity is controlled by the expression of the FoxP3 transcription factor. In contrast to pro-inflammatory T-cells, regulatory T-cells mediate immune homeostasis. Some regulatory T cells produce large quantities of regulatory cytokines (mainly IL-10). As a consequence, regulatory T-cells block the proliferation of pro-inflammatory T-cells, inhibit the production of pro-inflammatory cytokines and cooperate with local B-cells to enhance their production of IgA[34]. However, T-cell properties are dynamic[35-37] so their discrimination into pro-inflammatory and regulatory T-cells may be an oversimplification caused by cell density and/or cell contact inhibition[38].

In summary, the immune system in the GIT promotes immune tolerance against the encountered antigens, mainly derived from commensals and food, via GIT-DC which promote the generation of antigen specific Ig-A secreting B-cells and regulatory T-cells which together maintain immune homeostasis. Nevertheless, in some pathologies like CD, DC "are confused" and fail to recognize gluten as a harmless dietary antigen. When that happens, DC promote the development of gluten-specific pro-inflammatory T-cells which control progression of the disease. In the following sections, we will therefore discuss the properties of GIT-DC and try to understand some of the causes which may cause their malfunction in CD.

3. Dendritic Cells Biology

DC are potent APC. In contrast to other APCs such as B-lymphocytes (excluding already activated B cells) or Mφ, DCs are unique in their capacity to initiate a primary immune response by stimulating naïve T-cells; they also control the outcome (tolerogenic or proinflammatory) of the immune responses[16,39-41].

DC precursors migrate from the bone marrow to virtually all tissues in the body, including the mucosa in the GIT. Once in the tissues, DC become sentinels and sensors of the immune system. DC are sentinels as they are highly effective capturing and processing antigens[42,43] and hence sampling the surrounding environment. DC are also sensors given their capacity to discriminate the nature (harmful/harmless) of the sampled antigen via their high expression of pattern recognition receptor (PRR) molecules [including Toll-like receptors (TLRs)[44-46]] but also given their capacity to become activated in the presence of an innate immune stress (e.g. pro-inflammatory cytokines or oxidative stress)[47,48]. Therefore, DC occupy the interface between the innate and the highly specialized antigen-specific adaptive immune system.

When DC capture a "danger antigen", as recognized via their PRR and/or following maturation induced by an innate immune response, tissue DC lose their high antigen-processing capacity and migrate to secondary lymphoid organs in a CCR7-dependent manner[49,50] in a process of maturation which will promote their capacity to present the antigens to T-cells. Within the lymph nodes, mature DC will deliver three different signals to the naïve T-cells which will control their differentiation into antigen-specific pro-inflammatory T-cells. Such signals include i) an increased expression of the processed antigens on the surface of the HLA-II molecules; ii) increased expression of co-stimulatory molecules CD80(B7.1)/CD86(B7.2) (T-cell CD28/CTLA4 ligands) and/or CD40 (T-cell CD40L ligand); and iii) increased capacity to produce pro-inflammatory cytokines, like IL-12[51,52]. Therefore, lymph node mature DC have lost their antigen-capturing ability but are efficient for antigen presentation and lymphocyte stimulation controlling their differentiation into antigen-specific effector (pro-inflammatory) T-cells. However, DC can also drive development of non-inflammatory (tolerogenic or regulatory) lymphocytes if, at the time of the antigen presentation, they display a decreased expression of the first two signals coupled with an increased capacity to produce regulatory cytokines, like IL-10. In this manner, DC control the development of pro-inflammatory responses against foreign

harmful antigens whilst maintaining immune tolerance against harmless antigens.

3.1. Dendritic Cells and Migration Markers: Connecting Induction and Effector Areas

Antigen specific B- and T-cells express tissue-specific homing markers which control their migration back to the target tissues where the antigen is found. Lymphocytes migrating back to the GIT express on their surface the $\alpha 4\beta 7$ integrin[53] and/or the chemokine receptor CCR9[54]. The ligand for the $\alpha 4\beta 7$ heterodimer is the MAdCAM-1 molecule which is expressed by endothelial cells in the LP post-capillary venules of both the small and large bowels[55,56]. On the contrary, the ligand for CCR9 is the CCL25/TECK chemoattractant expressed by small-bowel epithelial cells[57,58]; there is a gradient of expression which is maximal at the proximal end of the small bowel and gradually decreases in the ileum to become undetectable in the colon[59]. Therefore, while $\alpha 4\beta 7^+$ lymphocytes have general mucosal tropism, those co-expressing $CCR9^+$ are specifically directed towards the small intestine, like pro-inflammatory gluten-specific T-cells in CD.

T-cell expression of such homing markers is controlled by DC. Thus, DC not only control the outcome (proinflammatory/toregonic) of the immune responses but also the location of that response via homing marker imprinting on antigen-specific lymphocytes[60]. Prior to stimulation, naïve T-cells express migration markers that lead them to lymphoid tissues[61]. DC entering tissues from the blood gain specificity induced by their tissue of residence. DC within the tissues, particularly after exposure to antigens, will migrate to the draining lymph nodes and deliver a fourth signal to the T-cells as they induce the expression of homing or migration markers on the responding lymphocytes[62-65]. Therefore, antigen specific responding lymphocytes are directed back to the target tissues where the antigens were found so that immune responses are performed in a compartmentalized tissue-specific way. The mechanisms through which DC induce the expression of tissue-specific homing markers on responding T-cells remain elusive but seem

to involve –among other components– fat soluble vitamins like vitamin A and D. The 25-OHD molecule (generated in the skin following the ultraviolet light-dependent activation of vitamin D) induces the expression of skin-homing markers on DC and hence on the T cells they stimulate T-cells[66]. Retinoic acid (RA), which is a metabolite of dietary vitamin A, induces the expression of gut-homing markers $\alpha 4\beta 7$ and/or CCR9 on DC which then stimulate T-cells with similar properties[62,65,67,68]. DC from the GIT –but not from other tissues-possess the enzymatic machinery necessary to synthesize RA[69-71] providing the mechanism by which GIT-DC gain gut specificity that will then control the migration of the antigen specific lymphocytes back to the GIT effector compartments[62,65,68]. Moreover, DC themselves also express tissue-specific homing markers which vary according to their location[65]. Circulating myeloid DC from CD patients (both untreated at diagnosis and after clinical remission following gluten-free diet) display an altered expression of migration markers with very high expression of CCR9[72] suggesting an increased small bowel migratory capacity which may correlate with a higher infiltration of DCs in target tissues[12]. Nevertheless, the mechanisms producing changes in homing capacity of circulating DC are unknown since it is generally accepted that DC normally die within lymph nodes and do not recirculate[73].

4. Dendritic Cells and Oral Tolerance

GIT-DC are exposed to a large amount of foreign, but harmless, antigens mainly derived from the commensal bacteria and the food. Therefore, in contrast to DC from other tissues, GIT-DC promote the immune tolerance against such antigens[74-76].

The lower immunogenic capacity of intestinal DC results from a number of factors. One of them is that GIT-DC have lower expression of PRRs -including TLR molecules-[77] which confers on them a lower capacity to recognize bacterial antigens in such microbiota-rich environment. In addition to decreased TLR expression, GIT-DC also display an immature phenotype as compared with DC

from other tissues; they have lower expression of both HLA-II molecules and surface co-stimulatory molecules, increased phagocytic capacity and higher capacity to produce regulatory cytokines such as IL-10[77-79]. Such a tolerogenic profile confers GIT-DC with a reduced stimulatory capacity when compared with DC from other tissues[65] which is key in preventing inflammatory processes in the absence of invading pathogens. In addition to their decreased stimulatory capacity, GIT-DC also promote the differentiation of both T-cells with antigen-specific regulatory properties and IgA-secreting B-cells which mediate immune tolerance in the GIT[80-84]. Last, but not least, GIT-DC also imprint gut-homing markers ($\alpha 4\beta 7$ and/or CCR9) on both Ig-A secreting B-cells and regulatory T-cells[81,85] so trafficking of such tolerogenic T-cells and IgA secreting B-cells is restricted to the gastrointestinal compartment. GIT-DC tolerogenic properties are dependent on RA which is essential for intestinal immune tolerance; it is only intestinal DCs (but not DC from other tissues) that possess the enzymatic machinery necessary to convert vitamin A into RA[69-71] and therefore provide the capacity to generate gut-homing regulatory T-cells and IgA-secreting B-cells[81,85-89]. Nevertheless, GIT-also maintains the capacity to trigger an active immune response against invading pathogens. Given that plasticity to maintain immune tolerance against food/commensals while triggering active immune responses against invading pathogens, it has been recently suggested that the GIT contains different DC subsets, each of them being responsible for different outcomes of the immune responses as discussed in the next section.

4.1. DC Subsets in the GIT

Intestinal DCs were originally classified into two mutually exclusive subsets: tolerogenic ($CD103^+$) and proinflammatory ($CX3CR1^+$) DC which respectively control immune tolerance against food and commensals or trigger immune responses against invading pathogens respectively[90-92]. Tolerogenic $CD103^+$DC, are derived from newly arrived DC, have the capacity to migrate to the lymph nodes in a CCR7 dependent manner, and possess the machinery (enzyme RALDH2) necessary to metabolize vitamin A and generate RA generation which mediates several GIT-DC properties. On the contrary, $CX3CR1^+$DC are

derived from newly arrived monocytes and lack both the enzymatic machinery to synthesize RA and the capacity to migrate to the lymph nodes; they would elicit a pro-inflammatory effect against invading pathogens.

4.1.1. CX3CR1$^+$ APC

CX3CR1$^+$DC were originally identified as the GIT-DC subset with capacity to send their dendrites through the IEC, establishing tight-junctions with them, and accessing luminal antigens[25]. Although originally defined as DC, CX3CR1 is virtually absent on colonic DC and CX3CR1$^+$ APC have been recently redefined as Mφ[20,28,93]. Their pro-inflammatory role has also been revisited given their capacity to expand T-cells with regulatory properties on an IL-10 dependent manner[20,94]. Moreover, CX3CR1$^+$Mφ also contribute to immune homeostasis given their capacity to extend their projections between the IEC and migrate towards the lumen in the presence of an infection while becoming loaded with bacterial antigens, thus limiting their access the LP[18,95].

4.1.2. CD103$^+$ DC

Intestinal CD103$^+$ DC can migrate to the lymph nodes, in a CCR7 dependent manner. Within them, the subset co-expressing CD11b$^+$ (murine analog of human CD1c, which identifies type 1 myeloid DC) is unique to the gut controlling the immune tolerance mainly via retinaldehyde dehydrogenase type 2 (RALDH-2) required to generate retinoic acid which mediates several GIT-DC properties[28,96,97].

CD103$^+$DC are decreased in the duodenum of CD patients[14] suggesting that they are related with the lack of oral tolerance against dietary gluten in such patients. However, most our knowledge about the tolerogenic GIT CD103$^+$DC subset have been obtained from murine models which, although essential to further our understanding on DC biology, may not always be translated into the human context[93,98]. Thus, although a majority of human GIT-DC have a regulatory profile[65,77,78,99] that is not restricted to the CD103$^+$ population which are not the main DC subset in the human GIT[14,93,100]. RALDH2 expression is

not restricted to human $CD103^+$ subset as it is also found on $CD103^-$ DC and even Mφ[100]. Moreover, recent evidence suggests that the system is more dynamic that originally described; "tolerogenic" $CD103^+CD11b^+$DC can also drive pro-inflammatory Th17 responses[28], $CD103^-$DC can also generate RA and migrate to the lymph nodes[101] and, finally, DC subsets and function also depend on the mouse strain and GIT location[102] proving GIT-DC plasticity.

Together, and although different DC subsets may exist in the GIT, it seems that the distinction between different DC subsets with different functions may be an oversimplification; DC properties are dynamic and depend on the surrounding microenvironment in which they are found.

4.2. Intestinal DC Plasticity

Tissue DC express different migration markers which are modulated by the local microenvironment[65,103] as DC acquire tissue-specific migration markers and the capacity to imprint them on lymphocytes they stimulate[62,65,68,104]. However, the tissue microenvironment does not only modulate DC homing marker expression but also their maturation status as innate immune factors induce DC maturation. In the absence of inflammation, GIT-DC acquire a regulatory profile following exposure to various "sedative" signals mainly secreted by the IEC[105-108] including thymic stromal lymphopoietin (TSLP), regulatory cytokines like TGF-β and IL-10 and RA[65,81,107,108] (Figure 2). Under such a sedative environment, and in the absence of external immune insults, GIT-DC acquire an immature phenotype characterized by decreased expression of PRR, but also of HLA-Class II molecules, co-stimulatory molecules and also an increased capacity to secrete regulatory cytokines. Given their capacity to metabolize vitamin A and generate RA, GIT-DC in such a calming environment will generate antigen-specific gut-homing T-cells with regulatory function and IgA-secreting B-cells which will in turn promote and maintain the mechanisms of immune tolerance against dietary and commensal antigens.

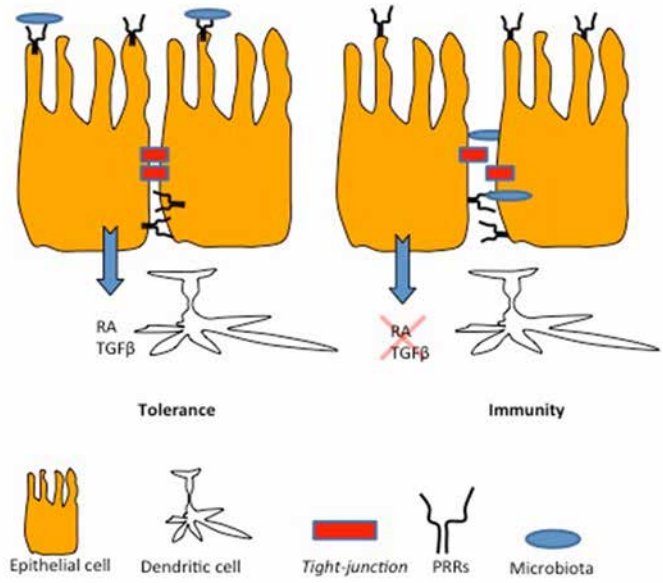


Figure 2. Epithelial cells and dendritic cell crosstalk.

Left: In resting conditions, in healthy controls, intestinal epithelial cells (IEC) recognize microbiota antigens in their apical membrane via pattern recognition receptors (PRR). When that happens, IEC secrete TGF-β and retinoic acid (RA) hence modulating lamina propria dendritic cells towards a tolerogenic phenotype.

Right: In the presence of invading bacteria, tight-junction integrity is compromised and pathogens get access through being recognized by PRR located on the basolateral membrane of the IEC. In this setting, IEC block the secretion of inhibitory signals and, conversely, of DC modulation towards tolerance.

The intestinal immune system is, however, dynamic. In the presence of danger signals its regulatory profile disappears as IEC stop secreting "sedative" signals. This is partly due to the fact that IEC can recognize the presence of invading bacteria. IEC are programmed to secrete TGF-β and RA when recognizing bacteria in their apical membrane by means of their PRRs; however, in the presence of invading bacteria tight junction integrity is affected so pathogens access through and are recognized by the PRRs located on the basolateral membrane of the IEC[109-112]. In this setting, IEC block the secretion of inhibitory signals and, conversely, of DC modulation towards

tolerance. Furthermore, the presence of an innate immune response against invading bacteria involves the secretion of different pro-inflammatory cytokines and/or oxygen reactive species with the ability to induce DC maturation[47,48]. Under such conditions, DC recognize captured antigens as invading pathogens, blocking immune tolerance and triggering active immune responses (Figure 2). This capacity of DCs to respond rapidly and efficiently to their microenvironment grants them the ability to control the immune system and the balance between immunity and tolerance. Nevertheless, the system is not perfect and factors altering the balance can lead to malfunctioning DC as in CD.

5. Dendritic Cells in Celiac Disease

DC maintain immune homeostasis in the GIT while in CD, they trigger an antigen-specific immune response against dietary gluten. DC themselves are the cell type expressing the HLA-DQ2/8 molecules (the main susceptibility genes in CD), a type of HLA-II molecule unique in their capacity to accommodate gluten antigens and perform antigen presentation. Nevertheless, the reason why gluten is recognized as a harmful antigen by DC remains elusive. Increased expression of TLR molecules on GIT-DC and MyD88 signalling has been reported in some pathologies like IBD[77,113]. Although GIT-DC have not been extensively studied in CD, tissue PRR expression is altered in the celiac mucosa[10,114,115] and gluten antigens are also recognized in a MyD88 dependent manner[116,117] so a potential role of PRR on gluten recognition in CD cannot be discarded.

Another possibility, however, suggests that DC do not recognize gluten as harmful antigen directly but only as a consequence of an innate immune response triggered in the GIT. As discussed in other chapters of this book, gluten antigens have a dual effect on the GIT mucosa of the CD patients as it triggers an innate immune response followed by a secondary antigen specific adaptive immune response. The second is triggered by the DC, which, as previously discussed, fail to recognize gluten as a harmless dietary antigen.

The reason for DC "confusion" could be a consequence of the first non-specific innate immune response. Such innate response[118] is characterized by the production of IL-15 by IEC in a NF-kB dependent manner following gluten recognition[119,120]. IL-15 has a direct effect disrupting the epithelial barrier as it increases tight-junction permeability[121,122] and induces apoptosis of IEC[123-126]. Under such immunological stress, IEC stop secreting their sedative signals (Figure 2). IL-15 also has the capacity to activate DC directly and the DC would then mature towards a pro-inflammatory phenotype (Figure 3). Gluten-induced IL-15 production by IEC is central in the first steps of CD pathogenesis and it also elicits co-adjuvant effects with RA exacerbating inflammatory responses to dietary antigens[127]. Therefore, gluten antigens sampled by DC are recognized as harmful and DC promote the differentiation of gluten-specific gut-homing pro-inflammatory T-cells; once back in the effector tissue (lamina propria) these T- cells will promote development and progression of the disease. DC, are therefore responsible for the incapacity of CD patients to establish immune tolerance against ingested gluten proteins; instead, they cause development of antigen-specific immune response.

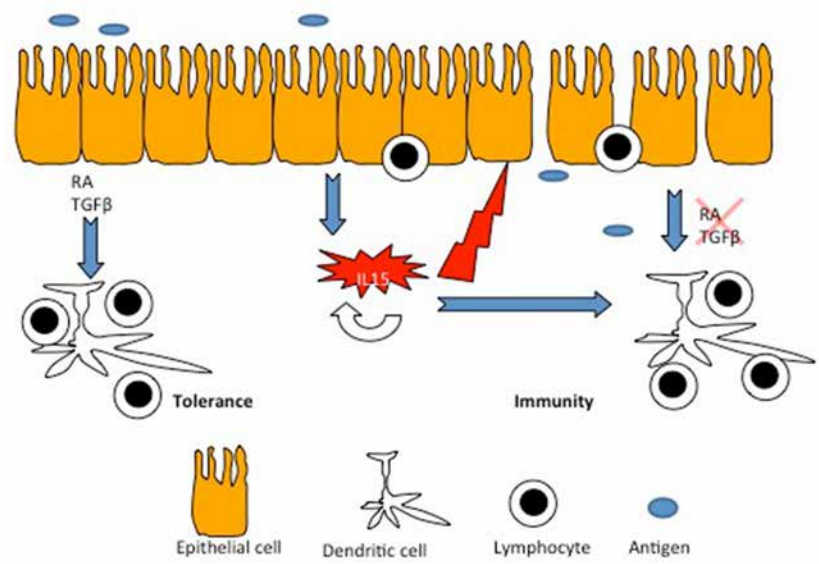


Figure 3. Dendritic cells and celiac disease. In resting condition, in healthy controls, intestinal epithelial cells (IEC) secrete sedative signals, including TGF-β and retinoic acid (RA), which modulate lamina propria dendritic cells (DC) towards a tolerogenic phenotype. In celiac disease, dietary gluten antigens induce an innate immune response characterized by IL-15 production by IEC. Pro-inflammatory IL-15 increases tight-junction permeability and induces IEC apoptosis. In such stressful environment, IEC stop the secretion of the sedative signals and therefore of DC modulation towards tolerance. Pro-inflammatory cytokines like IL-15 also have a direct maturation effect on DC. As a consequence, gluten antigens reaching to the lamina propria are now recognized as harmful so DC trigger the development of an antigen-specific immune response and hence the development of celiac disease pathogenesis.

References

1. Hooper LV, Midtvedt T, Gordon JI. *How host-microbial interactions shape the nutrient environment of the mammalian intestine.* Annu Rev Nutr. 2002; 22: 283-307.

   http://dx.doi.org/10.1146/annurev.nutr.22.011602.092259
   PMid:12055347

2. Hooper LV, Littman DR, Macpherson AJ. *Interactions between the microbiota and the immune system.* Science. 2012; 336: 1268-73.

   http://dx.doi.org/10.1126/science.1223490
   PMid:22674334

3. Shan M, Gentile M, Yeiser JR, Walland AC, Bornstein VU, Chen K et al. *Mucus enhances gut homeostasis and oral tolerance by delivering immunoregulatory signals.* Science. 2013; 342: 447-53.

   http://dx.doi.org/10.1126/science.1237910
   PMid:24072822 PMCid:PMC4005805

4. Van Den Bogaerde J, Cahill J, Emmanuel AV, Vaizey CJ, Talbot IC, Knight SC et al. *Gut mucosal response to food antigens in Crohn's disease.* Aliment Pharmacol Ther. 2002; 16: 1903-15.

   http://dx.doi.org/10.1046/j.1365-2036.2002.01360.x
   PMid:12390099

5. Ciacci C, Di Vizio D, Seth R, Insabato G, Mazzacca G, Podolsky DK et al. *Selective reduction of intestinal trefoil factor in untreated coeliac disease.* Clin Exp Immunol. 2002; 130: 526-31.

   http://dx.doi.org/10.1046/j.1365-2249.2002.02011.x
   PMid:12452845 PMCid:PMC1906543

6. Forsberg G, Fahlgren A, Hörstedt P, Hammarström S, Hernell O, Hammarström ML. *Presence of bacteria and innate immunity of intestinal epithelium in childhood celiac disease.* Am J Gastroenterol. 2004; 99: 894-904.

   http://dx.doi.org/10.1111/j.1572-0241.2004.04157.x
   PMid:15128357

7. Sánchez E, Nadal I, Donat E, Ribes-Koninckx C, Calabuig M, Sanz Y. *Reduced diversity and increased virulence-gene carriage in intestinal enterobacteria of coeliac children.* BMC Gastroenterol. 2008; 8: 50.

   http://dx.doi.org/10.1186/1471-230X-8-50
   PMid:18983674 PMCid:PMC2615025

8. Collado MC, Donat E, Ribes-Koninckx C, Calabuig M, Sanz Y. *Specific duodenal and faecal bacterial groups associated with paediatric coeliac disease.* J Clin Pathol. 2009; 62: 264-9.

http://dx.doi.org/10.1136/jcp.2008.061366
PMid:18996905

9. Nistal E, Caminero A, Herrán AR, Arias L, Vivas S, de Morales JM et al. *Differences of small intestinal bacteria populations in adults and children with/without celiac disease: effect of age, gluten diet, and disease.* Inflamm Bowel Dis. 2012; 18: 649-56.

http://dx.doi.org/10.1002/ibd.21830
PMid:21826768

10. Cheng J, Kalliomäki M, Heilig HG, Palva A, Lähteenoja H, de Vos WM et al. *Duodenal microbiota composition and mucosal homeostasis in pediatric celiac disease.* BMC Gastroenterol. 2013; 13: 113.

http://dx.doi.org/10.1186/1471-230X-13-113
PMid:23844808 PMCid:PMC3716955

11. Caminero A, Herrán AR, Nistal E, Pérez-Andrés J, Vaquero L, Vivas S et al. *Diversity of the cultivable human gut microbiome involved in gluten metabolism: isolation of microorganisms with potential interest for coeliac disease.* FEMS Microbiol Ecol. 2014.

12. Ráki M, Tollefsen S, Molberg Ø, Lundin KE, Sollid LM, Jahnsen FL. *A unique dendritic cell subset accumulates in the celiac lesion and efficiently activates gluten-reactive T cells.* Gastroenterology. 2006; 131: 428-38.

http://dx.doi.org/10.1053/j.gastro.2006.06.002
PMid:16890596

13. Di Sabatino A, Pickard KM, Gordon JN, Salvati V, Mazzarella G, Beattie RM et al. *Evidence for the role of interferon-alfa production by dendritic cells in the Th1 response in celiac disease.* Gastroenterology. 2007; 133: 1175-87.

http://dx.doi.org/10.1053/j.gastro.2007.08.018
PMid:17919493

14. Beitnes AC, Ráki M, Lundin KE, Jahnsen J, Sollid LM, Jahnsen FL. *Density of CD163+ CD11c+ dendritic cells increases and CD103+ dendritic cells decreases in the coeliac lesion.* Scand J Immunol. 2011; 74: 186-94.

http://dx.doi.org/10.1111/j.1365-3083.2011.02549.x
PMid:21392045

15. Beitnes AC, Ráki M, Brottveit M, Lundin KE, Jahnsen FL, Sollid LM. *Rapid accumulation of CD14+CD11c+ dendritic cells in gut mucosa of celiac disease after in vivo gluten challenge.* PLoS One. 2012; 7: e33556.

http://dx.doi.org/10.1371/journal.pone.0033556
PMid:22438948 PMCid:PMC3306402

16. Banchereau J, Steinman RM. *Dendritic cells and the control of immunity.* Nature. 1998; 392: 245-52.

http://dx.doi.org/10.1038/32588
PMid:9521319

17. Pabst O, Mowat AM. *Oral tolerance to food protein.* Mucosal Immunol. 2012; 5: 232-9.

http://dx.doi.org/10.1038/mi.2012.4
PMid:22318493 PMCid:PMC3328017

18. Arques JL, Hautefort I, Ivory K, Bertelli E, Regoli M, Clare S et al. *Salmonella induces flagellin- and MyD88-dependent migration of bacteria-capturing dendritic cells into the gut lumen.* Gastroenterology. 2009; 137: 579-87.

http://dx.doi.org/10.1053/j.gastro.2009.04.010
PMid:19375423

19. Platt AM, Mowat AM. *Mucosal macrophages and the regulation of immune responses in the intestine.* Immunol Lett. 2008; 119: 22-31.

http://dx.doi.org/10.1016/j.imlet.2008.05.009
PMid:18601952

20. Hadis U, Wahl B, Schulz O, Hardtke-Wolenski M, Schippers A, Wagner N et al. *Intestinal tolerance requires gut homing and expansion of FoxP3+ regulatory T cells in the lamina propria.* Immunity. 2011; 34: 237-46.

http://dx.doi.org/10.1016/j.immuni.2011.01.016
PMid:21333554

21. Jiang C, Ting AT, Seed B. *PPAR-gamma agonists inhibit production of monocyte inflammatory cytokines.* Nature. 1998; 391: 82-6.

http://dx.doi.org/10.1038/35154

22. Pull SL, Doherty JM, Mills JC, Gordon JI, Stappenbeck TS. *Activated macrophages are an adaptive element of the colonic epithelial progenitor niche necessary for regenerative responses to injury.* Proc Natl Acad Sci USA. 2005; 102: 99-104.

http://dx.doi.org/10.1073/pnas.0405979102
PMid:15615857 PMCid:PMC544052

23. Macdonald TT, Monteleone G. *Immunity, inflammation, and allergy in the gut.* Science. 2005; 307: 1920-5.

http://dx.doi.org/10.1126/science.1106442
PMid:15790845

24. Ng SC, Kamm MA, Stagg AJ, Knight SC. *Intestinal dendritic cells: their role in bacterial recognition, lymphocyte homing, and intestinal inflammation.* Inflamm Bowel Dis. 2010; 16: 1787-807.

http://dx.doi.org/10.1002/ibd.21247
PMid:20222140

25. Rescigno M, Urbano M, Valzasina B, Francolini M, Rotta G, Bonasio R et al. *Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria.* Nat Immunol. 2001; 2: 361-7.

http://dx.doi.org/10.1038/86373
PMid:11276208

26. Karlsson M, Lundin S, Dahlgren U, Kahu H, Pettersson I, Telemo E. "*Tolerosomes" are produced by intestinal epithelial cells.* Eur J Immunol. 2001; 31: 2892-900.

http://dx.doi.org/10.1002/1521-4141(2001010)31:10<2892::AID-IMMU2892>3.0.CO;2-I

27. Ostman S, Taube M, Telemo E. *Tolerosome-induced oral tolerance is MHC dependent.* Immunology. 2005; 116: 464-76.

http://dx.doi.org/10.1111/j.1365-2567.2005.02245.x

28. Persson EK, Scott CL, Mowat AM, Agace WW. *Dendritic cell subsets in the intestinal lamina propria: ontogeny and function.* Eur J Immunol. 2013; 43: 3098-107.

http://dx.doi.org/10.1002/eji.201343740
PMid:23966272 PMCid:PMC3933733

29. Abadie V, Discepolo V, Jabri B. *Intraepithelial lymphocytes in celiac disease immunopathology.* Semin Immunopathol. 2012; 34: 551-66.

http://dx.doi.org/10.1007/s00281-012-0316-x
PMid:22660791

30. Meresse B, Malamut G, Cerf-Bensussan N. *Celiac disease: an immunological jigsaw.* Immunity. 2012; 36: 907-19.

http://dx.doi.org/10.1016/j.immuni.2012.06.006
PMid:22749351

31. Suzuki K, Ha SA, Tsuji M, Fagarasan S. *Intestinal IgA synthesis: a primitive form of adaptive immunity that regulates microbial communities in the gut.* Semin Immunol. 2007; 19: 127-35.

http://dx.doi.org/10.1016/j.smim.2006.10.001
PMid:17161619

32. Nilsen EM, Jahnsen FL, Lundin KE, Johansen FE, Fausa O, Sollid LM et al. *Gluten induces an intestinal cytokine response strongly dominated by interferon gamma in patients with celiac disease.* Gastroenterology. 1998; 115: 551-63.

http://dx.doi.org/10.1016/S0016-5085(98)70134-9

33. Forsberg G, Hernell O, Melgar S, Israelsson A, Hammarström S, Hammarström ML. *Paradoxical coexpression of proinflammatory and down-regulatory cytokines in intestinal T cells in childhood celiac disease.* Gastroenterology. 2002; 123: 667-78.

http://dx.doi.org/10.1053/gast.2002.35355
PMid:12198691

34. Mills KH. *Regulatory T cells: friend or foe in immunity to infection?* Nat Rev Immunol. 2004; 4: 841-55.

http://dx.doi.org/10.1038/nri1485
PMid:15516964

35. Thorpe PE, Knight SC. *Microplate culture of mouse lymph node cells. I. Quantitation of responses to allogeneic lymphocytes endotoxin and phytomitogens.* J Immunol Methods. 1974; 5: 387-404.

http://dx.doi.org/10.1016/0022-1759(74)90022-2

36. Farrant J, Knight SC. *Help and suppression by lymphoid cells as a function of cellular concentration.* Proc Natl Acad Sci USA. 1979; 76: 3507-10.

http://dx.doi.org/10.1073/pnas.76.7.3507
PMid:158761 PMCid:PMC383856

37. Knight SC. *Control of lymphocyte stimulation in vitro: "help" and "suppression" in the light of lymphoid population dynamics.* J Immunol Methods. 1982; 50: R51-63.

http://dx.doi.org/10.1016/0022-1759(82)90297-6

38. Bernardo D, Al-Hassi HO, Mann ER, Tee CT, Murugananthan AU, Peake ST et al. *T-cell proliferation and forkhead box P3 expression in human T cells are dependent on T-cell density: physics of a confined space?* Hum Immunol. 2012; 73: 223-31.

http://dx.doi.org/10.1016/j.humimm.2011.12.017
PMid:22248741

39. Knight SC, Balfour BM, O'Brien J, Buttifant L, Sumerska T, Clarke J. *Role of veiled cells in lymphocyte activation.* Eur J Immunol. 1982; 12: 1057-60.

http://dx.doi.org/10.1002/eji.1830121214
PMid:7160425

40. Knight SC, Mertin J, Stackpoole A, Clark J. *Induction of immune responses in vivo with small numbers of veiled (dendritic) cells.* Proc Natl Acad Sci USA. 1983; 80: 6032-5.

http://dx.doi.org/10.1073/pnas.80.19.6032
PMid:6604279 PMCid:PMC534354

41. Banchereau J, Briere F, Caux C, Davoust J, Lebecque S, Liu YJ et al. *Immunobiology of dendritic cells.* Annu Rev Immunol. 2000; 18: 767-811.

http://dx.doi.org/10.1146/annurev.immunol.18.1.767
PMid:10837075

42. Jiang W, Swiggard WJ, Heufler C, Peng M, Mirza A, Steinman RM et al. *The receptor DEC-205 expressed by dendritic cells and thymic epithelial cells is involved in antigen processing.* Nature. 1995; 375: 151-5.

http://dx.doi.org/10.1038/375151a0
PMid:7753172

43. Sallusto F, Cella M, Danieli C, Lanzavecchia A. *Dendritic cells use macropinocytosis and the mannose receptor to concentrate macromolecules in the major histocompatibility complex class II compartment: downregulation by cytokines and bacterial products.* J Exp Med. 1995; 182: 389-400.

http://dx.doi.org/10.1084/jem.182.2.389
PMid:7629501

44. Kabelitz D, Wesch D, Oberg HH. *Regulation of regulatory T cells: role of dendritic cells and toll-like receptors.* Crit Rev Immunol. 2006; 26: 291-306.

http://dx.doi.org/10.1615/CritRevImmunol.v26.i4.10
PMid:17073555

45. Benko S, Magyarics Z, Szabó A, Rajnavölgyi E. *Dendritic cell subtypes as primary targets of vaccines: the emerging role and cross-talk of pattern recognition receptors.* Biol Chem. 2008; 389: 469-85.

http://dx.doi.org/10.1515/BC.2008.054
PMid:18953714

46. Granucci F, Zanoni I, Ricciardi-Castagnoli P. *Central role of dendritic cells in the regulation and deregulation of immune responses.* Cell Mol Life Sci. 2008; 65: 1683-97.

http://dx.doi.org/10.1007/s00018-008-8009-2
PMid:18327662

47. Reis e Sousa C. *Activation of dendritic cells: translating innate into adaptive immunity.* Curr Opin Immunol. 2004; 16: 21-5.

http://dx.doi.org/10.1016/j.coi.2003.11.007
PMid:14734106

48. Perera PY, Lichy JH, Waldmann TA, Perera LP. *The role of interleukin-15 in inflammation and immune responses to infection: implications for its therapeutic use.* Microbes Infect. 2012; 14: 247-61.

http://dx.doi.org/10.1016/j.micinf.2011.10.006
PMid:22064066 PMCid:PMC3270128

49. Sallusto F, Schaerli P, Loetscher P, Schaniel C, Lenig D, Mackay CR et al. *Rapid and coordinated switch in chemokine receptor expression during dendritic cell maturation.* Eur J Immunol. 1998; 28: 2760-9.

http://dx.doi.org/10.1002/(SICI)1521-4141(199809)28:09<2760::AID-IMMU2760>3.0.CO;2-N

50. Banchereau J. *The long arm of the immune system.* Sci Am. 2002; 287: 52-9.

http://dx.doi.org/10.1038/scientificamerican1102-52
PMid:12395726

51. Cella M, Scheidegger D, Palmer-Lehmann K, Lane P, Lanzavecchia A, Alber G. *Ligation of CD40 on dendritic cells triggers production of high levels of interleukin-12 and enhances T cell stimulatory capacity: T-T help via APC activation.* J Exp Med. 1996; 184: 747-52.

http://dx.doi.org/10.1084/jem.184.2.747
PMid:8760829

52. Cella M, Engering A, Pinet V, Pieters J, Lanzavecchia A. Inflammatory stimuli induce accumulation of MHC class II complexes on dendritic cells. Nature. 1997; 388: 782-7.

http://dx.doi.org/10.1038/42030
PMid:9285591

53. Lefrançois L, Parker CM, Olson S, Muller W, Wagner N, Schön MP et al. *The role of beta7 integrins in CD8 T cell trafficking during an antiviral immune response.* J Exp Med. 1999; 189: 1631-8.

http://dx.doi.org/10.1084/jem.189.10.1631
PMid:10330442 PMCid:PMC2193647

54. Butcher EC, Williams M, Youngman K, Rott L, Briskin M. *Lymphocyte trafficking and regional immunity.* Adv Immunol. 1999; 72: 209-53.

http://dx.doi.org/10.1016/S0065-2776(08)60022-X

55. Berlin C, Berg EL, Briskin MJ, Andrew DP, Kilshaw PJ, Holzmann B et al. *Alpha 4 beta 7 integrin mediates lymphocyte binding to the mucosal vascular addressin MAdCAM-1.* Cell. 1993; 74: 185-95.

http://dx.doi.org/10.1016/0092-8674(93)90305-A

56. Berg EL, McEvoy LM, Berlin C, Bargatze RF, Butcher EC. *L-selection-mediated lymphocyte rolling on MAdCAM-1.* Nature. 1993; 366: 695-8.

http://dx.doi.org/10.1038/366695a0
PMid:7505053

57. Wurbel MA, Philippe JM, Nguyen C, Victorero G, Freeman T, Wooding P et al. *The chemokine TECK is expressed by thymic and intestinal epithelial cells and attracts double- and single-positive thymocytes expressing the TECK receptor CCR9.* Eur J Immunol. 2000; 30: 262-71.

http://dx.doi.org/10.1002/1521-4141(200001)30:1<262::AID-IMMU262>3.0.CO;2-0

58. Johansson-Lindbom B, Agace WW. *Generation of gut-homing T cells and their localization to the small intestinal mucosa.* Immunol Rev. 2007; 215: 226-42.

http://dx.doi.org/10.1111/j.1600-065X.2006.00482.x
PMid:17291292

59. Ericsson A, Kotarsky K, Svensson M, Sigvardsson M, Agace W. *Functional characterization of the CCL25 promoter in small intestinal epithelial cells suggests a regulatory role for caudal-related homeobox (Cdx) transcription factors.* J Immunol. 2006; 176: 3642-51.

http://dx.doi.org/10.4049/jimmunol.176.6.3642
PMid:16517733

60. Hart AL, Ng SC, Mann E, Al-Hassi HO, Bernardo D, Knight SC. *Homing of immune cells: role in homeostasis and intestinal inflammation.* Inflamm Bowel Dis. 2010; 16: 1969-77.

http://dx.doi.org/10.1002/ibd.21304
PMid:20848507

61. von Andrian UH, Mackay CR. *T-cell function and migration. Two sides of the same coin.* N Engl J Med. 2000; 343: 1020-34.

http://dx.doi.org/10.1056/NEJM200010053431407
PMid:11018170

62. Stagg AJ, Kamm MA, Knight SC. *Intestinal dendritic cells increase T cell expression of alpha4beta7 integrin.* Eur J Immunol. 2002; 32: 1445-54.

http://dx.doi.org/10.1002/1521-4141(200205)32:5<1445::AID-IMMU1445>3.0.CO;2-E

63. Mora JR, Bono MR, Manjunath N, Weninger W, Cavanagh LL, Rosemblatt M et al. *Selective imprinting of gut-homing T cells by Peyer's patch dendritic cells.* Nature. 2003; 424: 88-93.

http://dx.doi.org/10.1038/nature01726
PMid:12840763

64. Johansson-Lindbom B, Svensson M, Wurbel MA, Malissen B, Márquez G, Agace W. *Selective generation of gut tropic T cells in gut-associated lymphoid tissue (GALT): requirement for GALT dendritic cells and adjuvant.* J Exp Med. 2003; 198: 963-9.

http://dx.doi.org/10.1084/jem.20031244
PMid:12963696 PMCid:PMC2194196

65. Mann ER, Bernardo D, Al-Hassi HO, English NR, Clark SK, McCarthy NE et al. *Human gut-specific homeostatic dendritic cells are generated from blood precursors by the gut microenvironment.* Inflamm Bowel Dis. 2012; 18: 1275-86.

http://dx.doi.org/10.1002/ibd.21893
PMid:21987473

66. Sigmundsdottir H, Pan J, Debes GF, Alt C, Habtezion A, Soler D et al. *DCs metabolize sunlight-induced vitamin D3 to 'program' T cell attraction to the epidermal chemokine CCL27.* Nat Immunol. 2007; 8: 285-93.

http://dx.doi.org/10.1038/ni1433
PMid:17259988

67. Iwata M, Hirakiyama A, Eshima Y, Kagechika H, Kato C, Song SY. *Retinoic acid imprints gut-homing specificity on T cells. Immunity.* 2004; 21: 527-38.

http://dx.doi.org/10.1016/j.immuni.2004.08.011
PMid:15485630

68. Bernardo D, Mann ER, Al-Hassi HO, English NR, Man R, Lee GH et al. *Lost therapeutic potential of monocyte-derived dendritic cells through lost tissue homing: stable restoration of gut specificity with retinoic acid.* Clin Exp Immunol. 2013; 174: 109-19.

http://dx.doi.org/10.1111/cei.12118
PMid:23607934 PMCid:PMC3784218

69. Johansson-Lindbom B, Svensson M, Pabst O, Palmqvist C, Marquez G, Förster R et al. *Functional specialization of gut CD103+ dendritic cells in the regulation of tissue-selective T cell homing.* J Exp Med. 2005; 202: 1063-73.

http://dx.doi.org/10.1084/jem.20051100
PMid:16216890 PMCid:PMC2213212

70. Coombes JL, Siddiqui KR, Arancibia-Cárcamo CV, Hall J, Sun CM, Belkaid Y et al. *A functionally specialized population of mucosal CD103+ DCs induces Foxp3+ regulatory T cells via a TGF-beta and retinoic acid-dependent mechanism.* J Exp Med. 2007; 204: 1757-64.

http://dx.doi.org/10.1084/jem.20070590
PMid:17620361 PMCid:PMC2118683

71. Jaensson E, Uronen-Hansson H, Pabst O, Eksteen B, Tian J, Coombes JL et al. *Small intestinal CD103+ dendritic cells display unique functional properties that are conserved between mice and humans.* J Exp Med. 2008; 205: 2139-49.

http://dx.doi.org/10.1084/jem.20080414
PMid:18710932 PMCid:PMC2526207

72. Comino I, Al-Hassi HO, Suligoj T, Lee GH, Sousa C, Landy J et al. *Constitutive gut-homing capacity on circulating myeloid dendritic cells in coeliac disease.* Rev Esp Enferm Dig. 2014; 106: 64-65.

http://dx.doi.org/10.4321/S1130-01082014000100013
PMid:24689721

73. Milling S, Yrlid U, Cerovic V, MacPherson G. *Subsets of migrating intestinal dendritic cells.* Immunol Rev. 2010; 234: 259-67.

http://dx.doi.org/10.1111/j.0105-2896.2009.00866.x
PMid:20193024

74. Mowat AM. *Anatomical basis of tolerance and immunity to intestinal antigens.* Nat Rev Immunol. 2003; 3: 331-41.

http://dx.doi.org/10.1038/nri1057
PMid:12669023

75. Chirdo FG, Millington OR, Beacock-Sharp H, Mowat AM. *Immunomodulatory dendritic cells in intestinal lamina propria.* Eur J Immunol. 2005; 35: 1831-40.

http://dx.doi.org/10.1002/eji.200425882
PMid:16010704

76. Coombes JL, Maloy KJ. *Control of intestinal homeostasis by regulatory T cells and dendritic cells.* Semin Immunol. 2007; Apr;19(2):116-26.

http://dx.doi.org/10.1016/j.smim.2007.01.001

77. Hart AL, Al-Hassi HO, Rigby RJ, Bell SJ, Emmanuel AV, Knight SC et al. *Characteristics of intestinal dendritic cells in inflammatory bowel diseases.* Gastroenterology. 2005; 129: 50-65.

http://dx.doi.org/10.1053/j.gastro.2005.05.013
PMid:16012934

78. Bell SJ, Rigby R, English N, Mann SD, Knight SC, Kamm MA et al. *Migration and maturation of human colonic dendritic cells.* J Immunol. 2001; 166: 4958-67.

http://dx.doi.org/10.4049/jimmunol.166.8.4958
PMid:11290774

79. Steinman RM, Hawiger D, Liu K, Bonifaz L, Bonnyay D, Mahnke K et al. *Dendritic cell function in vivo during the steady state: A role in peripheral tolerance.* Ann N Y Acad Sci. 2003; 987: 15-25.

http://dx.doi.org/10.1111/j.1749-6632.2003.tb06029.x
PMid:12727620

80. Macpherson AJ, Uhr T. *Induction of protective IgA by intestinal dendritic cells carrying commensal bacteria.* Science. 2004; 303(5664): 1662-5.

http://dx.doi.org/10.1126/science.1091334
PMid:15016999

81. Mora JR, Iwata M, Eksteen B, Song SY, Junt T, Senman B et al. *Generation of gut-homing IgA-secreting B cells by intestinal dendritic cells.* Science. 2006; 314: 1157-60.

http://dx.doi.org/10.1126/science.1132742
PMid:17110582

82. Benson MJ, Pino-Lagos K, Rosemblatt M, Noelle RJ. *All-trans retinoic acid mediates enhanced T reg cell growth, differentiation, and gut homing in the face of high levels of co-stimulation.* J Exp Med. 2007; 204: 1765-74.

http://dx.doi.org/10.1084/jem.20070719
PMid:17620363 PMCid:PMC2118687

83. Sun CM, Hall JA, Blank RB, Bouladoux N, Oukka M, Mora JR et al. *Small intestine lamina propria dendritic cells promote de novo generation of Foxp3 T reg cells via retinoic acid.* J Exp Med. 2007; 204: 1775-85.

http://dx.doi.org/10.1084/jem.20070602
PMid:17620362 PMCid:PMC2118682

84. Suzuki K, Maruya M, Kawamoto S, Sitnik K, Kitamura H, Agace WW et al. *The sensing of environmental stimuli by follicular dendritic cells promotes immunoglobulin A generation in the gut.* Immunity. 2010; 33: 71-83.

http://dx.doi.org/10.1016/j.immuni.2010.07.003
PMid:20643338

85. Strober W. *Vitamin A rewrites the ABCs of oral tolerance.* Mucosal Immunol. 2008; 1: 92-5.

http://dx.doi.org/10.1038/mi.2007.22
PMid:19079166

86. Denning TL, Wang YC, Patel SR, Williams IR, Pulendran B. *Lamina propria macrophages and dendritic cells differentially induce regulatory and interleukin 17-producing T cell responses.* Nat Immunol. 2007; 8: 1086-94.

http://dx.doi.org/10.1038/ni1511
PMid:17873879

87. Kang SG, Lim HW, Andrisani OM, Broxmeyer HE, Kim CH. *Vitamin A metabolites induce gut-homing FoxP3+ regulatory T cells.* J Immunol. 2007; 179: 3724-33.

http://dx.doi.org/10.4049/jimmunol.179.6.3724
PMid:17785809

88. Mora JR, Iwata M, von Andrian UH. *Vitamin effects on the immune system: vitamins A and D take centre stage.* Nat Rev Immunol. 2008; 8: 685-98.

http://dx.doi.org/10.1038/nri2378
PMid:19172691 PMCid:PMC2906676

89. Sigmundsdottir H, Butcher EC. *Environmental cues, dendritic cells and the programming of tissue-selective lymphocyte trafficking.* Nat Immunol. 2008; 9: 981-7.

http://dx.doi.org/10.1038/ni.f.208
PMid:18711435 PMCid:PMC3171274

90. Varol C, Vallon-Eberhard A, Elinav E, Aychek T, Shapira Y, Luche H et al. *Intestinal lamina propria dendritic cell subsets have different origin and functions.* Immunity. 2009; 31: 502-12.

http://dx.doi.org/10.1016/j.immuni.2009.06.025
PMid:19733097

91. Bogunovic M, Ginhoux F, Helft J, Shang L, Hashimoto D, Greter M et al. *Origin of the lamina propria dendritic cell network.* Immunity. 2009; 31: 513-25.

http://dx.doi.org/10.1016/j.immuni.2009.08.010
PMid:19733489 PMCid:PMC2778256

92. Schulz O, Jaensson E, Persson EK, Liu X, Worbs T, Agace WW et al. *Intestinal CD103+, but not CX3CR1+, antigen sampling cells migrate in lymph and serve classical dendritic cell functions.* J Exp Med. 2009; 206: 3101-14.

http://dx.doi.org/10.1084/jem.20091925
PMid:20008524 PMCid:PMC2806467

93. Mann ER, Landy JD, Bernardo D, Peake ST, Hart AL, Al-Hassi HO et al. *Intestinal dendritic cells: their role in intestinal inflammation, manipulation by the gut microbiota and differences between mice and men.* Immunol Lett. 2013; 150: 30-40.

http://dx.doi.org/10.1016/j.imlet.2013.01.007
PMid:23352670

94. Bain CC, Scott CL, Uronen-Hansson H, Gudjonsson S, Jansson O, Grip O et al. *Resident and pro-inflammatory macrophages in the colon represent alternative context-dependent fates of the same Ly6Chi monocyte precursors.* Mucosal Immunol. 2013; 6: 498-510.

http://dx.doi.org/10.1038/mi.2012.89
PMid:22990622 PMCid:PMC3629381

95. Nicoletti C, Arques JL, Bertelli E. *CX$_3$CR1 is critical for Salmonella-induced migration of dendritic cells into the intestinal lumen.* Gut Microbes. 2010; May-Jun;1(3):131-4.

http://dx.doi.org/10.4161/gmic.1.3.11711

PMid:21327020 PMCid:PMC3023593

96. Scott CL, Aumeunier AM, Mowat AM. *Intestinal CD103+ dendritic cells: master regulators of tolerance?* Trends Immunol. 2011; 32: 412-9.

http://dx.doi.org/10.1016/j.it.2011.06.003
PMid:21816673

97. Kinnebrew MA, Buffie CG, Diehl GE, Zenewicz LA, Leiner I, Hohl TM et al. *Interleukin 23 production by intestinal CD103(+)CD11b(+) dendritic cells in response to bacterial flagellin enhances mucosal innate immune defense.* Immunity. 2012; 36: 276-87.

http://dx.doi.org/10.1016/j.immuni.2011.12.011
PMid:22306017 PMCid:PMC3288454

98. Gibbons DL, Spencer J. *Mouse and human intestinal immunity: same ballpark, different players; different rules, same score.* Mucosal Immunol. 2011; 4: 148-57.

http://dx.doi.org/10.1038/mi.2010.85
PMid:21228770

99. Worthington JJ, Czajkowska BI, Melton AC, Travis MA. *Intestinal dendritic cells specialize to activate transforming growth factor-b and induce Foxp3+ regulatory T cells via integrin αvβ8.* Gastroenterology. 2011; 141: 1802-12.

http://dx.doi.org/10.1053/j.gastro.2011.06.057
PMid:21723222 PMCid:PMC3507624

100. Sanders TJ, McCarthy NE, Giles EM, Davidson KL, Haltalli ML, Hazell S et al. *Increased production of retinoic Acid by intestinal macrophages contributes to their inflammatory phenotype in patients with Crohn's disease.* Gastroenterology. 2014; 146: 1278-1288.

http://dx.doi.org/10.1053/j.gastro.2014.01.057
PMid:24503130

101. Cerovic V, Houston SA, Scott CL, *Aumeunier A, Yrlid U, Mowat AM, Milling SW. Intestinal CD103(-) dendritic cells migrate in lymph and prime effector T cells.* Mucosal Immunol. 2013; 6: 104-13.

http://dx.doi.org/10.1038/mi.2012.53
PMid:22718260

102. Denning TL, Norris BA, Medina-Contreras O, Manicassamy S, Geem D, Madan R et al. *Functional specializations of intestinal dendritic cell and macrophage subsets that control Th17 and regulatory T cell responses are dependent on the T cell/APC ratio, source of mouse strain, and regional localization.* J Immunol. 2011; 187: 733-47.

http://dx.doi.org/10.4049/jimmunol.1002701
PMid:21666057 PMCid:PMC3131424

103. Dudda JC, Lembo A, Bachtanian E, Huehn J, Siewert C, Hamann A et al. *Dendritic cells govern induction and reprogramming of polarized tissue-selective homing receptor patterns of T cells: important roles for soluble factors and tissue microenvironments.* Eur J Immunol. 2005; 35: 1056-65.

http://dx.doi.org/10.1002/eji.200425817
PMid:15739162

104. Edele F, Molenaar R, Gütle D, Dudda JC, Jakob T, Homey B et al. *Cutting edge: instructive role of peripheral tissue cells in the imprinting of T cell homing receptor patterns.* J Immunol. 2008; 181: 3745-9.

http://dx.doi.org/10.4049/jimmunol.181.6.3745
PMid:18768825

105. Rimoldi M, Chieppa M, Salucci V, Avogadri F, Sonzogni A, Sampietro GM et al. *Intestinal immune homeostasis is regulated by the crosstalk between epithelial cells and dendritic cells.* Nat Immunol. 2005; 6: 507-14.

http://dx.doi.org/10.1038/ni1192
PMid:15821737

106. Butler M, Ng CY, van Heel DA, Lombardi G, Lechler R, Playford RJ et al. *Modulation of dendritic cell phenotype and function in an in vitro model of the intestinal epithelium.* Eur J Immunol. 2006; 36: 864-74.

http://dx.doi.org/10.1002/eji.200535497
PMid:16544275

107. Iliev ID, Spadoni I, Mileti E, Matteoli G, Sonzogni A, Sampietro GM et al. *Human intestinal epithelial cells promote the differentiation of tolerogenic dendritic cells.* Gut. 2009A; 58: 1481-9.

108. Iliev ID, Mileti E, Matteoli G, Chieppa M, Rescigno M. *Intestinal epithelial cells promote colitis-protective regulatory T-cell differentiation through dendritic cell conditioning.* Mucosal Immunol. 2009B; 2: 340-50.

http://dx.doi.org/10.1038/mi.2009.13
PMid:19387433

109. Lee J, Mo JH, Katakura K, Alkalay I, Rucker AN, Liu YT et al. *Maintenance of colonic homeostasis by distinctive apical TLR9 signalling in intestinal epithelial cells.* Nat Cell Biol. 2006; 8: 1327-36.

http://dx.doi.org/10.1038/ncb1500
PMid:17128265

110. Lee J, Gonzales-Navajas JM, Raz E. *The "polarizing-tolerizing" mechanism of intestinal epithelium: its relevance to colonic homeostasis.* Semin Immunopathol. 2008; 30: 3-9.

http://dx.doi.org/10.1007/s00281-007-0099-7
PMid:18026955

111. Wells JM, Rossi O, Meijerink M, van Baarlen P. *Epithelial crosstalk at the microbiota-mucosal interface.* Proc Natl Acad Sci USA. 2011; 108. Suppl 1: 4607-14.

http://dx.doi.org/10.1038/embor.2012.96
PMid:22801555 PMCid:PMC3410395

112. Pott J, Hornef M. *Innate immune signalling at the intestinal epithelium in homeostasis and disease.* EMBO Rep. 2012; 13: 684-98.

http://dx.doi.org/10.1038/embor.2012.96
PMid:22801555 PMCid:PMC3410395

113. Fukata M, Breglio K, Chen A, Vamadevan AS, Goo T, Hsu D et al. *The myeloid differentiation factor 88 (MyD88) is required for CD4+ T cell effector function in a murine model of inflammatory bowel disease.* J Immunol. 2008; 180: 1886-94.

http://dx.doi.org/10.4049/jimmunol.180.3.1886
PMid:18209086 PMCid:PMC2828823

114. Szebeni B, Veres G, Dezsofi A, Rusai K, Vannay A, Bokodi G et al. *Increased mucosal expression of Toll-like receptor (TLR)2 and TLR4 in coeliac disease.* J Pediatr Gastroenterol Nutr. 2007; 45: 187-93.

http://dx.doi.org/10.1097/MPG.0b013e318064514a
PMid:17667714

115. Kalliomäki M, Satokari R, Lähteenoja H, Vähämiko S, Grönlund J, Routi T et al. *Expression of microbiota, Toll-like receptors, and their regulators in the small intestinal mucosa in celiac disease.* J Pediatr Gastroenterol Nutr. 2012; 54: 727-32.

http://dx.doi.org/10.1097/MPG.0b013e318241cfa8
PMid:22134550

116. Thomas KE, Sapone A, Fasano A, Vogel SN. *Gliadin stimulation of murine macrophage inflammatory gene expression and intestinal permeability are MyD88-dependent: role of the innate immune response in Celiac disease.* J Immunol. 2006; 176: 2512-21.

http://dx.doi.org/10.4049/jimmunol.176.4.2512
PMid:16456012

117. Palová-Jelínková L, Dáňová K, Drašarová H, Dvořák M, Funda DP, Fundová P et al. *Pepsin digest of wheat gliadin fraction increases production of IL-1β via TLR4/MyD88/TRIF/MAPK/NF-κB signaling pathway and an NLRP3 inflammasome activation.* PLoS One. 2013; 8: e62426.

http://dx.doi.org/10.1371/journal.pone.0062426
PMid:23658628 PMCid:PMC3639175

118. Maiuri L, Ciacci C, Ricciardelli I, Vacca L, Raia V, Auricchio S et al. *Association between innate response to gliadin and activation of pathogenic T cells in coeliac disease.* Lancet. 2003; 362: 30-7.

http://dx.doi.org/10.1016/S0140-6736(03)13803-2

119. Di Sabatino A, Ciccocioppo R, Cupelli F, Cinque B, Millimaggi D, Clarkson MM et al. *Epithelium derived interleukin 15 regulates intraepithelial lymphocyte Th1 cytokine production, cytotoxicity, and survival in coeliac disease.* Gut. 2006; 55: 469-77.

http://dx.doi.org/10.1136/gut.2005.068684
PMid:16105889 PMCid:PMC1856172

120. Bernardo D, Garrote JA, Fernández-Salazar L, Riestra S, Arranz E. *Is gliadin really safe for non-coeliac individuals? Production of interleukin 15 in biopsy culture from non-coeliac individuals challenged with gliadin peptides.* Gut. 2007; 56: 889-90.

http://dx.doi.org/10.1136/gut.2006.118265
PMid:17519496 PMCid:PMC1954879

121. Matysiak-Budnik T, Candalh C, Dugave C, Namane A, Cellier C, Cerf-Bensussan N et al. *Alterations of the intestinal transport and processing of gliadin peptides in celiac disease.* Gastroenterology. 2003; 125: 696-707.

http://dx.doi.org/10.1016/S0016-5085(03)01049-7

122. Clemente MG, De Virgiliis S, Kang JS, Macatagney R, Musu MP, Di Pierro MR et al. *Early effects of gliadin on enterocyte intracellular signalling involved in intestinal barrier function.* Gut. 2003; 52: 218-23.

http://dx.doi.org/10.1136/gut.52.2.218
PMid:12524403 PMCid:PMC1774976

123. Maiuri L, Ciacci C, Auricchio S, Brown V, Quaratino S, Londei M. *Interleukin 15 mediates epithelial changes in celiac disease.* Gastroenterology. 2000; 119: 996-1006.

http://dx.doi.org/10.1053/gast.2000.18149
PMid:11040186

124. Meresse B, Chen Z, Ciszewski C, Tretiakova M, Bhagat G, Krausz TN et al. *Coordinated induction by IL15 of a TCR-independent NKG2D signaling pathway converts CTL into lymphokine-activated killer cells in celiac disease.* Immunity. 2004; 21: 357-66.

http://dx.doi.org/10.1016/j.immuni.2004.06.020
PMid:15357947

125. Hüe S, Mention JJ, Monteiro RC, Zhang S, Cellier C, Schmitz J et al. *A direct role for NKG2D/MICA interaction in villous atrophy during celiac disease.* Immunity. 2004; 21: 367-77.

http://dx.doi.org/10.1016/j.immuni.2004.06.018
PMid:15357948

126. Ebert EC. *IL-15 converts human intestinal intraepithelial lymphocytes to CD94 producers of IFN-gamma and IL-10, the latter promoting Fas ligand-mediated cytotoxicity.* Immunology. 2005; 115: 118-26.

http://dx.doi.org/10.1111/j.1365-2567.2005.02132.x
PMid:15819704 PMCid:PMC1782126

127. DePaolo RW, Abadie V, Tang F, Fehlner-Peach H, Hall JA, Wang W et al. *Co-adjuvant effects of retinoic acid and IL-15 induce inflammatory immunity to dietary antigens.* Nature. 2011; 471: 220-4.

http://dx.doi.org/10.1038/nature09849
PMid:21307853 PMCid:PMC3076739

Chapter 3

Cereal Proteins: Immunostimulatory and Toxic Peptides

Fernando G. Chirdo[1], Eduardo Arranz[2]

[1] Instituto de Estudios Inmunológicos y Fisiopatológicos-IIFP. (UNLP-CONICET). Facultad de Ciencias Exactas. Universidad Nacional de La Plata. La Plata. Argentina.

[2] Mucosal Immunology Laboratory. Institute of Biology and Molecular Genetics (IBGM).

University of Valladolid, Spanish National Research Council (CSIC), Valladolid, Spain.

fchirdo@gmail.com, earranz@med.uva.es

Doi: http://dx.doi.org/10.3926/oms.251

How to cite this chapter

Chirdo FG, Arranz E. *Cereal Proteins: Immunostimulatory and Toxic Peptides*. In Arranz E, Fernández-Bañares F, Rosell CM, Rodrigo L, Peña AS, editors. *Advances in the Understanding of Gluten Related Pathology and the Evolution of Gluten-Free Foods*. Barcelona, Spain: OmniaScience; 2015. p. 141-162.

Abstract

Storage proteins from wheat kernels are the base of a wide variety of homemade and industrial food products. Nonetheless, for a group of individuals (celiac disease (CD) patients), these proteins are toxic. Gliadins and glutenins from wheat, as well as their counterparts in barley and rye, also called prolamins, are evolutionary related, and present a high degree of homology.

Polyclonal and monoclonal antibodies raised against prolamins have been a very useful tool to characterise structural and conformational features of prolamins, and particularly, for gluten analysis based on immunochemical techniques. Complete adherence to a gluten-free diet is required to recover the normal histology of the small intestine in CD patients. To this end, the use of certified gluten-free products is mandatory.

Aqueous solvents such as 60-70% ethanol, have been used for extraction of prolamins from flours and food. This method is not selective and, therefore, results in complex mixtures of proteins which together with their low solubility in aqueous solutions, high degree of homology, and consequently crossreactivity, produce some drawbacks in gluten analysis by immunoassays.

Prolamins drive an exacerbated immune response in intestinal mucosa of CD patients. T lymphocytes are a central piece in CD pathogenesis. However, new insights in the knowledge of innate immunity point out to some gliadin peptides which can also produce structural changes in the intestine as well as inflammatory reactions.

Keywords

Gliadins, glutenins, prolamins, toxic proteins, gluten analysis, immune response.

1. Introduction

Cereal grains are one of the most important sources of protein in human nutrition. Wheat and rice comprise over 70% of the cereal grains worldwide consumed Most of the wheat cultivars used correspond to the hexaploid (three genomes coded AABBDD) *Tritricum aestivum L.* varieties which are commonly known as bread wheat. *Tritricum durum,* tetraploid (genomes A and B), is primarily used for pasta production. Particularly, the massive use of wheat proteins is due to their physicochemical properties, i.e., their ability to form a particular structure called gluten. This structure is obtained from wheat flour through washing in the presence of water and elimination of some soluble components, mainly starch. As a result, an elastic and cohesive dough is obtained which is capable of retaining gas, a product of fermentation by microorganisms, usually yeast. Therefore, gluten is possibly the oldest known food and the one that is most widely distributed amongst different cultures. Due to its ability to form dough, gluten is extensively used also in the formulation of other foods and is central to the development of many products in food industry[1-3].

Storage proteins from wheat kernels are the base of a wide variety of homemade and industrial food products. Nonetheless, for one group of individuals (celiac disease (CD) patients), these proteins are toxic. In this chapter, we will go over the structural aspects of these toxic proteins to understand their role in the pathogenesis of CD as well as the principles involved in methods for the certification of gluten-free food.

2. Classification of Cereal Proteins

Wheat, barley (*Hordeum vulgare L.)* and rye (*Secale cereale L.*) are evolutionary related, and are members of the Triticeae tribe. They all contain protein groups with a high degree of homology and share some physicochemical properties. Oats, although found in the same sub-family, belong to the Aveneae tribe and present some different characteristics (Figure 1)[1-3].

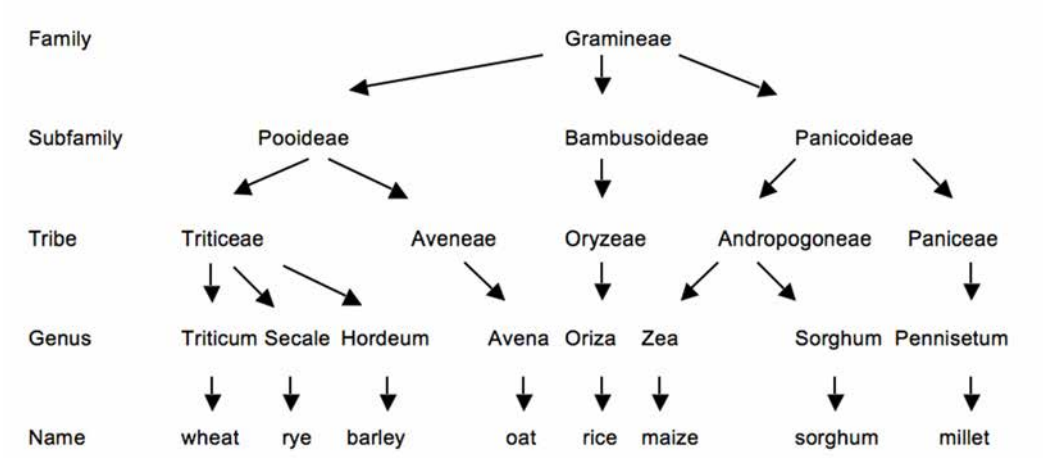


Figure 1. Taxonomic relationships in grains[4].

Proteins from endosperm of wheat grains are complex mixtures. These proteins, which were originally classified by T.B. Osborne (1907) into four fractions according to their solubility in the following: albumins (soluble in water); globulins (soluble in saline solutions), gliadins (soluble in 60-70% ethanol) and glutenins (only soluble under stronger conditions, i.e. acids, reducing agents and detergents, urea, etc).

Gliadins and glutenins, as well as their counterparts in barley and rye, are also called prolamins. This name is due to their high content of the aminoacids proline and glutamine, which along with phenylalanine explain for 60 to 80% of their aminoacid content. Prolamins are synthesised and deposited in the endosperm of the grain as primary source of nitrogen for protein synthesis, which occurs later during germination. Milling process produces wheat flour, the essential primary ingredient in food manufacturing. Gliadins and glutenins, as storage proteins, comprise almost half of the protein content in wheat flour[2]. Gliadins are found as monomers with molecular weights ranging from 30 up to 60kDa, whilst glutenins form polymers through interchain disulfide bonds with molecular weights from 80.000Da to several millions. As consequence of this crosslinking, glutenins are

poorly extracted when aqueous ethanol is used. Gliadins have been further classified into α-, β-, γ- and ω-gliadins, based on their electrophoretic mobility at acid pH (pH = 3, A-PAGE)[5]. The same procedure has been used to describe the homologous components of barley and rye.

3. Structural Characteristics and Physicochemical Properties

The primary structure of prolamins shows long regions of repeated sequences generated by insertion and duplication along the evolution, resulting in a high degree of polymorphism. The repetitive regions are formed by units of 4 to 9 aminoacid length. These units include one or more proline and glutamine, which explains for the high content of these two aminoacids in the prolamins.

Table 1 shows a prolamin classification which takes into account their composition and aminoacid sequence[1,3].

Table 1. Classification of Wheat, Barley and Rye Prolamins.

Prolamins					
	Gliadins (monomers)		**Glutenins (aggregates)**		
Wheat	ω-gliadins	α-,β-gliadins	γ-gliadins	LMW glu-	HMW glu-
	S-Poor	S-Rich			
Barley	C hordeins	–	γ-hordeins	B hordeins	D hordeins
Rye	ω-secalins	–	γ-secalins	LMW-secalins	HMW-secalins

The aminoacid sequence for α-gliadin, a 30kDa ethanol-soluble protein, was the first to be reported[6]. Further investigations revealed the overall structure of prolamins, consisting of typical sequences in the N-terminal end, conserved domains and repetitive regions. These characteristics are found in the homologous components of wheat, barley and rye. For example, the N-terminal regions of ω-gliadins and ω-secalins show a high degree of homology, and repetitive sequences account for 80% of the molecule[7]. Two consensus sequences were found: PQQPY and PQQPFPQQ explaining for the high proline (P) and glutamine (Q) content observed in these proteins. Sequence analysis of avenins revealed that, although some consensus sequences of repetitive units do exist, these are different from those found in wheat, barley and rye.

Based on their molecular weight, prolamins can be divided into: High Molecular Weight (HMW), Medium Molecular Weight (MMW) and Low Molecular Weight (LMW). Proteins from the HMW group include HWM-glutenins (wheat), HMW- secalins (rye) and D hordeins (barley), with molecular weights in the range of 70-90 kDa. The sequence motif QQPGQG is very frequent in the repetitive region.

The MMW group, molecular weight ranges between 50-70 kDa, includes ω-gliadins, ω-secalins (rye) and C-hordeins (barley). Sequences are typically formed by QPQQPFP and QQQFP repetitions. The LMW group, molecular weights ranges between 30-45 kDa, includes α-/β-gliadins and γ-gliadins (wheat), γ-secalins (rye) and γ-hordeins (barley); these contain cysteines forming intrachain disulfide bonds. It should be pointed out that proteins homologous to α-/β-gliadins are not found in rye and barley[1,3]. The typical repetitive sequence in these proteins is QPQQPFP. In this same group, there are other proteins with interstrand disulfide bridges: LMW-GS (wheat), γ75k-secalins (rye) and B-hordeins (barley) (Figure 2 and 3A).

The secondary structure of prolamins contains α-helix regions at the N- and C-terminal ends, and in some interspersed sequences. The repetitive regions adopt a structure called β-turn. The β-turn structural unit is composed of four residues; hydrogen bridge bonds are found between the first

carbonyl group and the amide group of the fourth residue[10]. The regularity of repetitive sequences and of the β-turn structure determines the formation of a cylindrical structure with 13 residues per turn, called a β-spiral. β-turns are predominant in ω-gliadins. They are also found in HMW glutenins and, to a lesser degree, in γ-gliadins. In these cases, the distribution of β-turns is irregular. In contrast, in α-gliadins, this structure is restricted to only a few domains near the N-terminal, the ones which are more irregular and can contain interspersed sequences with α-helix structure[10]. Prolamins are compact protein structures with high physicochemical stability[11]. Their rigid secondary structure is preserved, even under mild denaturalising conditions[12] and only aggressive denaturing conditions, such as 4M urea, may alter their structure[13].

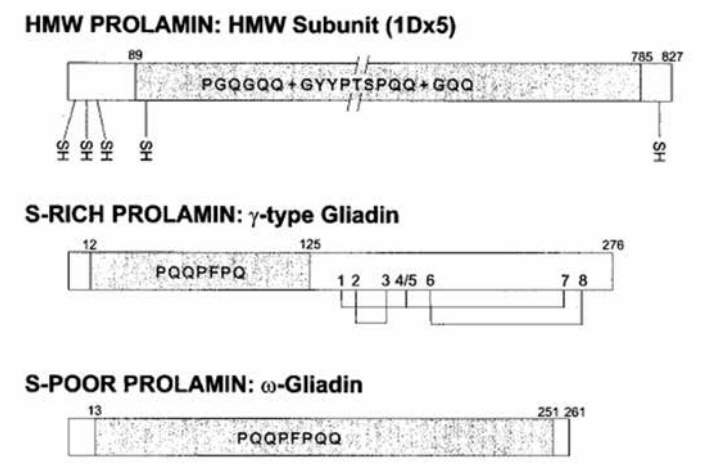


Figure 2. Outline of the structures of HMW glutenins and rich and poor sulphur gliadins. Connecting lines 1 to 8 indicate disulfide bridges between cysteines, while SH indicates the cysteine residue positions[1].

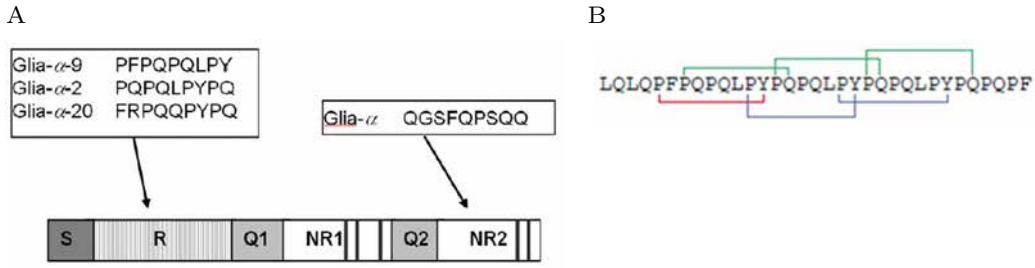


Figure 3. Toxic peptides in α-gliadins. A. Outline of the α-gliadin structure. S: short N-terminal sequence. R: repetitive domain. NR: non-repetitive domains separated by polyglutamine regions (Q). Some of the sequences reported as toxic peptides are indicated[8]*. B. Sequence from α-gliadins from aminoacids 57 to 89 (known as 33mer) including three overlapping toxic epitopes which are present three, two and one times)*[9]*.*

4. Extraction Procedures to Obtain Prolamin Fractions

Aqueous solvents such as 60-70% ethanol, 0.01 M acetic acid or 1 M urea, among others, have been used for extraction of prolamins from flours. This method is not selective and, therefore, results in complex mixtures of proteins[14]. Since prolamins have high tendency to aggregate in the presence of aqueous solvents, biochemical techniques to separate and purify them are not efficient. Reversed-phase high-performance liquid chromatography (RP-HPLC) is the recommended method for prolamin analysis. However, for preparative purposes only a limited amount of proteins can be purified using HPLC.

In order to obtain larger amount of these proteins, medium-pressure liquid chromatography has been used (FPLC, Fast Protein Liquid Chromatography)[15]. In this case, some enriched fractions can be obtained but they are commonly contaminated with component from other fractions. In conclusion, given their biochemical characteristics, it is not possible to obtain gliadins or other prolamins in a pure form using conventional analytic techniques. This is why, applying genetic engineering techniques, several

gliadins have been cloned and produced in a recombinant form. These recombinant proteins have been used in functional studies[16] and in the assessment of their role in the pathogenesis of the CD[17,18]. Although studies carried out using individual protein used as a model, it should be take in mind that most of the processes or mechanisms to be analysed depend more on the interaction among proteins than on properties of one single component.

5. Characterisation of Prolamins by Immunochemical Methods

Polyclonal and monoclonal antibodies were raised against wheat, barley, and rye proteins were a very useful tool to characterise structural and conformational features of prolamins. Though, the low solubility of prolamins in aqueous solutions, the difficulty of obtaining highly purified components and the high degree of homology, and consequently crossreactivity, produce some drawbacks in this kind of studies, the information obtained by immunochemical techniques was relevant to increase the knowledge of this particular protein system. Immunochemical analysis using polyclonal antibodies obtained in rabbits immunized with α-/β- and γ-gliadins, B-hordeins or C-hordeins, showed the immunogenicity of repetitive sequences and, in particular, that regions composed of beta-turns mostly determine the high cross-reactivity[19]. Those results revealed partial homology and the presence of similar conformational and/or lineal epitopes in α- and γ-gliadins, B-hordeins and C-hordeins. In the same studies, ω-gliadins showed much less reactivity, and no recognition of oat and rice proteins was observed.

Monoclonal antibodies were produced using different strategies for immunization and hybridoma selection. These antibodies have increased our knowledge on the structural characteristics of prolamins but also they were useful for the development of quantitative assays to determine gliadin concentration in foodstuffs[14,20-23]. One of these monoclonal

antibodies, called R5, has been extensively characterised and is one of the antibodies worldwide used in commercial ELISA tests for gluten control in foodstuff[24-26].

Characterisation of immunoreactivity and, in particular, the identification of the epitope recognised by a monoclonal antibody in this protein system is difficult. The complex protein system consisting of multiple antigen-antibody interactions with a broad range of affinities, and high crossreactivity makes difficult the interpretation of the immunochemical results. To identify the epitope recognised by monoclonal antibodies, synthetic peptides or phage display libraries were used. In the case of the R5 antibody, the core sequence of the epitopes was identified as QQPFP, QQQFP, LQPFP and QLPFP[24]. These sequences are found in wheat, rye and barley but not in oats or rice.

In addition to the immunochemical tests, modern techniques have been developed for gluten analysis more recently. Different kind of sensors based on physical properties, electrochemical[27], and magnetic[28] have been proposed to detect gluten peptides. Using a different approach, detection of DNA fragments of wheat genome by PCR has also been proposed to detect the presence of wheat components in foodstuff[29]. Though, these are all powerful techniques, they could not replace the massive use of the quantitative ELISA.

6. Commonly Used Gliadins in Research of CD Pathogenesis or Gluten Analysis

To assess the role of gliadins in the pathogenic mechanisms in CD or in the development of food certification assays, the most commonly used gliadin sources have so far been: commercial gliadins, enzymatic digestion of whole gliadins and, more recently, the standard prepared by the European Working Group in Prolamin Analysis and Toxicity (PWG)[30].

- Commercial gliadins are supplied by different companies. Essentially they consist of gliadins obtained from wheat flour following conventional protocols on elimination of the albumin-globulin fraction and later extraction with aqueous ethanol. The protein fraction extracted with aqueous ethanol is then freeze dried and distributed as a lyophilizated powder. It has been the most commonly used gliadin source, but it is not completely soluble. This is a relevant disadvantage when this gliadin preparation is used as standard in quantitative methods. In addition, due to the production procedure, the conformation of the proteins can be altered, and consequently their interaction with antibodies can be modified.
- Gliadin fragments obtained by enzymatic digestion of commercial gliadins have often been used in characterisation of the immune response in CD patients. They are obtained, in general, through treating commercial gliadins with trypsin and pepsin, and usually called PT-gliadin. This enzymatic digestion produces a mixture of peptides of varying size. For biological assays, this preparation is used as a model of gluten-derived peptides found in the intestinal lumen after the physiological process of digestion. The disadvantage of this preparation is the high variability between batches.
- The PWG gliadin was developed as part of an international multi-centre project. This preparation is an international reference material which allows the validation of quantitative tests. For the preparation, flour of 28 varieties of European wheat varieties were mixed, and the gliadin fraction was obtained following a conventional protocol for prolamin extraction. The optimization of the preparation procedure produced a high amount of gliadins. The PWG gliadin was characterized by the most wide-ranging methodology available (RP-HPLC, polyacrylamide gel electrophoresis, capillary electrophoresis, MALDI-TOF, immunoassays). Its stability and solubility were also evaluated. Thus, the PWG gliadin is a highly

stable and completely soluble reagent, which can be used as a reference material for quantitative assays in gluten analysis[30].

7. Prolamins and Toxicity. Induction of Innate and Adaptive Immune Response

Pioneer studies by the group of Dr. Sollid (Oslo, Sweden) at the beginning of the 1990s, demonstrated the specificity of lamina propria T lymphocytes isolated from the intestinal mucosa of untreated CD patients. Those experiments demonstrated the role of HLA alleles in CD pathogenesis[31,32]. Following studies, using panels of T lymphocytes isolated from the intestinal mucosa allowed a deep analysis of the peptides bound to the susceptibility HLA alleles (HLA-DQ2/DQ8)[33]. Because all the information collected through these studies, the mechanism of CD pathogenesis have been defined in detail, perhaps even more than is known for other immune-mediated pathologies.

Due to their particular sequences, gluten peptides are resistant to enzymatic degradation. Consequently, partially degraded and long gluten peptides are present in the intestinal lumen. These peptides are traslocated to the *lamina propria* where they are uptaken and processed by dendritic cells. There, tranglutaminase 2 (TG2), a multitask enzyme, mediates deamidation of glutamine residues at selected positions of the gluten peptides[35,36]. This modification renders peptides with higher affinity for the HLA susceptibility alleles[17,37-39]. Taking together, the selection of peptides able to interact with the HLA susceptibility alleles and the requirement for glutamine deamidation by TG2, algorithms for prediction of toxic sequences were developed[40,41]. Thus, the adaptive response is mainly restricted to certain gluten peptides which fulfil requirement of HLA binding and TG2 modification[42,43].

Though T cell reactivity seems to be heterogeneous, reactivity to α-gliadin predominates to other gliadins. Immunodominant peptides, such as α-gliadin p56-89[44], induce specific immune responses in virtually all patients with celiac

disease[17,45]. The major epitopes on α- and γ-gliadins, as well as on glutenins, have been identified; many bind to HLA-DQ2 and DQ8. In most cases, TG2 deaminated peptides show a higher binding affinity and increase induction of T cell proliferation[36,37,44].

A nomenclature for relevant gluten epitopes has been proposed based on the definition of the reactivity by at least one specific T cell clone, the HLA restriction element, and the nine aminoacid core of the epitope[41]. The list includes 31 epitopes recognized by CD4+ T cells, 24 HLA-DQ2 restricted (23 DQ2.5, 1 DQ2.2) and 7 HLA-DQ8 restricted (4 DQ8, 3 DQ8.5), from α-gliadin, γ-gliadin, ω-gliadin, LMW and HMW glutenins, hordeins, secalins and avenins. (Tabla 2).

It is known that gluten peptides may induce damage in cultured intestinal duodenal biopsies[46], or after being administered in vivo on the proximal or distal intestine[47]. Early effects, i.e. induction of cells stress pathways and stimulation of the local innate immunity, have been described for the α-gliadin fragments p31-49 or p31-43. Peptide 31-43 may induce the upregulation of stress inducible MHC-class I molecules MIC[48], epithelial cell death[48], and may potentiate the effect of Epidermal Growth Factor (EGF) by interference in the inactivation of its receptor[50], as well as the upregulation of mitogen-activated protein (MAP) kinase p38, CD83 and IL-15 production by mononuclear lamina propria cells[51]. It has been also reported that peptide 31-43, unlike other peptides, accumulates in the intracellular lysosomes where it induces TG2 activation and degradation of Peroxisome Proliferator-Activated Receptor (PPAR) gamma, a modulator of intestinal inflammation[52]. Other gliadin peptides have been involved in the expression of non-classical MHC-class II molecules HLA-E[53], and the activation of antigen presenting cells by TLR4[54], and the CXC-chemokine receptor 3 (CXCR3)[55].

Table 2. List of relevant peptides recognized by CD4 T cells.*

EPITOPE		
Current Nomenclature	**Former Nomenclature**	**sequence***
DQ2.5 restricted		
DQ2.5-glia-α1a	DQ2-α-I,α9	PFPQP**E**LPY
DQ2.5-glia-α1b	DQ2-α-III	PYPQP**E**LPY
DQ2.5-glia-α2	DQ2-α-II,α2	PQP**E**LPYPQ
DQ2.5-glia-α3	glia-α20	FRP**E**QPYPQ
DQ2.5-glia-γ1	DQ2-γ-I	PQQSFP**E**Q**Q**
DQ2.5-glia-γ2	DQ2-γ-II,γ30	IQP**E**QPAQL
DQ2.5-glia-γ3	DQ2-γ-III	**Q**QP**E**QPYP**Q**
DQ2.5-glia-γ4a	DQ2-γ-IV	SQP**E**Q**E**FPQ
DQ2.5-glia-γ4b	DQ2-γ-VIIc	PQP**E**Q**E**FPQ
DQ2.5-glia-γ4c	DQ2-γ-VIIa	**Q**QP**E**QPFPQ
DQ2.5-glia-γ4d	DQ2-γ-VIIb	PQP**E**QPFC**Q**
DQ2.5-glia-γ5	DQ2-γ-VI	QQPFP**E**QPQ
DQ2.5-glia-ω1	DQ2-ω-I	PFPQP**E**QPF
DQ2.5-glia-ω2	DQ2-ω-II	PQP**E**QPFPW
DQ2.5-glut-L1	glutenin-17	PFS**E**Q**E**QPV
DQ2.5-glut-L2	glutenin-156	FS**Q**QQ**E**SPF
DQ2.5-hor-1	Hor-α9,Hα9	PFPQP**E**QPF
DQ2.5-hor-2	Hor-α2,Hα2	PQP**E**QPFPQ
DQ2.5-hor-3	hor-I-DQ2	PIP**E**QPQPY
DQ2.5-sec-1	Sec-α9,Sα9	PFPQP**E**QPF
DQ2.5-sec-2	Sec-α2,Sα2	PQP**E**QPFPQ
DQ2.5-ave-1a	Av-α9A	PYPEQ**E**EPF
DQ2.5-ave-1b	Av-α9B,1490	PYPEQ**E**QPF
DQ8 restricted		
DQ8-glia-α1	DQ8-α-I	**E**GSFQPSQ**E**
DQ8-glia-γ1a	DQ8-γ-Ia	**E**QP**Q**QPFPQ
DQ8-glia-γ1b	DQ8-γ-Ib	**E**QP**Q**QPYP**E**
DQ8-glut-H1	HMW-glutenin	**Q**GYYPTSP**Q**

*Aminoacid sequence in one letter code. In red: Glutamate residues (E) due to TG2 desamidation are important for the affinity to DQ molecule. In blue: other Glutamine residues (Q) potential substrates for TG2[41].

However, it remains to be confirmed whether these toxic peptides are produced in the intestinal lumen by digestive enzymes, and the specific receptor for p31-43 (or related peptides) should be identified in order to understand its interaction with enterocytes and how transepithelial transport of this peptide occurs. Transcytosis experiments performed *ex vivo* suggest that transferrin receptor CD71 can mediate the translocation of IgA-gliadin complexes, though this mechanism will not be effective in patients with IgA deficiency[56]. A high transepithelial transport from the apical to the basal membrane of enterocytes has been described in CD patients, mediated by an IFNγ-dependent mechanism[53]. (Table 2) (Figure 3).

The current picture of CD pathogenesis involves two classes of toxic peptides: those able of generating a very fast change in the mucosa through inflammatory and innate mechanisms and others which trigger the full adaptive response. Both pathways interact and potentiate each other to sustain the chronic process of the intestinal damage[42,57].

In conclusion, studies aiming to increase our knowledge on toxic sequences derived from gliadins and glutenins, as well as from other toxic cereals have a great importance in many aspects of celiac disease. The development of analytical tools for the detection of gliadins and glutenins in food to be consumed by CD patients requires a precise immunochemical information on the reactivity of the antibodies used in quantitative techniques. Furthermore, the development of new methods requires also the identification of appropriate sequences from these proteins as target for detection by immunochemical and non immunochemical techniques. In addition, gliadin peptides can be used for the detection of specific antibodies against deamidated peptides, which are a useful tool in serology and screening strategies to detect CD patients. Besides, different gluten peptides have been reported to have a role in the pathogenesis of CD, as they are involved in both the induction of innate and adaptive immune responses. The mechanisms and sequences responsible for the induction of inflammatory reactions are still poorly understood. Some of these inflammatory pathways might also have a role in the new entity Non-Celiac Gluten Sensitivity.

References

1. Shewry PR, Halford NG. *Cereal seed storage proteins: structures, properties and role in grain utilization.* J Exp Botany 2002; 53(370): 947-58.
   http://dx.doi.org/10.1093/jexbot/53.370.947
2. Shewry PR, Tatham AS. *The prolamin storage proteins of cereal seeds: structure and evolution.* Biochem J. 1990; 267: 1-12.
   PMid:2183790 PMCid:PMC1131235
3. Delcour JA, Joye IJ, Pareyt B, Wilderjans E, Brijs K, Lagrain B. *Wheat gluten functionality as a quality determinant in cereal-based food products.* Annu Rev Food Sci Technol. 2012; 3: 469-92.
   http://dx.doi.org/10.1146/annurev-food-022811-101303
   PMid:22224557
4. Kagnoff MF. *Overview and pathogenesis of celiac disease.* Gastroenterology 2005; 128(2 Suppl 1): S10-8.
   http://dx.doi.org/10.1053/j.gastro.2005.02.008
   PMid:15825116
5. Bushuk W, Zillman RR. *Wheat cultivar identification by gliadin electrophoregrams.* Can J Plant Sci. 1978; 58: 505-15.
   http://dx.doi.org/10.4141/cjps78-076
6. Kasarda DD, Okita TW, Bernardin JE, Baecker PA, Nimmo CC, Lew EJ et al. *Nucleic acid (cDNA) and amino acid sequences of a-type gliadins from wheat (Triticum aestivum).* Proc Natl Acad Sci (USA). 1984; 81: 4712-6.
   http://dx.doi.org/10.1073/pnas.81.15.4712
7. Kasarda DD, Autran JC, Lew EJ, Nimmo CC, Shewry PR. *N-terminal amino acid sequences of [omega]-gliadins and w-secalins. Implications for the evolution of prolamin genes.* Biochim Biophys Acta. 1983; 747: 138-50.
   http://dx.doi.org/10.1016/0167-4838(83)90132-2
8. van Herpen TW, Goryunova SB, van der Schoot J, Mitreva M, Salentijin E, Vorst et al. *Alpha-gliadin genes from the A, B and D genomes of wheat contain different sets of celiac disease epitopes.* BMC Genomics. 2006; 7: 1-13.
   http://dx.doi.org/10.1186/1471-2164-7-1
   PMid:16403227 PMCid:PMC1368968
9. Sollid LM. *Coeliac disease: dissecting a complex inflammatory disorder.* Nat Rev Immunol. 2002; 2(9): 647-55.
   http://dx.doi.org/10.1038/nri885
   PMid:12209133
10. Tatham AS, Miflin BJ, Shewry PR. *The beta-turn conformation in wheat gluten proteins: relationships to gluten elasticity.* Cereal Chem. 1985; 62: 405-12.

11. Schofield JD, Bottomley RC, Timms MF, Booth MR. *The effect of heat on wheat gluten and the involvement of sulphydryl-disulphide interchange reactions.* J. Cereal Sci. 1983; 1: 241-53.
http://dx.doi.org/10.1016/S0733-5210(83)80012-5
12. Andrews JL, Skerritt JH. *Quality-related epitopes of High Mr. subunits of wheat glutenin.* J Cereal Sci. 1994; 19: 219-29.
http://dx.doi.org/10.1006/jcrs.1994.1029
13. Goldsbrough AP, Bulleid NJ, Freedman RB, Flavell RB. *Conformational differences between two wheat HMW-glutenin subunits are due to a short region containing six amino acid differences.* Biochem J. 1989; 263: 837-42.
PMid:2597130 PMCid:PMC1133506
14. Skerritt JH, Hill AS. *Enzyme immunoassay for determination of gluten in foods.* J Assoc Off Anal Chem. 1991; 74: 257-64.
PMid:2050607
15. Chirdo FG, Fossati CA, Añón MC. *Fractionation of wheat, barley and rye prolamins by cation-exchange FPLC.* J Agric Food Chem. 1994; 42: 2460-5.
http://dx.doi.org/10.1021/jf00047a018
16. Shimoni Y, Blechl AE, Anderson OD, Galili G. *A recombinant protein of two high molecular weight glutenins alters gluten polymer formation in transgenic wheat.* J Biol Chem. 1997; 272(24): 15488-9.
http://dx.doi.org/10.1074/jbc.272.24.15488
PMid:9182582
17. Arentz-Hansen H, Korner R, Molberg O, Quarsten H, Vader W, Kooy Y et al. *The intestinal T cell response to alpha gliadin in adult celiac disease is focused on a single deamidated glutamine targeted by tissue transglutaminase.* J Exp Med. 2000A; 191: 603-12.
http://dx.doi.org/10.1084/jem.191.4.603
PMid:10684852 PMCid:PMC2195837
18. Arentz-Hansen EH, McAdam SN, Molberg O, Kristiansen C, Sollid LM. *Production of a panel of recombinant gliadins for the characterisation of T cell reactivity in coeliac disease.* Gut. 2000B; 46: 46-51.
http://dx.doi.org/10.1136/gut.46.1.46
PMid:10601054 PMCid:PMC1727782
19. Festenstein GN, Hay FC, Shewry PR. *Immunochemical relationships of the prolamin storage proteins of barley, wheat, rye and oats.* Biochim Biophys Acta. 1987; 912: 371-83.
http://dx.doi.org/10.1016/0167-4838(87)90042-2
20. Ellis HJ, Doyle AP, Wieser H, Sturgess RP, Day P, Ciclitira PJ. *Measurement of gluten using a monoclonal antibody to a sequenced peptide of alpha-gliadin from the coeliac-activating domain I.* J Biochem Biophys Methods. 1994; 28(1): 77-82.
http://dx.doi.org/10.1016/0165-022X(94)90066-3

21. Chirdo FG, Añón MA, Fossati CA. *Optimization of a competitive ELISA for quantification of prolamins in food.* Food and Agricultural Immunology. 1995; 7(4): 333-43.
http://dx.doi.org/10.1080/09540109509354893

22. Chirdo FG, Añón MC, Fossati CA. *Development of high sensitive enzyme immunoassays for gliadin quantification using the streptavidin-biotin amplification system.* Food Agric Immunol. 1998; 10(2): 143-55.
http://dx.doi.org/10.1080/09540109809354977

23. Sánchez D, Tucková L, Burkhard M, Plicka J, Mothes T, Hoffmanová I et al. *Specificity analysis of anti-gliadin mouse monoclonal antibodies used for detection of gliadin in food for gluten-free diet.* J Agric Food Chem. 2007; 4; 55(7): 2627-32.

24. Kahlenberg F, Sánchez D, Lachmann I, Tuckova L, Tlaskalova H, Méndez E et al. *Monoclonal antibody R5 for detection of putatively celiac-toxic gliadin peptides.* Eur Food Res Technol. 2006; 222: 78-82.
http://dx.doi.org/10.1007/s00217-005-0100-4

25. Moron B, Bethune MT, Comino I, Manyani H, Ferragud M, López MC et al. *Toward the assessment of food toxicity for celiac patients: Characterization of monoclonal antinboides to a main immunogenic gluten peptide.* PloS One. 2008 May 28; 3(5):e2294.
http://dx.doi.org/10.1371/journal.pone.0002294
PMid:18509534 PMCid:PMC2386552

26. Mujico JR, Dekking L, Kooy-Winkelaar Y, Verheijen R, van Wichen P, Streppel L et al. Validation of a new enzyme-linked immunosorbent assay to detect the triggering proteins and peptides for celiac disease: interlaboratory study. J AOAC Int. 2012; 95(1): 206-15.
http://dx.doi.org/10.5740/jaoacint.11-042
PMid:22468361

27. Nassef HM, Bermudo-Redondo MC, Ciclitira PJ, Ellis HJ, Fragoso A, O'Sullivan CK. *Electrochemical immunosensor for detection of celiac disease toxic gliadin in foodstuff.* Anal Chem. 2008; 80(23): 9265-71.
http://dx.doi.org/10.1021/ac801620j
PMid:19551990

28. Laube T, Kergaravat SV, Fabiano SN, Hernández SR, Alegret S, Pividori MI. *Magneto immunosensor for gliadin detection in gluten-free foodstuff: towards food safety for celiac patients.* Biosens Bioelectron. 2011; 27(1): 46-52.
http://dx.doi.org/10.1016/j.bios.2011.06.006
PMid:21764291

29. Hernández M, Esteve T, Pla M. *Real-time polymerase chain reaction based assays for quantitative detection of barley, rice, sunflower, and wheat.* J Agric Food Chem. 2005; 53(18): 7003-9.
http://dx.doi.org/10.1021/jf050797j
PMid:16131102

30. Van Eckert R, Berghofer E, Ciclitira PJ, Chirdo FG, Denery-Papini S, Ellis J et al. *Towards a new gliadin reference material- Isolation and characterisation.* J Cereal Sc. 2006; 43: 331-41.

http://dx.doi.org/10.1016/j.jcs.2005.12.009

31. Lundin KE, Scott H, Hansen T, Paulsen G, Halstensen TS, Fausa O et al. *Gliadin-specific, HLA-DQ(alpha 1*0501,beta 1*0201) restricted T cells isolated from the small intestinal mucosa of celiac disease patients.* J Exp Med. 1993; 178(1): 187-96.

http://dx.doi.org/10.1084/jem.178.1.187
PMid:8315377

32. Johansen BH, Buus S, Vartdal F, Viken H, Eriksen JA, Thorsby E et al. *Binding of peptides to HLA-DQ molecules: peptide binding properties of the disease-associated HLA-DQ(alpha 1*0501, beta 1*0201) molecule.* Int Immunol. 1994; 6(3): 453-61.

http://dx.doi.org/10.1093/intimm/6.3.453
PMid:8186196

33. Van de Wal Y, Kooy YM, van Veelen PA, Peña SA, Mearin LM, Molberg Ø et al. *Small intestinal T cells of celiac disease patients recognize a natural pepsin fragment of gliadin.* Proc Natl Acad Sci USA. 1998; 95(17): 10050-4.

http://dx.doi.org/10.1073/pnas.95.17.10050
Pmid:9707598 PMCid:PMC21459

34. Qiao SW, Bergseng E, Molberg Ø, Xia J, Fleckenstein B, Khosla C et al. *Antigen presentation to celiac lesion-derived T cells of a 33-mer gliadin peptide naturally formed by gastrointestinal digestion.* J Immunol. 2004; 173(3): 1757-62.

http://dx.doi.org/10.4049/jimmunol.173.3.1757
PMid:15265905

35. Ráki M, Schjetne KW, Stamnaes J, Molberg Ø, Jahnsen FL, Issekutz TB et al. *Surface expression of transglutaminase 2 by dendritic cells and its potential role for uptake and presentation of gluten peptides to T cells.* Scand J Immunol. 2007; 65(3): 213-20.

http://dx.doi.org/10.1111/j.1365-3083.2006.01881.x
PMid:17309775

36. Van de Wal Y, Kooy Y, Van Veelen P, Pena S, Mearin L, Papadopoulos G et al. *Selective deamidation by tissue transglutaminase strongly enhances gliadin-specific T cell reactivity.* J Immunol. 1998; 161: 1585-8.

PMid:9712018

37. Moldberg O, McAdam SN, Korner R, Quarsten H, Kristiansen C, Scott H et al. *Tissue transglutaminase selectively modifies gliadin peptides that are recognized by gut –derived T cells in coeliac disease.* Nature Med. 1998; 4(6): 713-7.

http://dx.doi.org/10.1038/nm0698-713

38. Anderson RP, Degano P, Godkin AJ, Jewell DP, Hill AVS. *In vivo antigen challenge in celiac disease identifies a single transglutaminase-modified peptide as the dominant A-gliadin T cell epitope.* Nature Med. 2000; 6: 337-42.
http://dx.doi.org/10.1038/73200
PMid:10700238

39. Molberg O, Uhlen AK, Jensen T, Flaete NS, Fleckenstein B, Arentz-Hansen H et al. *Mapping of gluten T-cell epitopes in the bread wheat ancestors: implications for celiac disease.* Gastroenterology. 2005; 128(2): 393-401.
http://dx.doi.org/10.1053/j.gastro.2004.11.003
PMid:15685550

40. Sollid LM, Qiao SW, Anderson RP, Gianfrani C, Koning F. *Nomenclature and listing of celiac disease relevant gluten T-cell epitopes restricted by HLA-DQ molecules.* Immunogenetics. 2012; 64(6): 455-60.
http://dx.doi.org/10.1007/s00251-012-0599-z
PMid:22322673 PMCid:PMC3349865

41. Abadie V, Sollid LM, Barreiro LB, Jabri B. *Integration of genetic and immunological insights into a model of celiac disease pathogenesis.* Annu Rev Immunol. 2011; 29: 493-525.
http://dx.doi.org/10.1007/s00251-012-0599-z
PMid:22322673 PMCid:PMC3349865

42. Meresse B, Malamut G, Cerf-Bensussan N. *Celiac disease: an immunological jigsaw.* Immunity. 2012; 36(6): 907-19.
http://dx.doi.org/10.1016/j.immuni.2012.06.006
PMid:22749351

43. Shan L, Molberg O, Parrot I, Hausch F, Filiz F, Gray GM et al. *Structural basis for gluten intolerance in celiac sprue.* Science. 2002; 297(5590): 2275-9.
http://dx.doi.org/10.1126/science.1074129
PMid:12351792

44. Vader W, Kooy Y, Van Veelen P, De Ru A, Harris D, Benckhuijsen W et al. *The gluten response in children with celiac disease is directed toward multiple gliadin and glutenin peptides.* Gastroenterology. 2002; 122(7): 1729-37.
http://dx.doi.org/10.1053/gast.2002.33606
PMid:12055577

45. Howdle PD, Corazza GR, Bullen AW, Losowsky MS. *Gluten sensitivity of small intestinal mucosa in vitro: quantitative assessment of histologic change.* Gastroenterology. 1981; 80(3): 442-50.
PMid:7450439

46. Ellis HJ, Ciclitira PJ. *In vivo gluten challenge in celiac disease.* Can J Gastroenterol. 2001; 15(4): 243-7.
PMid:11331926

47. Hue S, Mention JJ, Monteiro RC, Zhang S, Collier C, Schmitz J et al. *A direct role for NKG2D/MICA interaction in villous atrophy during celiac disease.* Immunity. 2004; 21, 367-77.

http://dx.doi.org/10.1016/j.immuni.2004.06.018
PMid:15357948

48. Giovannini C, Sanchez M, Straface E, Scazzocchio B, Silano M, De Vincenzi M. *Induction of apoptosis in caco-2 cells by wheat gliadin peptides.* Toxicology. 2000; 145(1): 63-71.

http://dx.doi.org/10.1016/S0300-483X(99)00223-1

49. Barone M, Gimigliano A, Castoria G, Paolella G, Maurano F et al. *Growth factor-like activity of gliadin, an alimentary protein: implications for celiac disease.* Gut 2007; 56: 480-88.

http://dx.doi.org/10.1136/gut.2005.086637
PMid:16891357 PMCid:PMC1856836

50. Maiuri L, Ciacci C, Ricciardelli I, Vacca L, Raia V, Auricchio S et al. *Association between innate response to gliadin and activation of pathogenic T cells in coeliac disease.* Lancet. 2003; 362(9377): 30-7.

http://dx.doi.org/10.1016/S0140-6736(03)13803-2

51. Luciani A, Villella VR, Vasaturo A, Giardino I, Pettoello-Mantovani M, Guido S et al. *Lysosomal accumulation of gliadin p31-43 peptide induces oxidative stress and tissue transglutaminase-mediated PPARgamma downregulation in intestinal epithelial cells and coeliac mucosa.* Gut. 2010; 59(3): 311-9.

http://dx.doi.org/10.1136/gut.2009.183608
PMid:19951908

52. Terrazzano G, Sica M, Gianfrani C, Mazzarella G, Maurano F, De Giulio B et al. *Gliadin regulates the NK-dendritic cell cross-talk by HLA-E surface stabilization.* J Immunol. 2007; 179: 372-81.

http://dx.doi.org/10.4049/jimmunol.179.1.372
PMid:17579058

53. Junker Y, Zeissig S, Kim S-J, Barisari D, Wieser H, Leffler DA et al. *Wheat amylase trypsin inhibitors drive intestinal inflammation via activation of toll-like receptor 4.* J Exp Med. 2012; 209(13): 2395-2408.

http://dx.doi.org/10.1084/jem.20102660
PMid:23209313 PMCid:PMC3526354

54. Lammers KM, Lu R, Brownley J, Lu B, Gerard C, Thomas K et al. *Gliadin induces an increase in intestinal permeability and zonulin release by binding to the chemokine receptor CXCR3.* Gastroenterology. 2008; 135(1): 194-204 e3.

55. Matysiak-Budnik T, Moura IC, Arcos-Fajardo M, Lebreton C, Menard S, Candalh C et al. *Secretory IgA mediates retrotranscytosis of intact gliadin peptides via the transferrin receptor in celiac disease.* J Exp Med. 2008; 205(1): 143-54.

http://dx.doi.org/10.1084/jem.20071204
PMid:18166587 PMCid:PMC2234361

56. Zimmer KP, Fischer I, Mothes T, Weissen-Plenz G, Schmitz M, Wieser H et al. *Endocytotic segregation of gliadin peptide 31-49 in enterocytes*. Gut. 2010; 59(3): 300-10.

http://dx.doi.org/10.1136/gut.2008.169656
PMid:19654123

57. Sollid LM, Jabri B. Triggers and drivers of autoimmunity: lessons from celiac disease. Nat Rev Immunol. 2013 Apr; 13(4): 294-302.

http://dx.doi.org/10.1038/nri3407
PMid:23493116 PMCid:PMC3818716

Chapter 4

Pathogenesis of Celiac Disease

Celia Escudero-Hernández[1], Jose Antonio Garrote[1,2], Eduardo Arranz[1]

[1] Mucosal Immunology Laboratory. Institute of Biology and Molecular Genetics (IBGM).

University of Valladolid – Spanish National Research Council (CSIC), Consejo Superior de Investigaciones Científicas, Valladolid, Spain.

[2] Laboratorio de Genética, Servicio de Análisis Clínicos, Hospital Universitario Río Hortega, Valladolid, Spain.

cescuder@ibgm.uva.es, jagarrote@saludcastillayleon.es, earranz@med.uva.es

Doi: http://dx.doi.org/10.3926/oms.252

How to cite this chapter

Escudero-Hernández C, Garrote JA, Arranz E. *Pathogenesis of Celiac Disease*. In Arranz E, Fernández-Bañares F, Rosell CM, Rodrigo L, Peña AS, editors. *Advances in the Understanding of Gluten Related Pathology and the Evolution of Gluten-Free Foods*. Barcelona, Spain: OmniaScience; 2015. p. 163-191.

Abstract

Celiac disease is a chronic, immune-mediated inflammatory disorder of the small intestine that affects genetically susceptible individuals after ingestion of gluten proteins in wheat, barley and rye cereals. The interaction of genetic and environmental factors leads to loss of tolerance to these proteins and to the development of intestinal lesions characterised by intraepithelial lymphocytosis, enterocyte destruction, mucosal remodelling and the presence of auto-antibodies to the enzyme tissue transglutaminase (TG2). The most widely-accepted pathogenic model includes altered digestion and transport of gluten across the epithelium. This focuses on adaptive immunity mechanisms that depend on stimulation of gluten-reactive CD4+ T cells, which are capable of recognising TG2-deamidated gluten peptides presented by HLA-DQ2/DQ8 molecules, and proinflammatory cytokine production, especially interferon (IFN)-γ. Furthermore, in the innate immune response, gluten has a direct toxic effect on the epithelium, in which the main mediator is interleukin (IL)-15. This is manifested by the expression of stress molecules in enterocytes and activation of CD8+ intraepithelial T-cell cytotoxic function. Some aspects still need to be clarified, especially regarding the nonspecific interaction between gluten and epithelial cells, passage of gluten peptides into the *lamina propria* mucosa, TG2 activation, mechanisms that regulate IL-15 expression, and auto-antibody production.

Keywords

Tolerance breakage, transepithelial transport, IL15, IFNγ, intraepithelial lymphocytosis, CD8+ T lymphocytes, TG2, HLA-DQ.

1. Introduction

Celiac disease (CD) is an inflammatory disorder with autoimmune features that affects genetically predisposed individuals. It is triggered by the ingestion of gluten and other related proteins in barley, rye and possibly oats. The interaction of genetic and environmental factors leads to loss of gluten tolerance and the development of intestinal lesions characterised by increased number of lymphocytes in the epithelium and *lamina propria* (LP), villi loss, destruction of epithelial cells and mucosal remodelling, in addition to the presence of auto-antibodies to the enzyme tissue transglutaminase type 2 (TG2). The lesion and inflammatory bowel changes resolve when gluten is removed from the diet[1]. Patients with CD have also been found to have other changes that affect gut lumen digestion[2,3], the direct action of the gluten peptides on the epithelium and gluten protein transport across the epithelium to the LP mucosa[4,5].

The inappropriate immune response to gluten proteins observed in celiac patients involves both innate and adaptive immunity[6,7]. The key element in the pathogenesis of CD is the activation of the CD4+ T-cells in the LP mucosa after the recognition of TG2-deamidated gluten peptides bound to major histocompatibility complex class II (MHC-II) molecules, called HLA-II in humans. TG2 action consists of transforming certain glutamine residues into glutamic acid, resulting in the exposure of negative charges and enhanced affinity between HLA-DQ2 and/or HLA-DQ8 molecules and these peptide fragments that are resistant to proteolytic digestion by digestive enzymes. CD4+ T-cell activation triggers a pro-inflammatory Th1 cytokine response, with a predominance of interferon (IFN)-γ, other cytokines such as tumour necrosis factor [TNF]-α, interleukin [IL]-18 and IL-21, with the absence of IL-12, together with a proportionate decrease in the expression of immunoregulatory cytokines IL-10 and transforming growth factor (TGF)-β[8,9]. Accordingly, a lesion occurs in the mucosa of the proximal small intestine that causes malabsorption and reduced uptake of nutrients. The clinical and functional consequences vary depending on the degree of mucosal atrophy and transformation[10,11.]

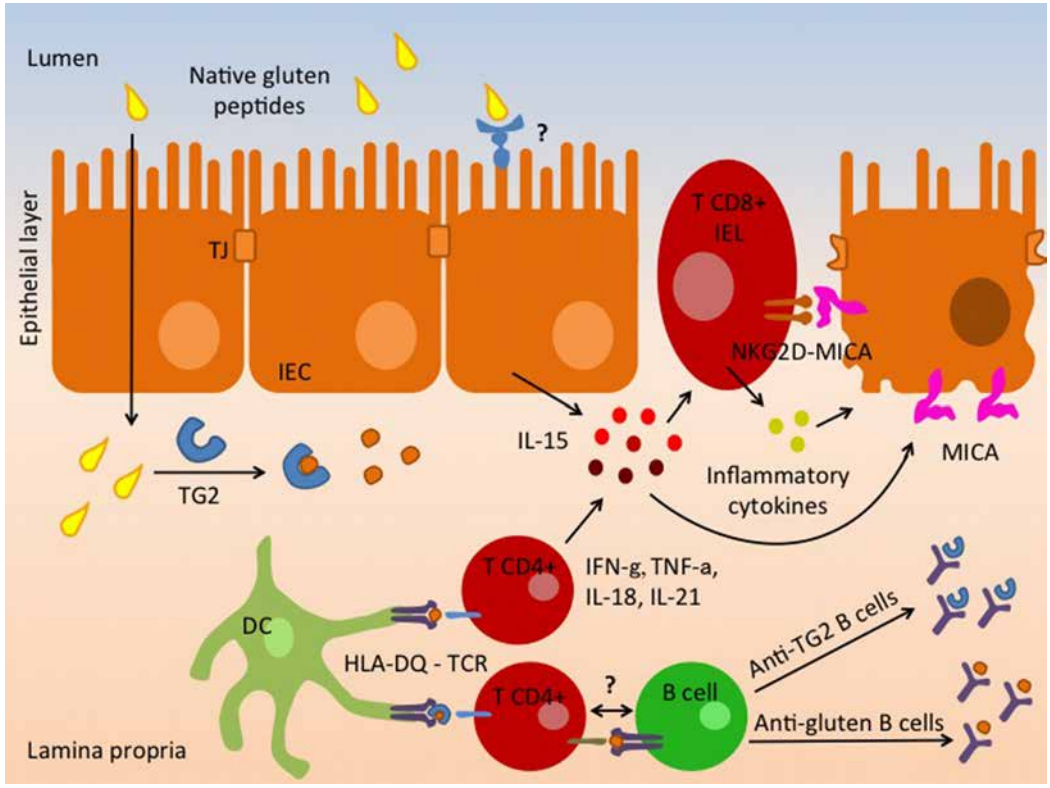


Figure 1. Immunological response to gluten peptides. TG2 modifies gluten peptides by deamidation, thus, HLA-DQ molecules are more likely to bind peptides and these are presented to LP T CD4+ lymphocytes for a longer period of time. T CD4+ lymphocytes are activated and committed to produce Th1 cytokines (IFN-γ, TNF-α, IL-18 and IL-21); they could also help to antibody synthesis by B cells. B cells differentiate into plasmatic cells and secrete specific antibodies against TG2 or gliadin. IECs can produce IL-15 after exposure to other gliadin peptides. Altogether, inflammatory cytokines induce IECs to express stress molecules (MICA), the ligand of NKG2D receptors on activated IELs. Finally, IELs destroy IECs, increasing intestinal permeability. IECs, intestinal epithelial cells; TJ, tight-junctions; TG2, tissue transglutaminase 2; DC, dendritic cell; IELs, intraepithelial lymphocytes; LP, lamina propria; TCR, T-cell receptor; IFN-γ, interferon-γ; TNF-α, tumor necrosis factor-α; IL, interleukin; MICA, MHC class I polypeptide-related sequence A; NKG2D, natural killer cell activating factor 2D.

However, the activation of a gluten-specific CD4+ T-cell response (adaptive immunity) is not sufficient to trigger the mucosal lesion that is characteristic of CD. Some gluten peptides, such as α-gliadin p31-43 and p31-49, induce changes in the innate immunity by acting directly on the epithelium, irrespective of the CD4+ T-cells and HLA-DQ2/DQ8 molecule restriction. This is manifested through an increase in expression of IL-15, cyclooxygenase (COX)-2 and CD25 and CD83 activation markers in the mononuclear cells of the LP[12]. In CD, intestinal intraepithelial lymphocytes in the intestine lose the expression of inhibitory CD94/NKG2A receptors, while increasing the expression of the activating receptors NKG2D and CD94/NKG2C. At the same time, epithelial cells increase the expression of ligands MIC and HLA- E, respectively[13,14]. Epithelial damage leads to increased gut permeability, which may permit the passage of larger, partly-digested gliadin peptides, thereby triggering a positive feedback loop that maintains the inflammatory reaction and intestinal lesion[1] (Figure 1).

2. Intestinal Epithelium

The intestinal epithelium lines the gastrointestinal tract. It is the body's largest mucosal surface and it separates the intestinal lumen from the underlying tissue, where the gut-associated lymphoid tissue (GALT) is located. This physical barrier consists of a single layer of polarised columnar cells (intestinal epithelial cells [IECs]), held together by tight junctions, which prevent the activation of systemic immune responses that can promote the progression of chronic infections and metabolic diseases[15]. Furthermore, the intestinal epithelium has self-protecting and self-regulating properties, not only because it controls new cell growth and old cell replacement, but also because some IECs are specialised to secrete mucus (which is mainly composed of MUC2 protein) and antimicrobial peptides[16], which regulate the levels of commensal and pathogenic bacteria, at the same time as limiting their resistance to an antimicrobial response[15].

The intestinal epithelium may also be directly involved in the immune response due to the ability of microfold cells (M cells) and goblet cells to sample luminal contents and regulate responses through membrane expression of different pattern recognition receptors (PRRs) such as Toll-like receptors (TLRs)[17], which recognise common patterns in pathogenic micro-organisms; NOD-like receptors (NLRs)[18], which detect foreign molecules or cell damage markers in the cytosol; and RIG-I-like receptors (RLRs)[19], which recognise viral ribonucleic acid (RNA). However, the need to tolerate commensal micro-organisms and harmless dietary antigens means that immune responses depend more on the presence of danger signals in infection and stress induced by invasive microorganisms. The term vita-PAMP has been coined to refer to viability receptors and pathogen-associated molecular pattern receptors involved in these processes[20]. Under normal conditions (absence of infection and/or danger signals), the epithelium expresses a repertoire of molecules that maintain homeostasis in the intestinal mucosa. These molecules include thymic stromal lymphopoietin (TSLP)[21,22], TGF-β [21,22], retinoic acid[21], IL-25[23], B-cell activating factor (BAFF)[24] and the B-cell proliferation-inducing ligand (APRIL)[25].

2.1 Gluten Transport Across the Epithelium

Under normal conditions, proteins are mostly hydrolysed by gastric and pancreatic peptidases in the gastrointestinal tract, resulting in smaller peptides or isolated amino acids, which then cross the intestinal epithelium through hydrogen ion-dependent co-transport and sodium-coupled secondary active transport[26]. In CD, gluten proteins are not fully digested. Residual fragments are resistant to enzymatic proteolysis[3] and due to their size, they are not readily absorbed and accumulate in the gut lumen to cross the epithelium through four alternative routes: (1) the paracellular pathway, through the tight junctions between enterocytes[4]; (2) the transcellular pathway, by a mechanism involving enterocyte endocytosis and lysosome degradation during their transit to the basement membrane (a pathway that appears to be altered in CD because intact peptides are allowed to cross the

epithelium to reach the LP)[5,27-29]; (3) retrotranscytosis, a mechanism that depends on gliadin fragments binding to secretory immunoglobulin A1 (sIgA1-peptide) and then CD71, which is a transferrin receptor that is overexpressed in the apical region of the mucosa in active CD[30]; or (4) direct access through extensions of dendritic cells (DCs) derived from monocytes (phenotype CD11c^{low} F4/80+ CX3CR1^{high}), which are sandwiched between epithelial cells[31,32] (Figure 2).

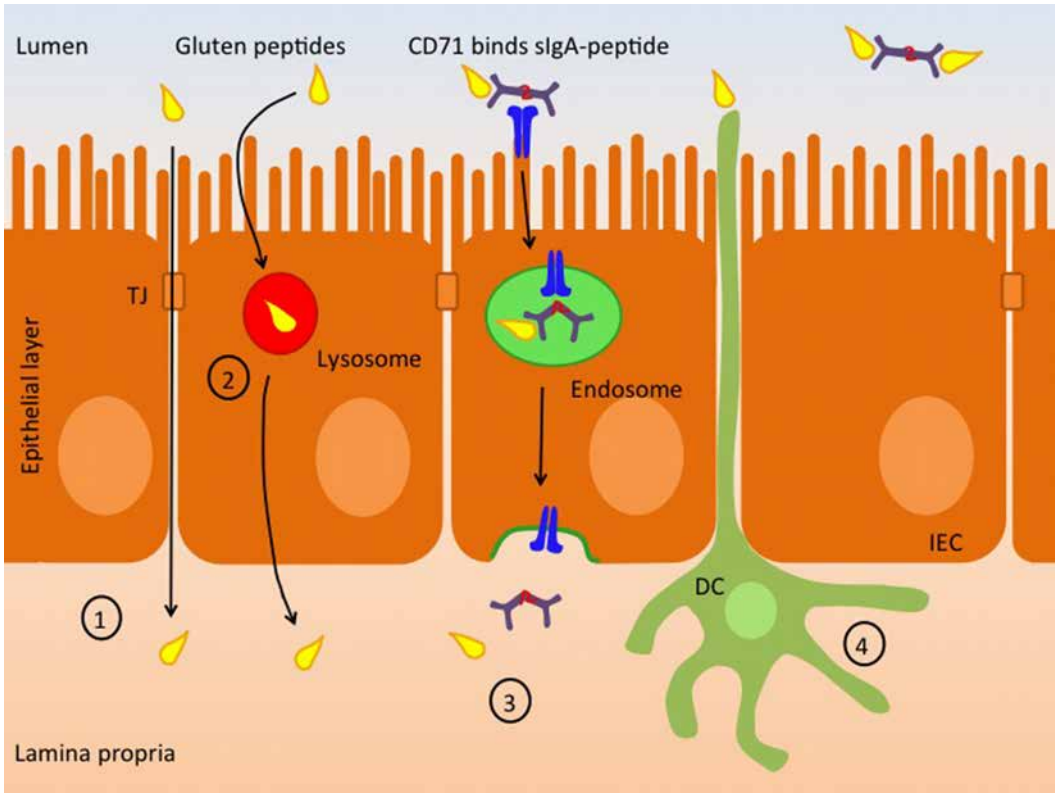


Figure 2. Gliadin transport across the epithelial layer. 1) Paracellular route: gluten cross the epithelial layer through the tight-junctions between enterocytes. 2) Transcellular route: Enterocytes perform endocytosis and degrade proteins in the lysosomes; this route is altered in coeliac disease patients. 3) Retrotranscytosis: secretory IgA binds gliadin peptides, by interaction with the transferrin receptor, CD71, in the apical zone of enterocytes. 4) Dendritic cells can sample antigens directly from the intestinal lumen through dendrites. TJ, tight-junctions; IEC, intestinal epithelial cell; DC, dendritic cell; sIgA, secretory immunoglobulin A.

The passage of gluten peptides across the epithelium not only affects intestinal barrier function, but also the profiles of gene expression and the phosphorylation cascades of metabolic processes, cell proliferation and adhesion, among others[33,34]. Using two *in vitro* culture models and gluten-sensitive macaques, it has been observed that the IFN-γ secreted by activated T-cells in the LP increases gut permeability and promotes immunoreactive α-gliadin (p57-89) peptide 33-mer passage across the epithelium[27,35,36].

Depending on the degree of intestinal inflammation, paracellular transport may also influence peptide transport across the epithelium, because gliadin is able to bind to chemokine receptor CXCR3 and this activates the MyD88 adapter, resulting in the release of zonulin, a protein that rearranges the cell cytoskeleton and modifies tight junctions[37,38]. An increase in mRNA expression of CXCL10 and CXCL11 has been observed in biopsies of patients with active phase CD, as well as elevated serum levels of CXCL10 in these patients[39]. The same study confirmed that CXCL10 is produced by plasma cells and enterocytes, and that its expression increases in the presence of IL-15. It also found increased CXCR3 expression in cells that infiltrate gut mucosa (T-cells in the epithelium and LP, and plasma cells)[39].

3. Adaptive Response to Gluten

Tissue transglutaminase (TG2) is the key component that explains the activation of the adaptive immune response to gluten. TG2 plays a fundamental role in the pathogenic mechanism because it induces enzymatic modification of immunodominant gliadin peptides, leading to the expression of negative charges in amino acid residues in certain positions, thereby increasing affinity for the HLA-DQ2/DQ8 molecules[40]. In addition, TG2 is the main self-antigen of the specific serum antibodies that are of great value in diagnosing CD[41] (Figure 2).

TG2 is found throughout the body. This enzyme catalyses the formation of covalent bonds between glutamine carboxyl groups and lysine amino groups.

It is involved in cell apoptosis because it prevents the exit of cytoplasmic material and, when secreted outside the cell, it collaborates in the remodelling of the extracellular matrix during tissue repair[42]. It is mostly located intracellularly, but appears extracellularly in response to tissue injury. In the normal gut, TG2 is expressed in subepithelial areas, in the LP mucosa and in connective tissue around the crypts; however, in CD, TG2 is also expressed on the apical surface of enterocytes, which may be a gluten-dependent effect[43]. In addition, this enzyme may play a role in the retrotranscytosis mechanism and in gliadin peptide passage through the epithelium, because it has been demonstrated that TG2 can interact with CD71 and sIgA on the apical surface of enterocytes in biopsies of patients with CD. Furthermore, TG2 inhibitors appear to block the transport of gliadin peptide p31-49 via this pathway[44].

TG2 effects on gluten peptides take place under non-physiological conditions (more donor than acceptor molecules) or at a pH of less than 7.0. In these situations, gliadin, which has a glutamine content of more than 30%, is susceptible to TG2-induced changes[42,45]. This is highly relevant in CD, because deamidated peptides have a higher affinity for HLA-DQ molecules, and HLA-DQ2 in particular[1,46,47]. The core structure of the HLA-DQ2 peptide pocket binds these negatively charged amino acids at positions P4, P6 and P7, whereas the HLA-DQ8 molecule does so more externally, at positions P1, P4 and P9[1,46,47]. The fact that the deamidated residues are positioned differently in each gluten peptide suggests that the specific immune response to gluten may be activated for several different pathogenic reasons. The TG2-induced enzymatic change that unmasks the most immunogenic epitopes of gliadin and other prolamines, or that leads to new epitopes due to interaction with proteins in the extracellular matrix may be responsible for the loss of tolerance and onset of autoimmune diseases[1].

However, although the deamidation of gluten peptides is not an absolute requirement, this reaction helps potentiate the adaptive response not only by increasing immunogenic peptide binding to HLA-DQ molecules, but also by improving their stimulatory capacity to present the antigen and promote the

gluten-specific CD4+ T-cell activation[48]. Another possibility is that TG2 activation is not a primary phenomenon in the immune response to gluten, but is triggered by the presence of native (not deamidated) gluten, causing a local inflammatory reaction capable of activating TG2 and initiating its exit from the cytosol. This would amplify the proinflammatory signal and therefore the immune response to gluten[29,49,50] (Figure 1). Furthermore, the activation of TG2 and other enzymes in the gut mucosa may be the result of other environmental factors such as viral infections[51], previous inflammatory reactions[52] or a tissue damage process[53].

3.1. T-Cell Response to Gluten

The adaptive response mediated by LP specific T-cells requires antigen presentation by antigen-presenting cells (APCs) that carry the HLA-DQ2/DQ8 restriction element. In the normal duodenum, APCs that express HLA-DQ molecules in the membrane may be macrophages (accounting for about 80%) of phenotypes CD163+CD11c-; or DCs (the remaining 20%), which are characterised by having a tolerogenic phenotype CD103+CD11c+. However, in CD, most DCs appear to come from the recruitment of peripheral blood monocytes with subsequent maturation *in situ* and they have a proinflammatory phenotype (CD14+ CD11c+). Conversely, there are reduced cell populations with tolerogenic phenotypes (CD103+CD11c+ DCs and CD163+CD11c- macrophages)[54]. The presence of elevated IFN-α levels in the mucosa of patients with CD may be a critical factor in proinflammatory DC differentiation[55], as is suggested by the onset of CD in patients with hepatitis C treated with IFN-α[56], and the predisposition for CD observed in individuals with Down's syndrome (chromosome 21 contains the gene that codes for the IFN-α receptor)[57].

In addition to their involvement in gliadin epitope presentation in the mesenteric lymph nodes, the HLA-DQ2 and DQ8 molecules can also present neo-epitopes and TG2-gluten-peptide complexes to CD4+ T-cells in the LP mucosa[58,59]. These activated lymphocytes trigger a pro-inflammatory response characterised by the secretion of Th1 cytokines with a predominance of IFN-γ,

as well as TNF-α, IL-18 and IL-21, together with a decrease in regulatory cytokines IL-10 and TGF-β[8,60,61]. This cytokine profile and the production of metalloproteinases that break down extracellular matrix proteins, may contribute to the typical lesions observed in CD[1] (Figure 1).

In the healthy gut, the epithelium and LP mucosa express TGF-β1, but in CD TGF-β1is decreased in the epithelial surface and there is loss of crypts, thus increasing the number of macrophages and activated T-cells in the adjacent LP, where there is no tissue damage[62]. Furthermore, IFN-α may be involved in Th1 cell differentiation by enhancing IFN-γ production. It has been observed that IFN-α administration in susceptible individuals can induce a Th1 response leading to hyperplastic lesions[55]. Although as yet unconfirmed, IFN-α may be secreted by activated fibroblasts and macrophages and even DCs in the LP mucosa after an episode of intestinal infection[63], and that it could contribute to intestinal inflammation by rescuing activated T-cells from apoptosis, maintaining memory T-cells once the stimulus has disappeared, and increasing expression of co-stimulatory molecules in local APCs[55]. IL-18 is a cytokine produced by macrophages, DCs and epithelial cells that acts on memory cells and effector cells, enhancing expression of IL-12- or IFN-α-dependent IFN-γ. Under normal conditions, the intestine expresses IL-18, but this expression increases in CD at the expense of its mature form, which requires the involvement of the IL-1β converting enzyme (ICE) or local proteinases[60] (Figure 1).

3.2. B-Cell Response To Gluten

CD is characterised by the presence of a variety of serum antibodies against self and foreign molecules[64]. In 1997, TG2 was identified as the main self-antigen with anti-endomysial antibody reactivity[41]. Anti-TG2 IgA antibodies are produced by plasma cells that infiltrate the LP mucosa of the duodenum[65]. In active phase CD, a two- to three-fold increase in these antibodies has been observed in the lesion area. TG2-specific IgA deposits in the gut have also been described in all disease stages[66], even before the onset of symptoms or before the pathological intestinal lesion appears[67].

B-cells are professional APCs that interact with the antigen through the BCR receptor. Under normal conditions, the gut contains few virgin or memory B-cells and the majority are plasmablasts or plasma cells in the LP with low expression of HLA-II molecules[68]. B-cells probably play a more important role as APCs in the mesenteric lymph nodes, where they may amplify T-cell response to gluten. Although TG2-specific T-cells have not yet been identified, gluten-specific CD4+ T-cells may assist in B-cell differentiation into plasma cells that produce anti-TG2 IgA and IgG antibodies, which disappear when gluten is removed from the diet. One possible explanation is based on the ability of B-cells to act as APCs, as they may present TG2-gluten-peptide complexes via HLA-DQ to gluten-specific T-cells, which in turn would receive the necessary assistance for antibody synthesis[69]. Furthermore, anti-TG2 antibodies may amplify the inflammatory response by increasing gluten absorption and inducing the activation of Fc receptors on local granulocytes[30] (Figure 1).

In CD, other serum auto-antibodies have also been described that present specifically, for example, to actin, different types of collagen, members of the transglutaminase family (TG3, TG6) and clotting factor XIII[70]. It should be noted that IgA/TG3 complexes have been found in the skin of patients with dermatitis herpetiformis[71,72] and the presence of antibodies to neuronal enzyme TG6 has been associated with gluten ataxia[73]. These findings could explain how the extraintestinal manifestations of CD develop.

4. Innate Response to Gluten

Several gliadin peptides have been described with innate response stimulatory properties that act on IECs and DCs, although clarification is needed regarding how they interact with the epithelium and which signalling pathways they activate. These peptides are not recognised by gluten-specific CD4+ T-cells in the context of HLA-DQ2/DQ8 molecules (such as α-gliadin peptides p31-43 and p31-49), which could alter protein processing and intracellular trafficking in IECs and/or activate a stress pathway that has yet

to be identified[5,28,34]. Increased expression of IL-15, cyclooxygenase (COX)-2 and CD25 and CD83 activation markers in the mononuclear cells of the LP has been described using *ex vivo* culture models from biopsies of patients with CD[12]. An increase has also been observed in the expression of the molecules related to the MHC-class I (MIC) polypeptide in IECs[74]. Moreover, some of these gliadin peptides can behave similarly to epidermal growth factor (EGF) by delaying EGF receptor (EGFR) endocytosis and thus prolonging its activation[75]. Although it has been shown that patients with CD also express EGFR and have an activated EGFR signalling pathway, both EGFR and its signalling pathway are constitutively altered (through enhanced phosphorylation of the ERK kinase), i.e., independently of gluten ingestion, which could explain the highly specific damage that gliadin exerts in the epithelium[34]. Apart from these peptides, others may activate DCs by interacting with TLR4[76], as well as stabilising the non-classical MHC molecule HLA-E in the membrane[77], or they could increase gut permeability after binding to chemokine receptor CXCR3[37], an effect that could also be due to the weakening of the tight junctions between the enterocytes[4].

4.1. Role of the Intraepithelial Lymphocytes

Intraepithelial lymphocytes (IELs) form a heterogeneous population located in the basolateral zone of enterocytes, with varying distribution along the intestine. IELs are divided into two groups, natural IELs (T TCR$\alpha\beta$ and T TCR$\gamma\delta$) and induced IELs (T TCR$\alpha\beta$ CD4+ and T TCR$\alpha\beta$ CD8$\alpha\beta$+), defined by their activation mechanisms and the antigens that they recognise[1, 78] (Table 1). The functions of IELs are to defend against infectious agents, memory acquisition and to control responses to innocuous factors, as well as to maintain epithelial integrity[78] (Table 1).

Despite their tolerogenic and protective role, IELs can exacerbate the severity of pathologies such as CD and inflammatory bowel disease[78-80]. In CD, a correlation has been described between the number of TCR$\alpha\beta$ T-cells and villous atrophy[81]. It has also been observed that IELs undergo transformation, acquiring a cytotoxic phenotype[82]. There is also an increased proportion of

IELs with TCR$\gamma\delta$+, which is maintained even with a gluten-free diet, and this is one of the most characteristic changes of CD[83-85]. Natural IELs share some of the preactivation characteristics of CD4+ T-cells that are present in blood and in the LP mucosa and, although they have a higher activation threshold than the latter, in CD they could actually be activated in the gut in response to proinflammatory molecules, and even become autoreactive cells[78,86,87]. Under these conditions, cytotoxic IELs interact through the innate molecules NKG2D and CD94 with their corresponding ligands, MICA and HLA-E, expressed in the IECs[14]. Intraepithelial lymphocytosis occurs as a result, with enterocyte destruction and other alterations such as villous atrophy and crypt hyperplasia[12,78] (Figure 1).

Table 1. Classification of immune system cells that may be involved in the innate or nonspecific response to gluten in the epithelium. IELs, intraepithelial lymphocytes; NK, natural killer; NKT, NK T-cell; TCR, T-cell receptor; MHC, major histocompatibility complex; N/A, not applicable.

	TCR	Restriction	Differentiation	Functions
Natural IELs	$\alpha\beta$ or $\gamma\delta$	MHC	Thymus	Tolerance and protection against diet and microbiota in early life and later protection.
Induced IELs	$\alpha\beta$	MHC	Peripheral	Adaptation to diet and to microbiota: defence, memory and maintenance of integrity. Prevention of exaggerated responses to innocuous antigens.
NK cells	N/A	N/A	Bone marrow, lymph nodes, spleen, tonsils, thymus.	Response to viruses and tumour cells.
NKT cells	Semi-invariant (vα24β11 and others)	CD1d	Peripheral	Protection against tumour cells and autoimmune diseases. Oral tolerance.

Other cell populations that might be involved in the pathogenesis of CD are natural killer (NK) cells and NKT cells[88]. NK cells are involved in responses to virally infected cells and tumours, independently of MHC and antibody formation[89]. A reduction in the number of NK cells has been observed in patients with active CD compared with a control group or patients on a gluten-free diet[85]. Unlike NK cells, NKT cells are a heterogeneous group that have the TCR complex in the membrane, as well as CD3 and Ig receptors and, in some subsets, they also express a semi-invariant TCR receptor (including TCR vα24β11)[90]. They can be activated through TCRs, but independently of MHC[90], and they induce epithelial IL-10 production[91]. However, the role of NKT cells in CD and other diseases is still not fully understood, since these cells can produce cytokines of any pattern, including regulatory ones[92].

4.2. Role of Interleukin (IL)-15 and IL-21

IL-15 is the main mediator in the gluten-induced innate immune response in the gut. This pleiotropic cytokine binds to its specific receptor, related to the IL-2 receptor, by a high-affinity α chain (IL-15Rα). Binding between IL-15 and IL-15Rα, which is necessary for cytokine function, takes place before IL-15 expression in the membrane[93], and is one of the many processes involved in the complex regulation of IL-15[94]. In CD, IL-15 is produced in large quantities by the IECs in response to gluten, but also by mononuclear cells, macrophages and DCs in the LP mucosa[95]. In this context, IL-15 induces IEL reprogramming[13], as well as increasing the expression of MICA stress molecules in enterocytes[96], DC activation[97,98] and positive modulation of IL-21, a cytokine that also plays an important role in the pathogenesis of CD[99,100] (Figure 1). It has been observed that gliadin peptides increase the release of IL-15 in the gut mucosa not only in patients with CD, but also in non-celiac individuals. However, only the mucosa of patients with CD shows increased expression of the IL-15Rα receptor, which could confer these patients a lower threshold of response to IL-15[101].

The finding of an association between the *IL2/IL21* gene region and susceptibility to CD has focused interest on IL-21, a cytokine that is a key determinant in the onset and persistence of CD gut lesions[100]. Furthermore, an increase in IL-21 expression has been observed in biopsies of patients with active CD[61]. IL-21 production is located in lymphocytes in both LP mucosa and the epithelium alike and it is sometimes co-expressed with IFN-γ. Part of this production is also attributed to NKT cells[102]. As mentioned earlier, IL-21 expression is induced by IL-15[99] and both appear to be responsible for blocking the regulatory mechanisms in CD[103-105]. Although this cytokine is produced by Th17 cells, others that follow this pattern are not found to be increased in CD (except in a small group of adults with CD)[106,107].

The two cytokines, IL-15 and IL-21, can act together through different signalling pathways to enhance CD4+ T-cell resistance to regulatory T cells (Treg) in gut mucosa in patients with CD. It is known that IL-15 can interfere with the TGF-β1/Smad3[104] and PI3K[103] anti-inflammatory signals, but the mechanisms of action of IL-21 has yet to be clarified[105]. Finally, IL-15 may also play an important role in the development of refractory CD (RCD) and enteropathy-associated T-cell lymphoma (EATL), by inducing proliferation and resistance to apoptosis of cytotoxic IELs[95].

References

1. Jabri B, Sollid LM. *Tissue-mediated control of immunopathology in coeliac disease.* Nature reviews. Immunology. 2009; 9: 858-70.

   http://dx.doi.org/10.1038/nri2670
   PMid:19935805

2. Hausch F, Shan L, Santiago NA, Gray GM, Khosla C. *Intestinal digestive resistance of immunodominant gliadin peptides.* American journal of physiology. Gastrointestinal and liver physiology. 2002; 283: G996-G1003.

   http://dx.doi.org/10.1152/ajpgi.00136.2002
   PMid:12223360

3. Shan L, Molberg Ø, Parrot I, Hausch F, Filiz F, Gray GM et al. *Structural basis for gluten intolerance in celiac sprue.* Science. 2002; 297: 2275-9.

   http://dx.doi.org/10.1126/science.1074129
   PMid:12351792

4. Clemente MG, De Virgiliis S, Kang JS et al. *Early effects of gliadin on enterocyte intracellular signalling involved in intestinal barrier function.* Gut. 2003; 52: 218-23.

   http://dx.doi.org/10.1136/gut.52.2.218
   PMid:12524403 PMCid:PMC1774976

5. Menard S, Lebreton C, Schumann M, MatysiakBudnik T, Dugave C, Bouhnik Y, et al. *Paracellular versus transcellular intestinal permeability to gliadin peptides in active celiac disease.* The American Journal of Pathology. 2012; 180: 608-15.

   http://dx.doi.org/10.1016/j.ajpath.2011.10.019
   PMid:22119716

6. Sollid LM, Jabri B. *Triggers and drivers of autoimmunity: lessons from coeliac disease.* Nature reviews. Immunology. 2013; 13: 294-302.

   http://dx.doi.org/10.1038/nri3407
   PMid:23493116 PMCid:PMC3818716

7. Qiao SW, Iversen R, Raki M, Sollid LM. *The adaptive immune response in celiac disease.* Seminars in immunopathology. 2012; 34: 523-40.

   http://dx.doi.org/10.1007/s00281-012-0314-z
   PMid:22535446

8. Nilsen EM, Jahnsen FL, Lundin KE, Johansen FE, Fausa O, Sollid LM et al. *Gluten induces an intestinal cytokine response strongly dominated by interferon gamma in patients with celiac disease.* Gastroenterology. 1998; 115: 551-63.

   http://dx.doi.org/10.1016/S0016-5085(98)70134-9

9. Leon AJ, Garrote JA, Blanco-Quiros A et al. *Interleukin 18 maintains a long-standing inflammation in coeliac disease patients.* Clinical and experimental immunology. 2006; 146: 479-85.

http://dx.doi.org/10.1111/j.1365-2249.2006.03239.x
PMid:17100768 PMCid:PMC1810422

10. Marsh MN, Crowe PT. *Morphology of the mucosal lesion in gluten sensitivity.* Bailliere's clinical gastroenterology. 1995; 9: 273-93.

http://dx.doi.org/10.1016/0950-3528(95)90032-2

11. Oberhuber G, Granditsch G, Vogelsang H. *The histopathology of coeliac disease: time for a standardized report scheme for pathologists.* European journal of gastroenterology & hepatology. 1999; 11: 1185-94.

http://dx.doi.org/10.1097/00042737-199910000-00019

12. Maiuri L, Ciacci C, Ricciardelli I et al. *Association between innate response to gliadin and activation of pathogenic T cells in coeliac disease.* Lancet. 2003; 362: 30-7.

http://dx.doi.org/10.1016/S0140-6736(03)13803-2

13. Meresse B, Chen Z, Ciszewski C, Tretiakova M, Bhagat G, Krausz TN et al. *Coordinated induction by IL15 of a TCR-independent NKG2D signaling pathway converts CTL into lymphokine-activated killer cells in celiac disease.* Immunity. 2004; 21: 357-66.

http://dx.doi.org/10.1016/j.immuni.2004.06.020
PMid:15357947

14. Meresse B, Curran SA, Ciszewski C et al. *Reprogramming of CTLs into natural killer-like cells in celiac disease.* J Exp Med. 2006; 203: 1343-55.

http://dx.doi.org/10.1084/jem.20060028
PMid:16682498 PMCid:PMC2121214

15. Peterson LW, Artis D. *Intestinal epithelial cells: regulators of barrier function and immune homeostasis.* Nature reviews. Immunology. 2014; 14: 141-53.

http://dx.doi.org/10.1038/nri3608
PMid:24566914

16. Bevins CL, Salzman NH. *Paneth cells, antimicrobial peptides and maintenance of intestinal homeostasis.* Nature reviews. Microbiology. 2011; 9: 356-68.

http://dx.doi.org/10.1038/nrmicro2546
PMid:21423246

17. Abreu MT. *Toll-like receptor signalling in the intestinal epithelium: how bacterial recognition shapes intestinal function.* Nature reviews. Immunology. 2010; 10: 131-44.

http://dx.doi.org/10.1038/nri2707
PMid:20098461

18. Elinav E, Henao-Mejia J, Flavell RA. *Integrative inflammasome activity in the regulation of intestinal mucosal immune responses.* Mucosal immunology. 2013; 6: 4-13.

http://dx.doi.org/10.1038/mi.2012.115
PMid:23212196

19. Loo YM, Gale M Jr. *Immune signaling by RIG-I-like receptors.* Immunity. 2011; 34, 680-92.

http://dx.doi.org/10.1016/j.immuni.2011.05.003
PMid:21616437 PMCid:PMC3177755

20. Sander LE, Davis MJ, Boekschoten MV, Amsen D, Dascher CC, Ryffel B et al. *Detection of prokaryotic mRNA signifies microbial viability and promotes immunity.* Nature. 2011; 474: 385-9.

http://dx.doi.org/10.1038/nature10072
PMid:21602824 PMCid:PMC3289942

21. Rimoldi M, Chieppa M, Salucci V et al. *Intestinal immune homeostasis is regulated by the crosstalk between epithelial cells and dendritic cells.* Nature immunology. 2005; 6: 507-14.

http://dx.doi.org/10.1038/ni1192
PMid:15821737

22. Zaph C, Troy AE, Taylor BC et al. *Epithelial-cell-intrinsic IKK-beta expression regulates intestinal immune homeostasis.* Nature. 2007; 446: 552-6.

http://dx.doi.org/10.1038/nature05590
PMid:17322906

23. Zaph C, Du Y, Saenz SA et al. *Commensal-dependent expression of IL-25 regulates the IL-23-IL-17 axis in the intestine.* J Exp Med. 2008; 205: 2191-8.

http://dx.doi.org/10.1084/jem.20080720
PMid:18762568 PMCid:PMC2556798

24. Xu W, He B, Chiu A, Chadburn A, Shan M, Buldys M et al. *Epithelial cells trigger frontline immunoglobulin class switching through a pathway regulated by the inhibitor SLPI.* Nature immunology. 2007; 8: 294-303.

http://dx.doi.org/10.1038/ni1434
PMid:17259987

25. He B, Xu W, Santini PA et al. *Intestinal bacteria trigger T cell-independent immunoglobulin A(2) class switching by inducing epithelial-cell secretion of the cytokine APRIL.* Immunity. 2007; 26: 812-26.

http://dx.doi.org/10.1016/j.immuni.2007.04.014
Pmid:17570691

26. Daniel H. *Molecular and integrative physiology of intestinal peptide transport.* Annual review of physiology. 2004; 66: 361-84.
http://dx.doi.org/10.1146/annurev.physiol.66.032102.144149
PMid:14977407

27. Schumann M, Richter, J.F, Wedell, I et al. *Mechanisms of epithelial translocation of the alpha(2)-gliadin-33mer in coeliac sprue.* Gut. 2008; 57: 747-754.
http://dx.doi.org/10.1136/gut.2007.136366
PMid:18305066

28. Zimmer KP, Fisher I, Mothes T, Plenz G, Schmitz M, Wieser H. *Endocytotic segregation of gliadin peptide 31-49 in enterocytes.* Gut. 2010; 59, 300-10.
http://dx.doi.org/10.1136/gut.2008.169656
PMid:19654123

29. Luciani A, Villella VR, Vasaturo A, Giardino I, Mantovani M, Guido S. *Lysosomal accumulation of gliadin p31-43 peptide induces oxidative stress and tissue transglutaminase-mediated PPARgamma downregulation in intestinal epithelial cells and coeliac mucosa.* Gut. 2010; 59, 311-9.
http://dx.doi.org/10.1136/gut.2009.183608
PMid:19951908

30. Matysiak-Budnik T, Moura IC, Arcos-Fajardo M, Lebreton C, Menard S, Candalh C et al. *Secretory IgA mediates retrotranscytosis of intact gliadin peptides via the transferrin receptor in celiac disease.* J Exp Med. 2008; 205: 143-54.
http://dx.doi.org/10.1084/jem.20071204
PMid:18166587 PMCid:PMC2234361

31. Rescigno M, Di Sabatino A. *Dendritic cells in intestinal homeostasis and disease.* The Journal of clinical investigation. 2009; 119: 2441-50.
http://dx.doi.org/10.1172/JCI39134
PMid:19729841 PMCid:PMC2735931

32. Visser J, Rozing J, Sapone A, Lammers K, Fasano A. *Tight junctions, intestinal permeability, and autoimmunity: celiac disease and type 1 diabetes paradigms.* Annals of the New York Academy of Sciences. 2009; 1165: 195-205.
http://dx.doi.org/10.1111/j.1749-6632.2009.04037.x
PMid:19538307 PMCid:PMC2886850

33. Bracken S, Byrne G, Kelly J, Jackson J, Feighery C. *Altered gene expression in highly purified enterocytes from patients with active coeliac disease.* BMC genomics. 2008; 9: 377.
http://dx.doi.org/10.1186/1471-2164-9-377
PMid:18691394 PMCid:PMC2533024

34. Nanayakkara M, Lania G, Maglio M, Kosova R, Sarno M et al. *Enterocyte proliferation and signaling are constitutively altered in celiac disease.* PloS one. 2013; 8: e76006.

http://dx.doi.org/10.1371/journal.pone.0076006
PMid:24204586 PMCid:PMC3799793

35. Bethune MT, Siegel M, Howles-Banerji S, Khosla C. *Interferon-gamma released by gluten-stimulated celiac disease-specific intestinal T cells enhances the transepithelial flux of gluten peptides.* The Journal of pharmacology and experimental therapeutics. 2009; 329: 657-68.

http://dx.doi.org/10.1124/jpet.108.148007
PMid:19218531 PMCid:PMC2672868

36. Mazumdar K, Alvarez X, Borda JT et al. *Visualization of transepithelial passage of the immunogenic 33-residue peptide from alpha-2 gliadin in gluten-sensitive macaques.* PloS one. 2010; 5: e10228.

http://dx.doi.org/10.1371/journal.pone.0010228
PMid:20419103 PMCid:PMC2856682

37. Lammers KM, Lu R, Brownley J, Lu B, Gerard C, Thomas K et al. *Gliadin induces an increase in intestinal permeability and zonulin release by binding to the chemokine receptor CXCR3.* Gastroenterology. 2008; 135: 194-204 e193.

38. Palova-Jelinkova L, Danova K, Drasarova H et al. *Pepsin digest of wheat gliadin fraction increases production of IL-1beta via TLR4/MyD88/TRIF/MAPK/NF-kappaB signaling pathway and an NLRP3 inflammasome activation.* PloS one. 2013; 8: e62426.

http://dx.doi.org/10.1371/journal.pone.0062426
PMid:23658628 PMCid:PMC3639175

39. Bondar C, Araya RE, Guzman L, Rua EC, Chopita N et al. *Role of CXCR3/CXCL10 Axis in Immune Cell Recruitment into the Small Intestine in Celiac Disease.* PloS one. 2014; 9: e89068.

http://dx.doi.org/10.1371/journal.pone.0089068
PMid:24586509 PMCid:PMC3930692

40. Arentz-Hansen H, Körner R, Molberg O, Quarsten H, Vader W, Kooy YM et al. *The intestinal T cell response to alpha-gliadin in adult celiac disease is focused on a single deamidated glutamine targeted by tissue transglutaminase.* J Exp Med. 2000; 191: 603-12.

http://dx.doi.org/10.1084/jem.191.4.603
PMid:10684852 PMCid:PMC2195837

41. Dieterich W, Ehnis, T., Bauer, M et al. *Identification of tissue transglutaminase as the autoantigen of celiac disease.* Nature medicine. 1997; 3: 797-801.

http://dx.doi.org/10.1038/nm0797-797
PMid:9212111

42. Bruce SE, Bjarnason I, Peters TJ. *Human jejunal transglutaminase: demonstration of activity, enzyme kinetics and substrate specificity with special relation to gliadin and coeliac disease.* Clin Sci (Lond). 1985; 68, 573-9.

43. Biagi F, Biagi F, Campanella J, Laforenza U, Gastaldi G, Tritto S et al. *Transglutaminase 2 in the enterocytes is coeliac specific and gluten dependent.* Digestive and liver disease: official journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver. 2006; 38: 652-8.

http://dx.doi.org/10.1016/j.dld.2006.05.021
PMid:16916632

44. Lebreton C, Ménard S, Abed J, Moura IC, Coppo R et al. *Interactions among secretory immunoglobulin A, CD71, and transglutaminase-2 affect permeability of intestinal epithelial cells to gliadin peptides.* Gastroenterology. 2012; 143: 698-707, e691-694.

45. Wieser H. *Chemistry of gluten proteins.* Food Microbiol. 2007; 24, 115-9.

http://dx.doi.org/10.1016/j.fm.2006.07.004
PMid:17008153

46. Kim CY, Quarsten H, Bergseng E, Khosla C, Sollid LM. *Structural basis for HLA-DQ2-mediated presentation of gluten epitopes in celiac disease.* Proceedings of the National Academy of Sciences of the United States of America. 2004; 101: 4175-9.

http://dx.doi.org/10.1073/pnas.0306885101
PMid:15020763 PMCid:PMC384714

47. Henderson KN, Tye-Din JA, Reid HH, Chen Z, Borg NA, Beissbarth T et al. *A structural and immunological basis for the role of human leukocyte antigen DQ8 in celiac disease.* Immunity. 2007; 27: 23-34.

http://dx.doi.org/10.1016/j.immuni.2007.05.015
PMid:17629515

48. van de Wal Y, Kooy Y, van Veelen P, Peña S, Mearin L, Papadopoulos G et al. *Selective deamidation by tissue transglutaminase strongly enhances gliadin-specific T cell reactivity.* J Immunol. 1998; 161: 1585-8.

PMid:9712018

49. Lundin KE, Scott H, Hansen T, Paulsen G, Halstensen TS, Fausa O et al. *Gliadin-specific, HLA-DQ(alpha 1*0501,beta 1*0201) restricted T cells isolated from the small intestinal mucosa of celiac disease patients.* J Exp Med. 1993; 178: 187-196.

http://dx.doi.org/10.1084/jem.178.1.187
PMid:8315377

50. Lundin KE, Scott H, Fausa O, Thorsby E, Sollid LM. *T cells from the small intestinal mucosa of a DR4, DQ7/DR4, DQ8 celiac disease patient preferentially recognize gliadin when presented by DQ8.* Human immunology. 1994; 41: 285-91.

http://dx.doi.org/10.1016/0198-8859(94)90047-7

51. Zanoni G, Navone R, Lunardi C et al. *In celiac disease, a subset of autoantibodies against transglutaminase binds toll-like receptor 4 and induces activation of monocytes.* PLoS medicine. 2006; 3: e358.

http://dx.doi.org/10.1371/journal.pmed.0030358
PMid:16984219 PMCid:PMC1569884

52. Farrace MG, Picarelli A, Di Tola M et al. *Presence of anti-"tissue" transglutaminase antibodies in inflammatory intestinal diseases: an apoptosis-associated event?* Cell death and differentiation. 2001; 8: 767-70.

http://dx.doi.org/10.1038/sj.cdd.4400880
PMid:11464221

53. Siegel M, Strnad P, Watts RE, Choi K, Jabri B, Omary MB et al. *Extracellular transglutaminase 2 is catalytically inactive, but is transiently activated upon tissue injury.* PloS one. 2008; 3: e1861.

54. Beitnes AC, Raki M, Lundin KE, Jahnsen J, Sollid LM et al. *Rapid accumulation of CD14+CD11c+ dendritic cells in gut mucosa of celiac disease after in vivo gluten challenge.* PloS one. 2012; 7: e33556.

http://dx.doi.org/10.1371/journal.pone.0033556
PMid:22438948 PMCid:PMC3306402

55. Monteleone G, Pender SLF, Alstead E, Hauer AC, Lionetti P,. McKenzie C et al. *Role of interferon alpha in promoting T helper cell type 1 responses in the small intestine in coeliac disease.* Gut. 2001; 48: 425-9.

http://dx.doi.org/10.1136/gut.48.3.425
PMid:11171837 PMCid:PMC1760133

56. Cammarota G, Cuoco L, Cianci R, Pandolfi F, Gasbarrini G. *Onset of coeliac disease during treatment with interferon for chronic hepatitis C.* Lancet. 2000; 356: 1494-5.

http://dx.doi.org/10.1016/S0140-6736(00)02880-4

57. George EK, Mearin ML, Bouquet J, von Blomberg BM, Stapel SO, van Elburg RM et al. *High frequency of celiac disease in Down syndrome. The Journal of pediatrics.* 1996; 128: 555-7.

http://dx.doi.org/10.1016/S0022-3476(96)70369-4

58. Qiao SW, Bergseng E, Molberg O, Jung G, Fleckenstein B, Sollid LM. *Refining the rules of gliadin T cell epitope binding to the disease-associated DQ2 molecule in celiac disease: importance of proline spacing and glutamine deamidation.* J Immunol. 2005; 175: 254-61.

http://dx.doi.org/10.4049/jimmunol.175.1.254
Pmid:15972656

59. Ciccocioppo R, Di Sabatino A, Ara C, Biagi F, Perilli M, Amicosante G. *Gliadin and tissue transglutaminase complexes in normal and coeliac duodenal mucosa.* Clinical and experimental immunology. 2003; 134: 516-24.
http://dx.doi.org/10.1111/j.1365-2249.2003.02326.x
PMid:14632760 PMCid:PMC1808891

60. Salvati VM, MacDonald TT, Bajaj-Elliott M et al. *Interleukin 18 and associated markers of T helper cell type 1 activity in coeliac disease.* Gut. 2002; 50: 186-90.
http://dx.doi.org/10.1136/gut.50.2.186
PMid:11788557 PMCid:PMC1773110

61. Fina D, Sarra M, Caruso R et al. *Interleukin 21 contributes to the mucosal T helper cell type 1 response in coeliac disease.* Gut. 2008; 57: 887-92.
http://dx.doi.org/10.1136/gut.2007.129882
PMid:17965065

62. Lionetti P, Pazzaglia A, Moriondo M, Azzari C, Resti M, Amorosi A, et al. *Differing patterns of transforming growth factor-beta expression in normal intestinal mucosa and in active celiac disease.* Journal of pediatric gastroenterology and nutrition. 1999; 29: 308-13.
http://dx.doi.org/10.1097/00005176-199909000-00013
PMid:10467997

63. Di Sabatino A, Pickard KM, Gordon JN, Salvati V, Mazzarella G, Beattie RM et al. *Evidence for the role of interferon-alfa production by dendritic cells in the Th1 response in celiac disease.* Gastroenterology. 2007; 133: 1175-87.
http://dx.doi.org/10.1053/j.gastro.2007.08.018
PMid:17919493

64. Dieterich W, Storch WB, Schuppan D. *Serum antibodies in celiac disease.* Clin Lab. 2000; 46: 361-4.
PMid:10934583

65. Di Niro R, Mesin L, Zheng NY, Stamnaes J, Morrissey M, Lee JH. *High abundance of plasma cells secreting transglutaminase 2-specific IgA autoantibodies with limited somatic hypermutation in celiac disease intestinal lesions.* Nature medicine. 2012; 18: 441-5.
http://dx.doi.org/10.1038/nm.2656
PMid:22366952

66. Brandtzaeg P. *The changing immunological paradigm in coeliac disease.* Immunology letters. 2006; 105: 127-39.
http://dx.doi.org/10.1016/j.imlet.2006.03.004
Pmid:16647763

67. Mesin L, Sollid LM, Di Niro R. *The intestinal B-cell response in celiac disease.* Frontiers in immunology. 2012; 3: 313.

http://dx.doi.org/10.3389/fimmu.2012.00313
PMid:23060888 PMCid:PMC3463893

68. Farstad IN, Carlsen H, Morton HC, Brandtzaeg P. *Immunoglobulin A cell distribution in the human small intestine: phenotypic and functional characteristics.* Immunology. 2000; 101: 354-63.

http://dx.doi.org/10.1046/j.1365-2567.2000.00118.x
PMid:11106939 PMCid:PMC2327091

69. Sollid LM, Molberg O, McAdam S, Lundin KE. *Autoantibodies in coeliac disease: tissue transglutaminase - guilt by association?* Gut. 1997; 41: 851-2.

http://dx.doi.org/10.1136/gut.41.6.851
PMid:9462222 PMCid:PMC1891617

70. Alaedini A, Green PH. *Autoantibodies in celiac disease.* Autoimmunity. 2008; 41: 19-26.

http://dx.doi.org/10.1080/08916930701619219
PMid:18176861

71. Sardy M, Karpati S, Merkl B, Paulsson M, Smyth N. *Epidermal transglutaminase (TGase 3) is the autoantigen of dermatitis herpetiformis.* J Exp Med. 2002; 195: 747-57.

http://dx.doi.org/10.1084/jem.20011299
PMid:11901200 PMCid:PMC2193738

72. Qiao SW, Iversen R, Raki M, Sollid LM. *The adaptive immune response in celiac disease.* Semin Immunopathol. 2012; 34: 523-40.

http://dx.doi.org/10.1007/s00281-012-0314-z
PMid:22535446

73. Hadjivassiliou M, Aeschlimann P, Strigun A et al. *Autoantibodies in gluten ataxia recognize a novel neuronal transglutaminase.* Ann Neurol. 2008; 64: 332-43.

http://dx.doi.org/10.1002/ana.21450
PMid:18825674

74. Hue S, Mention JJ, Monteiro RC, Zhang S, Cellier C, Schmitz J et al. *A direct role for NKG2D/MICA interaction in villous atrophy during celiac disease.* Immunity. 2004; 21: 367-77.

http://dx.doi.org/10.1016/j.immuni.2004.06.018
PMid:15357948

75. Barone MV, Gimigliano A, Castoria G, Paolella G, Maurano F, Paparo F et al. *Growth factor-like activity of gliadin, an alimentary protein: implications for coeliac disease.* Gut. 2007; 56: 480-8.

http://dx.doi.org/10.1136/gut.2005.086637
PMid:16891357 PMCid:PMC1856836

76. Junker Y, Zeissig S, Kim SJ, Barisani D, Wieser H, Leffler DA et al. *Wheat amylase trypsin inhibitors drive intestinal inflammation via activation of toll-like receptor 4.* J Exp Med. 2012; 209: 2395-408.
http://dx.doi.org/10.1084/jem.20102660
PMid:23209313 PMCid:PMC3526354

77. Terrazzano G, Sica M, Gianfrani C, Mazzarella G, Maurano F, De Giulio B, de Saint-Mezard S et al. *Gliadin regulates the NK-dendritic cell cross-talk by HLA-E surface stabilization.* J Immunol. 2007; 179: 372-81.
http://dx.doi.org/10.4049/jimmunol.179.1.372
PMid:17579058

78. Cheroutre H, Lambolez F, Mucida D. *The light and dark sides of intestinal intraepithelial lymphocytes.* Nature reviews. Immunology. 2011; 11, 445-56.
http://dx.doi.org/10.1038/nri3007
PMid:21681197 PMCid:PMC3140792

79. Qiu Y, Yang H. *Effects of Intraepithelial Lymphocyte-Derived Cytokines on Intestinal Mucosal Barrier Function.* Journal of interferon & cytokine research: the official journal of the International Society for Interferon and Cytokine Research. 2013.

80. Cheroutre H. *In IBD eight can come before four.* Gastroenterology. 2006; 131: 667-70.
http://dx.doi.org/10.1053/j.gastro.2006.06.041
PMid:16890619

81. Kutlu T, Brousse N, Rambaud C, Le Deist F, Schmitz J, Cerf-Bensussan N. *Numbers of T cell receptor (TCR) alpha beta+ but not of TcR gamma delta+ intraepithelial lymphocytes correlate with the grade of villous atrophy in coeliac patients on a long term normal diet.* Gut. 1993; 34: 208-14.
http://dx.doi.org/10.1136/gut.34.2.208
PMid:8432475 PMCid:PMC1373972

82. Spencer J, MacDonald TT, Diss TC, Walker-Smith JA, Ciclitira PJ, Isaacson PG. *Changes in intraepithelial lymphocyte subpopulations in coeliac disease and enteropathy associated T cell lymphoma (malignant histiocytosis of the intestine).* Gut. 1989; 30: 339-46.
http://dx.doi.org/10.1136/gut.30.3.339
PMid:2785074 PMCid:PMC1378456

83. Halstensen TS, Scott H, Brandtzaeg P. *Intraepithelial T cells of the TcR gamma/delta+ CD8- and V delta 1/J delta 1+ phenotypes are increased in coeliac disease.* Scandinavian journal of immunology. 1989; 30: 665-72.
http://dx.doi.org/10.1111/j.1365-3083.1989.tb02474.x
Pmid:2481336

84. Arranz E, Bode J, Kingstone K, Ferguson A. *Intestinal antibody pattern of coeliac disease: association with gamma/delta T cell receptor expression by intraepithelial lymphocytes, and other indices of potential coeliac disease.* Gut. 1994; 35: 476-82.
http://dx.doi.org/10.1136/gut.35.4.476
PMid:8174984 PMCid:PMC1374795

85. Leon F. *Flow cytometry of intestinal intraepithelial lymphocytes in celiac disease.* Journal of immunological methods. 2011; 363: 177-86.
http://dx.doi.org/10.1016/j.jim.2010.09.002
PMid:20833175

86. Guehler SR, Finch RJ, Bluestone JA, Barrett TA. *Increased threshold for TCR-mediated signaling controls self reactivity of intraepithelial lymphocytes. J Immunol.* 1998; 160: 5341-6.
PMid:9605133

87. Han A et al. *Dietary gluten triggers concomitant activation of CD4+ and CD8+ alphabeta T cells and gammadelta T cells in celiac disease.* Proceedings of the National Academy of Sciences of the United States of America. 2013; 110: 13073-8.
http://dx.doi.org/10.1073/pnas.1311861110
PMid:23878218 PMCid:PMC3740842

88. Calleja S, Vivas S, Santiuste M, Arias L, Hernando M, Nistal E et al. *Dynamics of non-conventional intraepithelial lymphocytes-NK, NKT, and gammadelta T-in celiac disease: relationship with age, diet, and histopathology.* Digestive diseases and sciences. 2011; 56: 2042-9.
http://dx.doi.org/10.1007/s10620-010-1534-5
PMid:21221796

89. Caligiuri MA. *Human natural killer cells.* Blood. 2008; 112: 461-9.
http://dx.doi.org/10.1182/blood-2007-09-077438
PMid:18650461 PMCid:PMC2481557

90. Middendorp S, Nieuwenhuis EE. *NKT cells in mucosal immunity.* Mucosal immunology. 2009; 2: 393-402.
http://dx.doi.org/10.1038/mi.2009.99
PMid:19587641

91. Colgan SP, Hershberg RM, Furuta GT, Blumberg RS. *Ligation of intestinal epithelial CD1d induces bioactive IL-10: critical role of the cytoplasmic tail in autocrine signaling.* Proceedings of the National Academy of Sciences of the United States of America. 1999; 96: 13938-43.
http://dx.doi.org/10.1073/pnas.96.24.13938
PMid:10570177 PMCid:PMC24169

92. Godfrey DI, Kronenberg M. *Going both ways: immune regulation via CD1d-dependent NKT cells.* The Journal of clinical investigation. 2004; 114: 1379-88.
http://dx.doi.org/10.1172/JCI200423594

93. Duitman EH, Orinska Z, Bulanova E, Paus R, Bulfone-Paus S. *How a cytokine is chaperoned through the secretory pathway by complexing with its own receptor: lessons from interleukin-15 (IL-15)/IL-15 receptor alpha.* Molecular and cellular biology. 2008; 28: 4851-61.
http://dx.doi.org/10.1128/MCB.02178-07
PMid:18505820 PMCid:PMC2493373

94. Budagian V, Bulanova E, Paus R, Bulfone-Paus S. *IL-15/IL-15 receptor biology: a guided tour through an expanding universe.* Cytokine & growth factor reviews. 2006; 17: 259-80.
http://dx.doi.org/10.1016/j.cytogfr.2006.05.001
PMid:16815076

95. Pagliari D, Cianci R, Frosali S, Landolfi R, Cammarota G, Newton EE. *The role of IL-15 in gastrointestinal diseases: A bridge between innate and adaptive immune response.* Cytokine & growth factor reviews. 2013.
http://dx.doi.org/10.1016/j.cytogfr.2013.05.004
PMid:23791986

96. Martin-Pagola A, Pérez-Nanclares G, Ortiz L, Vitoria JC, Hualde I et al. *MICA response to gliadin in intestinal mucosa from celiac patients.* Immunogenetics. 2004; 56: 549-54.
http://dx.doi.org/10.1007/s00251-004-0724-8
PMid:15490153

97. Ohteki T, Suzue K, Maki C, Ota T, Koyasu S. *Critical role of IL-15-IL-15R for antigen-presenting cell functions in the innate immune response.* Nature immunology. 2001; 2: 1138-43.
http://dx.doi.org/10.1038/ni729
PMid:11702064

98. Mattei F, Schiavoni G, Belardelli F, Tough DF. *IL-15 is expressed by dendritic cells in response to type I IFN, double-stranded RNA, or lipopolysaccharide and promotes dendritic cell activation.* J Immunol. 2001; 167: 1179-87.
http://dx.doi.org/10.4049/jimmunol.167.3.1179
PMid:11466332

99. Sarra M, Cupi ML, Monteleone I, Franzè E, Ronchetti G, Di Sabatino A et al. *IL-15 positively regulates IL-21 production in celiac disease mucosa.* Mucosal immunology. 2013; 6: 244-55.
http://dx.doi.org/10.1038/mi.2012.65
PMid:22785229

100. van Heel DA, Franke L, Hunt KA, Gwilliam R, Zhernakova A, Inouye M et al. *A genome-wide association study for celiac disease identifies risk variants in the region harboring IL2 and IL21.* Nature genetics. 2007; 39: 827-9.

http://dx.doi.org/10.1038/ng2058
PMid:17558408 PMCid:PMC2274985

101. Bernardo D, Garrote JA, Allegretti Y, León A, Gómez E, Bermejo-Martin JF et al. *Higher constitutive IL15R alpha expression and lower IL-15 response threshold in coeliac disease patients.* Clinical and experimental immunology. 2008; 154: 64-73.

http://dx.doi.org/10.1111/j.1365-2249.2008.03743.x
PMid:18821940 PMCid:PMC2561095

102. Coquet JM, Kyparissoudis K, Pellicci DG, Besra G, Berzins SP, Smyth MJ et al. *IL-21 is produced by NKT cells and modulates NKT cell activation and cytokine production.* J Immunol. 2007; 178: 2827-34.

http://dx.doi.org/10.4049/jimmunol.178.5.2827
PMid:17312126

103. Ben Ahmed M, Belhadj Hmida N, Moes N et al. *IL-15 renders conventional lymphocytes resistant to suppressive functions of regulatory T cells through activation of the phosphatidylinositol 3-kinase pathway.* J Immunol. 2009; 182: 6763-70.

http://dx.doi.org/10.4049/jimmunol.0801792
PMid:19454671

104. Benahmed, M., Meresse, B., Arnulf, B. et al. *Inhibition of TGF-beta signaling by IL-15: a new role for IL-15 in the loss of immune homeostasis in celiac disease.* Gastroenterology. 2007; 132: 994-1008.

http://dx.doi.org/10.1053/j.gastro.2006.12.025
PMid:17324400

105. Peluso I, Fantini MC, Fina D et al. *IL-21 counteracts the regulatory T cell-mediated suppression of human CD4+ T lymphocytes.* J Immunol. 2007; 178: 732-9.

http://dx.doi.org/10.4049/jimmunol.178.2.732
PMid:17202333

106. Bodd M, Raki M, Tollefsen S et al. *HLA-DQ2-restricted gluten-reactive T cells produce IL-21 but not IL-17 or IL-22.* Mucosal immunology. 2010; 3: 594-601.

http://dx.doi.org/10.1038/mi.2010.36
PMid:20571486

107. van Leeuwen MA, Lindenbergh-Kortleve DJ, Raatgeep HC et al. *Increased production of interleukin-21, but not interleukin-17A, in the small intestine characterizes pediatric celiac disease.* Mucosal immunology. 2013; 6: 1202-13.

PMid:23571506

Chapter 5

Intestinal Microbiota and Celiac Disease

Marta Olivares, Yolanda Sanz

Microbial Ecology, Nutrition & Health Research Group. Institute of Agrochemistry and Food Technology. National Research Council (IATA-CSIC), Valencia, Spain.

yolsanz@iata.csic.es

Doi: http://dx.doi.org/10.3926/oms.253

How to cite this chapter

Olivares M, Sanz Y. *Intestinal Microbiota and Celiac Disease.* In Arranz E, Fernández-Bañares F, Rosell CM, Rodrigo L, Peña AS, editors. *Advances in the Understanding of Gluten Related Pathology and the Evolution of Gluten-Free Foods.* Barcelona, Spain: OmniaScience; 2015. p. 193-221.

Abstract

Intestinal microbiota is constituted by a particular assembly of bacteria that develop symbiotic relationships with their host, contributing to diverse physiological functions and determining resilience to disease. Diverse environmental and intrinsic factors can upset this symbiotic relationship, shifting the ecosystem from a state of eubiosis to one of dysbiosis, which causes functional modifications and promotes disease. Indeed, immune dysfunction frequently coincides with intestinal dysbiosis and one can occur as a result of the other, creating a vicious circle. On this basis, hypotheses suggest that a dysbiotic gut microbiota could influence the onset and progression of celiac disease (CD). Epidemiological studies indicate that common perinatal and early postnatal factors influencing CD risk also affect the intestinal microbiota structure. A recent prospective study of healthy infants at family risk of developing CD has also revealed that the HLA-DQ genotype influences the microbiota composition. Several studies have also shown imbalances in the intestinal microbiota of CD patients, which are not fully normalized despite their adherence to a gluten-free diet, thus suggesting that such imbalances are not just a secondary consequence of CD. Furthermore, two small intervention studies have recently reported potential interest in the use of specific bifidobacteria to improve CD treatment, although larger human trials are required to confirm the benefits. Altogether, findings indicate that gut microbiota composition and function may be one of the missing pieces in the CD puzzle that could help to fully explain disease pathogenesis and risk. Thus, it is interesting to investigate new

strategies for CD management that target gut microbiota within this research field.

Keywords

Microbiota, celiac disease, *Bifidobacterium*, probiotics.

---

1. Introduction

Celiac disease (CD) is a chronic enteropathy triggered by cereal gluten proteins in genetically predisposed individuals. CD onset usually occurs in early childhood after the first exposure to dietary gluten; however, recent decades have witnessed an increase in the number of subjects experiencing gluten intolerance in their late adulthood[1]. This phenomenon is not fully explained by improvements in diagnosis and suggests changes in exposure to environmental factors that contribute to disease development.

The etiology of the disease is strongly associated with the genes of the human leukocyte antigen (HLA) that encode the HLA-DQ2 (HLA-DQ2.5 and HLA-DQ2.2) and HLA-DQ8 heterodimers expressed by antigen-presenting cells (APC). Gluten peptides bond to HLA heterodimers and are presented to T cells that trigger a complex immune response involving the innate and adaptive system. Most patients are carriers of the HLA-DQ2/DQ8 genes but this genotype is also present in about 40% of the general population and only a small percentage (2-5%) develops CD[2,3]. This indicates that the HLA-DQ genotype is necessary but not solely responsible for development of the disease. Gluten is the main environmental trigger of CD but its intake neither fully explains the onset nor its clinical manifestations. In recent years, other environmental factors that influence the early gut microbiota composition such as type of delivery at birth and milk-feeding, intestinal infections and antibiotic intake, have also been associated with the risk of developing CD[2-7].

Observational studies of children and adult patients with CD (untreated and treated with a gluten-free diet (GFD)) revealed imbalances in their intestinal microbiota as compared to control subjects, which could contribute to the pathogenesis of the disease[8,9]. This evidence suggests that the imbalances in gut microbiota are not only a secondary consequence of the inflammatory milieu characteristic of the active phase of CD but that they could also be a predisposing factor for disease development. However, the GFD *per se* also induced changes in gut microbiota composition of healthy adult subjects and could be partly responsible for the alterations detected in

treated CD patients[10]. Therefore, to understand whether gut microbiota imbalances could play a role in CD onset, a prospective study is underway to investigate the early features of the intestinal microbiome in infants at family risk of CD development.

Currently, CD is among the most prevalent chronic digestive disorders but the only treatment is life-long adherence to a GFD. However, compliance with this dietary restriction is complicated due to the presence of gluten in most processed foods and patients are continuously exposed to gluten. Therefore, the identification of modifiable environmental factors that contribute to CD onset is critical for the development of strategies that lead to a reduction in disease incidence. This may be the case for components of the intestinal microbiota, whose acquisition could be modulated by environmental and dietary factors.

Here, we summarize the current understanding of the role played by intestinal microbiota in the etiopathogenesis of CD. We also discuss the possibilities of contributing to disease prevention and treatment by modulating gut microbiota composition and function.

2. Gut Microbiota Acquisition in Infants and CD Risk

The primary colonization of the intestinal microbiota begins at birth with the acquisition of microbes from the environment, mainly from the maternal vagina and the skin. It is a dynamic process that involves interactions of co-occurrence and exclusion between intestinal bacteria, reflecting life events of the newborn and undergoing changes until the first two-three years of age when the microbiome starts to converge toward a generic adult-like profile[11,12]. The intestinal colonization process leads to the acquisition and establishment of a protective microbiota that could modulate the risk of developing immune-mediated diseases in adulthood[13]. This influence is mediated by early gut microbiota and immune system interactions that are crucial for the development of tolerance towards harmless antigens from the diet and the microbiota.

2.1. Type of Delivery and Breast Feeding Practices

Perinatal and early postnatal environmental factors influencing the microbiota composition have been associated with CD susceptibility[14]. The greater risk of children born by caesarean section developing CD[15] might be attributed to the delay in intestinal colonization by bifidobacteria and the reduced bacterial diversity observed in caesarean-born compared to naturally-delivered infants[16]. Longer breast-feeding and particularly maintenance of breast-feeding when gluten is introduced seems to reduce the risk of developing CD or, at least, delays its onset in most case-control retrospective studies included in the meta-analysis by Akobeng et al.(2006)[17]. Also, feeding practices involving the gradual introduction of gluten simultaneous to breastfeeding were proposed as the protective agent responsible for reducing CD prevalence in one birth cohort compared to the "Swedish CD epidemic" cohort[18]. However, other prospective epidemiological and intervention studies failed to find a protective effect of breast-feeding in either CD autoimmunity or biopsy proven CD[19,20]. These inconsistencies could be due to the implication of non-controlled variables (type of delivery, incidence of infections, amount of gluten in the diet, etc.) that confound the statistical analysis on breastfeeding effects. Duration of breast-feeding could be associated with a reduced or delayed exposure of the newborn to dietary gluten, which might contribute to the protective effect of breast milk. Plausibly bioactive breast milk components may also be involved in the potential protective effect of breast-feeding on CD development. For the infant's gut, breast milk is a source of bacteria[21,22] and of human milk oligosaccharides (HMOs), which promote gut colonization by *Bifidobacterium* spp., possibly explaining the differences observed between the intestinal microbiota of breast-fed and formula-fed infants[23-25]. The beneficial properties of bifidobacteria on infants' health is widely accepted[26], and scarcity of these bacteria have been associated with the onset of inflammatory bowel disease (IBD)[27], type 1 diabetes (T1D)[28] and infant allergies[29]. Besides human milk provides many bioactive substances involved in passive immune protection and in immunological development of the neonate[30]. A complex network of chemo-attractants and cytokines in

human milk are thought to play a role in compensating the developmental delay of the neonate immune system and in preventing the development of immune-mediated diseases[31]. Recent research has analyzed differences between breast-milk composition of healthy mothers and mothers with CD on a GFD[32]. Mothers with CD presented a decrease in several immune markers (interleukin (IL)-12p70, transforming growth factor (TGF)-β1 and secretory IgA (sIgA) and in numbers of *Bifidobacterium* spp. in breast-milk[32]. Likewise, these differences in the breast milk of CD mothers might influence the protective effects of breast-feeding on infant health, partly explaining the controversy across studies[32]. Similarly imbalances characterized by a lower content of immune mediators (interferon (IFN)-γ, TGF-β2, IL-10 and sIgA) have been described and interpreted as a health risk factor for infants of allergic mothers[33,34]. Furthermore, wheat gliadins and other gluten peptides have been detected in breast milk using specific IgA-antibodies against gliadin[35,36] and the presence of gluten in breast milk has been suggested to play a role in the induction of oral tolerance of the breastfed infants. Thus, breast milk of mothers with CD following a GFD will lack this stimulus, and this might influence the future gluten tolerance of their offspring. However, as yet there is no evidence to support this hypothesis.

A number of epidemiological studies indicate that several perinatal factors participate in conjunction to modulate CD risk. However, there are no prospective studies revealing how differences in breast milk composition and in intestinal microbiota acquisition early in life might ultimately protect or contribute to CD onset.

2.2. Genotype and Intestinal Microbiota

Murine models using diverse mice strains congenic for major histocompatibility complex (MHC) genes indicate that MHC influences the composition of the faecal microbiota[37]. Recently a fish model using *Gasterosteus aculeatus* (threespine stickleback) has shown that the presence of certain MHC polymorphism is associated with altered abundance of some microbial families[38].

Over 30 years ago, Van de Merwe et al.[39] described that the faecal microbiota of monozygotic human twins was much more similar than that of dizygotic twins. Later a similar observation was reported for adults with varying degrees of relatedness[40] and identical twins, fraternal twins and unrelated controls[41].The most recent study compared microbiota of 416 twin pairs and identified many microbial taxa whose abundances were influenced by host genetics. The family Christensenellaceae showed the highest heritability, which formed a co-occurrence network with other heritable bacteria and Archaea in lean individuals[42]. This evidence suggests that host genetics influence the composition of the human gut and that this influences the phenotype[42]. In the case of CD, a prospective study in a cohort of 164 infants with a family history of the disease reported associations between genetic risk (HLA-DQ genotype) and alterations in intestinal microbiota composition[43-45]. The HLA-DQ2/8 genotype and the type of feeding (maternal or formula) influenced in conjunction the intestinal colonization analyzed by fluorescence *in situ* hybridization (FISH), real time PCR and denaturing gradient gel electrophoresis (DGGE) techniques[43-45]. In addition, specific decreases in *Bifidobacterium* spp. and *B. longum* and increases in *Staphylococcus* spp. were associated with higher genetic risk of developing CD irrespective of milk-feeding type[44]. The recent pyrosequencing analysis of the microbiota of a sub-cohort of 22 infants, all breast-fed and naturally delivered, confirmed that the HLA-DQ genotype influences *per se* the intestinal microbiota composition[46]. The high risk (HLA-DQ2 genotype) infant group showed an increase in the proportions of Firmicutes (*Clostridium* sensu stricto and unclassified Clostridiaceace and *Gemella*) and Protebacteria (*Raoultella* and unclassified Enterobacteriacea) and a reduction in Actinobacteria (*Bifidobacterium*). Associations have also been made between some *Clostridium* species, such as *C. difficile,* in ileal samples of human subjects and the NOD2 genotype and the phenotype of inflammatory bowel diseases[47]. A prospective study also reported that a reduction in the ratio of *Bifidobacterium* to *Clostridium* counts was associated with subsequent development of atopic dermatitis[48]. Another small study characterized the longitudinal changes in the microbial communities of genetically predisposed infants (HLA-DQ2/8)[5] and compared the results with

the data from another study on non-genotyped healthy infants[12]. The microbiota of HLA-DQ2/8 carriers was characterized by higher abundance of Firmicutes and lower abundance of Bacteroidetes (1% to undetectable) compared to that of healthy infants. However, the differences attributed by the authors to the HLA-DQ genotype could be due to their use of different methodologies for sampling, storage and processing of stool samples and for the taxonomic analyses (small subunit (SSU) rDNA microarray *vs* 454 pyrosequencing). This makes indeed the data incomparable.

The mechanisms by which the HLA-DQ genotype could selectively influence colonization and composition of gut microbiota remain unknown. However, we can speculate that MHC II presents phagocytized antigens of intestinal bacteria, which may then be presented to T cells. Depending on the antigen presented, effector T-cell activation could contribute to regulating the gut microbes colonizing the gut by activating B-cells to secrete protective antibodies directly into the gut mucosa and lumen[49]. Bacterial antigens presented via MHC II molecules, could also lead to T cell maturation into effector cells (Th1, Th2 or Th17) or Foxp3$^+$Treg cells with immunosuppressive activity, which could contribute to developing tolerance towards the intestinal microbiota. In this context, studies in rodents indicate that the repertoire of thymus-derived Treg cells, which constitute most Treg cells in all lymphoid and intestinal organs including the colon, is heavily influenced by microbiota composition, thus supporting this hypothesis[50].

Regarding possible pathogenicity of the microbiota alterations found in the CD genotype, the increase in *Staphylococcus* spp. described by De Palma et al.[44] is of particular interest. Some staphylococcal superantigens preferentially interact with HLA-DQ molecules, activating an inflammatory response that could increase the risk of developing CD[51]. This cohort of infants is being followed-up to monitor whether the intestinal microbiota alterations detected in early life are ultimately associated with CD onset. There is a strong association between CD and the expression of HLA-DQ2/DQ8 molecules compared to other HLA-linked diseases[52], but several non-HLA genes also contribute to the disease[53] and their influence on the intestinal microbiota composition should not

be discarded. For instance, non expression of the *FUT2* gene coding for fucosyltransferase 2, leading to a non-secretor phenotype, has been associated with an increased susceptibility of developing CD[54]. Fucosyltranferase 2 is responsible for synthesising ABH antigens in the mucus and other secretions and its expression has also been associated with reduced diversity, richness and abundance of bifidobacteria in the human intestinal tract[55]. Therefore, both HLA-DQ2/8 molecules and the non-secretor phenotype due to *FUT2* gene dysfunction have been linked with CD onset and also with reduced intestinal numbers of *Bifidobacterium* spp. This evidence, together with the reduced bifidobacteria levels detected in CD patients (described below; 9, 10), indicate this bacterial genus plays a role in CD risk.

3. Influence of Intestinal Microbiota in CD Pathogenesis

Several observational studies in children and adults with CD have shown alterations in the intestinal microbiota composition compared to control subjects. Our studies using molecular quantitative methods, such as FISH and quantitative PCR, found reduced numbers of *Bifidobacterium* spp. and *B. longum* and increased numbers of *Bacteroides* spp. in stools and duodenal biopsies of CD patients untreated and treated with a GFD compared to control subjects[8,9]. Also enterobacteria and staphylococci numbers were higher in untreated CD patients than in controls, but these differences were almost restored in CD subjects on a long-term GFD[9]. Likewise, other studies in children reported increased prevalence of *Bacteroides vulgatus* and *E. coli* in CD biopsies before and after the GFD by temporal temperature gradient gel electrophoresis (TTGE) compared to controls[56] and lower numbers of *Lactobacillu*s and *Bifidobacterium* and higher numbers of *Bacteroides, Staphylococcus* and enterobacteria in stools of children with CD compared to healthy controls[57]. Other studies performed by DGGE of the microbiota of adults with CD clustered the dominant microbial communities of healthy individuals together and separate from those of untreated CD patients[58]. However, the above study reported an increased prevalence in

Bifidobacterium bifidum in patients with active CD as opposed to the lower bifidobacteria numbers detected in CD patients in our studies[9,57,59,60] or the absence of differences reported in another study[61]. The analysis of metabolites derived from intestinal microbiota activity has also revealed significant differences between treated CD patients and healthy controls and suggests there is a metabolic signature for the CD microbiome[58,59]. One of the most recent studies has also reported that CD patients with gastrointestinal symptoms had different microbiota composition when compared with controls and patients with dermatitis herpetiformis, suggesting that the microbiota may play a role in the manifestation of the disease[62]. In Sweden, an early study with samples collected between 1985-1996 revealed that rod-shaped bacteria were frequently associated with the mucosa of CD patients, both in the active phase and treated with a GFD, as detected by scanning electron microscopy (SEM)[63]. Later, these SEM analyses were complemented with 16S rDNA sequencing to identify the bacterial communities detected in the samples of the Swedish epidemic (1985-1996) and in a new cohort of patients (2004-2007)[64]. Only one CD biopsy collected during 2004-2007 contained rod-shaped bacteria in contrast to the frequency described in the samples of the Swedish epidemic, invalidating the initial theory that these bacteria were causative factors of the CD epidemic[64]. The characterization of the microbiota from biopsies of CD patients from the Swedish celiac epidemic showed that SEM positive biopsies were significantly enriched in *Clostridium*, *Prevotella* spp. and *Actinomyces* compared to the SEM negative biopsies also from CD patients[64]. We also carried out a deeper characterization of the CD microbiota by isolating bacterial strains and analyzing their pathogenic features[65-67]. Specifically, *E. coli* clones belonging to virulent phylogenetic groups (B2 and D) isolated from untreated and treated CD patients presented a higher number of virulence genes encoding P fimbriae, capsule K5 and hemolysin than those isolated from healthy controls[65]. Furthermore, the abundance of the species *Bacteroides fragilis* coding for metalloproteases was increased in both untreated and treated CD patients, and thus could presumably play a pathogenic role in CD[66]. In fact, *Bacteroides fragilis* and the strains producing metalloproteases are frequently involved in opportunistic infections

and aggravate colitis in animal models[68]. The isolation and identification of clones belonging to the genus *Staphylococcus* also revealed that the species *S. epidermidis* carrying the *mecA* gene (methicillin resistant gene) was more abundant in the CD patients (treated and untreated) than in controls[67].

4. Potential Mechanism of Action of Intestinal Microbiota in CD

The microbiota and its alteration could contribute to the etiopathogenesis of CD by providing proteolytic activities that influence the generation of toxic and immunogenic peptides from gluten[66,69]; and by mediating-host-microbe interactions, which could influence the intestinal barrier[70] and the immune function[71] (Figure 1).

Some gluten peptides (gliadin) withstand gastrointestinal digestion and disturb the intestinal integrity by altering tight junction proteins, increasing epithelial intestinal permeability[73]. These may facilitate the access of gliadin peptides to the lamina propria and its interaction with infiltrated lymphocytes and APCs responsible for triggering the immune response. *B. fragilis* clones isolated from the intestinal microbiota of CD patients showed gliadin-hydrolyzing activity, and some of them generated peptides that maintain their immunogenicity, eliciting inflammatory cytokine production by Caco-2 cell cultures, and showing a greater ability to permeate the Caco-2 cell monolayer[66]. In contrast, different bifidobacteria and, particularly, *B. longum* CECT 7347 (also named *B. longum* IATA-ES1) reduced the cytotoxic and inflammatory effects of gliadin peptides generated during gastrointestinal digestion[69]. Thus, *in vitro* studies indicate that the proteolytic activity of the intestinal microbiota may modify gliadin peptides differently, increasing or reducing their toxicity. Similarly, Fernandez-Feo et al.[74] and Caminero et al.[75] isolated species from the oral cavity and faeces able to hydrolyse gluten peptides; however, their physiological effects have not been evaluated.

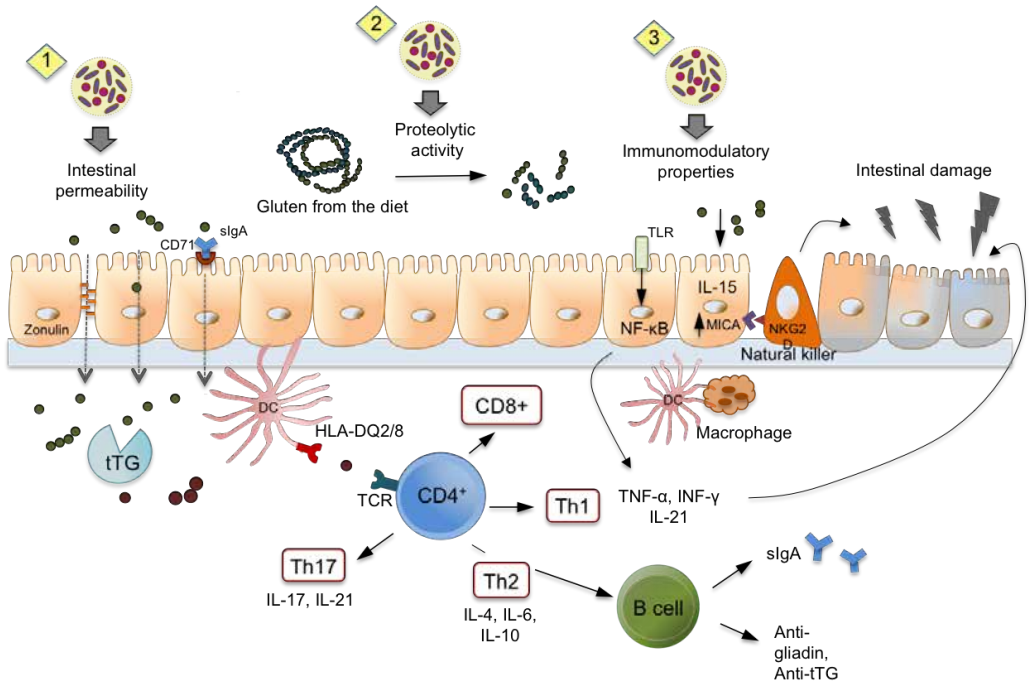


Figure 1. Schematic representation of CD pathogenesis and the potential role of intestinal dysbiosis. Some gluten peptides cross the intestinal epithelium and can be deamidated by the tissue transglutaminase (tTG), which increases their ability to bind the HLA-DQ2/8 molecules of antigen-presenting cells and to trigger an adaptive immune response, involving Th1, Th2 and Th17 cells that lead to the release of pro-inflammatory cytokines (IFN-γ, interleukin (IL)-21, etc.) and the production of CD antibodies; other gluten peptides activate the innate immune response by interacting with eptithelial cells and APCs and, thus, trigerring the activation of inflammatory pathways (NFκB) and the production of inflammatory cytokines such as IL-15. In particular, IL-15 increases the expression of the MICA molecule at epithelial cell surface and triggers activation of intraepithelial lymphocytes through engagement of NKG2D, leading to an innate-like cytotoxicity toward epithelial cells and enhanced CD8 T cell-mediated adaptive response, contributing to villous atrophy[72].The microbiota could contribute to the etiopathogenesis of CD by (2) providing proteolytic activities that influence the generation of toxic and immunogenic peptides from gluten[66,69] and by mediating host-microbe interactions which could influence (1) the intestinal barrier[70] and (3) immune function[71].

Regarding the mechanism of action related to the intestinal barrier function, CD-triggers (gliadin and IFN-γ) decreased the goblet cell numbers in intestinal loops of inbred Wistar-AVN rats, and research shows the presence of enterobacteria isolated from CD patients, such as *Escherichia coli* CBL2 and *Shigella* CBD8, aggravate this effect[71]. Furthermore, exposure to these enterobacteria causes increased mucin secretion and greater disruption of tight junctions. By contrast, *Bifidobacterium bifidum* CECT 7365 (also named *B. bifidum* IATA-ES2) increased the number of goblet cells and the production of inhibitors of metalloproteinases, and also reduces gliadin translocation to the lamina propria, which could contribute to gut mucosal protection[71]. Other probiotic bacteria such as *Lactobacillus rhamnosus* GG have been shown to contribute *in vitro* to the maintenance of normal intestinal permeability in Caco-2 cell cultures exposed to gliadin[76].

The composition of the gut microbiota could also influence the release of pro-inflammatory cytokines triggered by gluten peptides. For instance, a mixture of isolated bacteria from CD patients (*Prevotella* sp., *Lachnoanaerobaculum umeaense* and *Actinomyces graevenitzii*) induced IL-17A mRNA expression in *ex vivo* biopsies of intestinal mucosa of CD patients[77]. Thus it was hypothesized that those bacteria could modulate the IL-17 response by helping to breakdown gluten tolerance[77]. By contrast, in gliadin-sensitized HLA-DQ8 transgenic mice, a strain of *Lactobacillus casei* reduced the TNF-α levels in jejunal tissue sections[78]. In a model of newborn rats sensitized with IFN-γ and orally administered gliadin, *B. longum* CECT 7347 reduced TNF-α and increased IL-10 concentration in intestinal tissue samples[79].

On the one hand, *B. longum* CECT 7347 and *B. bifidum* CECT 7365 reduced the inflammatory cytokines (IFN-γ and TNF-α) produced by the microbiota of CD patients, and, on the other, they increased IL-10 production, with anti-inflammatory effects in peripheral blood mononuclear cell (PBMC) cultures[80]. *Escherichia coli* CBL2 and *Shigella* CBD8 isolated from CD patients, boosted the production of IL-12 and IFN-γ, and the expression of HLA-DR and CD40 in co-cultures of monocyte-derived dendritic

cells (MDDCs) and Caco-2 cells compared to *B. longum* CECT 7347 or *B. bifidum* CECT 7365[81]. These responses could be mediated by the activation of toll-like receptors (TLRs), which play an important role in the recognition of microbial components, driving different transcription pathways involved in the immune response. So far it has been reported that biopsies from CD patients display increased TLR2 expression, which is a receptor responding to bacterial lipopeptides, and of TLR9, which is a receptor responding to bacterial DNA[61]. We could hypothesize that this increased TLR expression in biopsies of CD patients may intensify gut microbiota signalling and host response to intestinal dysbiosis although direct evidence is not available.

5. Gluten Intake and Intestinal Microbiota

The only treatment for CD is adherence to a life-long GFD, which implies important dietary changes. Specifically, women on a GFD have a reduced dietary protein and fibre intake and an increased fat intake[82]. These dietary differences also seem to cause changes in the intestinal microbiota composition and in the immune response to the altered microbiota *in vitro*. After three months of adherence to the GFD, children with CD showed increases in the *B. fragilis* group and *Enterobacteriaceae* numbers and in sIgA levels in stools[83]. In healthy adults the GFD caused shifts in gut microbiota composition, characterized by reduced numbers of *Bifidobacterium* spp., *B. longum* and the *Lactobacillus* group, and increased numbers of *Enterobacteriaceae* and *E. coli*[10]. This led to the proposal that GFD should be considered as an environmental factor that may contribute to shaping the microbiota composition in treated CD patients[10].

In animal models, gut microbiota changes have also been related to the GFD but the data are not comparable to humans. For example, GFD-induced changes in the microbiota of NOD mice are characterized by higher numbers in *Bacteroides* and *Akkermansia* and a higher percentage of $CD4^+CD25^+$Foxp3 regulatory cells, and reduced T1D incidence[84]. By contrast, NOD mice fed a diet containing gluten had higher numbers of

Bifidobacterium, Tannerella and *Barnesiella* and increased T1D incidence[84]. Harsen et al., (2014)[85] also proposed that GFD-induced increases in *Akkermansia*, Protebacteria and TM7 abundance protected the offspring of NOD mice and reduced the incidence of diabetes[86]; however, direct evidence is lacking.

6. Role of Probiotics in CD: Human Studies

There are proposals to use of some probiotic bacteria in CD management based on the associations between CD and intestinal microbiota imbalances, and the role attributed to some bacterial strains in maintaining gut barrier function and regulating the immune response in certain chronic inflammatory diseases. To our knowledge, only two intervention trials have been conducted with probiotics in CD patients to date. Both were randomized, double-blind placebo-controlled trials, but differed in the aim, species and strain of bifidobacteria tested. In one of the interventions, *B. infantis* NLS was administered to untreated CD patients consuming gluten to evaluate the effect of the probiotic independently of the GFD[87]. The beneficial properties of *B. infantis* NLS included the reduction of some gastrointestinal symptoms, specifically indigestion, constipation and reflux with borderline significance. However, it did not improve diarrhoea or abdominal pain, nor modify intestinal permeability or the pro-inflammatory status, as reflected by the analysis of serum cytokines and chemokines[87]. Another study evaluated the influence of administering *B. longum* CECT 7347 to children with newly diagnosed CD following a GFD to assess whether it improved the efficacy of the GFD[83]. Inter-group comparisons revealed a decrease in peripheral $CD3^+$ T lymphocytes and TNF-α levels in the bifidobacterial group. The administration of *B. longum* CECT 7347 also reduced *Bacteroides fragilis* group numbers and sIgA in stools when compared to the placebo[83], which could presumably contribute to better recovery from the inflammatory status associated with the active phase of the disease. Despite the experimental differences, presumably the mechanism behind the effects of *B. infantis* NLS

differ from those of *B. longum* CECT 7347, as the latter influences inflammatory markers, gut microbiota and host-related defence mechanisms. Both studies suggest the potential interest of these probiotic bacterial strains for improving CD treatment, although larger human trials are required to confirm and strength of this evidence.

7. Conclusions

Most studies demonstrate associations between CD and shifts in the composition of intestinal microbiota. These alterations are not only consequence of the inflammatory status characteristic of the active phase of the disease because the ecological perturbations are not completely restored after adherence to a GFD, even though the GFD *per se* also influences the microbiota composition. In healthy infants at family risk of CD, prospective studies also indicate that alterations in gut microbiota composition are associated with the HLA-DQ genotype and could influence CD onset. The influence of gut microbiota composition on the etiopathogenesis of CD could be related to its proteolytic activity and ability to generate toxigenic and immunogenic peptides and, particularly, to its ability to regulate gut barrier function and the immune response to gluten. Further and larger studies are, however, necessary to confirm that gut microbiota modulation by the administration of specific bacterial strains could contribute to improving the health status of CD subjects, and to reducing the risk of CD development.

Acknowledgments

This work was supported by grants AGL2011-25169 from the Spanish Ministry of Economy and Competitiveness (MINECO). The scholarship to M. Olivares from Consejo Superior de Investigaciones Científicas (CSIC) is fully acknowledged

References

1. Catassi C, Kryszak D, Bhatti B, Sturgeon C, Helzlsouer K, Clipp SL et al. *Natural history of celiac disease autoimmunity in a USA cohort followed since 1974.* Ann Med. 2010; 42(7): 530-8.
   http://dx.doi.org/10.3109/07853890.2010.514285
   PMid:20868314
2. Ivarsson A, Hernell O, Stenlund H, Persson LA. *Breast-feeding protects against celiac disease.* The American journal of clinical nutrition. 2002; 75(5): 914-21.
   PMid:11976167
3. Sandberg-Bennich S, Dahlquist G, Kallen B. *Coeliac disease is associated with intrauterine growth and neonatal infections.* Acta paediatrica. 2002; 91(1): 30-3.
   http://dx.doi.org/10.1111/j.1651-2227.2002.tb01635.x
   PMid:11883814
4. Sanz Y, De Pama G, Laparra M. *Unraveling the ties between celiac disease and intestinal microbiota.* International reviews of immunology. 2011; 30(4): 207-18.
   http://dx.doi.org/10.3109/08830185.2011.599084
   PMid:21787226
5. Sellitto M, Bai G, Serena G, Fricke WF, Sturgeon C, Gajer P et al. *Proof of concept of microbiome-metabolome analysis and delayed gluten exposure on celiac disease autoimmunity in genetically at-risk infants.* PloS one. 2012; 7(3): e33387.
   http://dx.doi.org/10.1371/journal.pone.0033387
   PMid:22432018 PMCid:PMC3303818
6. Marild K, Stephansson O, Montgomery S, Murray JA, Ludvigsson JF. *Pregnancy outcome and risk of celiac disease in offspring: a nationwide case-control study.* Gastroenterology. 2012; 142(1): 39-45 e3.
7. Marild K, Ye W, Lebwohl B, Green PH, Blaser MJ, Card T et al. *Antibiotic exposure and the development of coeliac disease: a nationwide case-control study.* BMC gastroenterology. 2013; 13: 109.
   http://dx.doi.org/10.1186/1471-230X-13-109
   PMid:23834758 PMCid:PMC3720284
8. Nadal I, Donat E, Ribes-Koninckx C, Calabuig M, Sanz Y. *Imbalance in the composition of the duodenal microbiota of children with coeliac disease.* Journal of medical microbiology. 2007; 56(Pt 12): 1669-74.
   http://dx.doi.org/10.1099/jmm.0.47410-0
   PMid:18033837

9. Collado MC, Donat E, Ribes-Koninckx C, Calabuig M, Sanz Y. *Specific duodenal and faecal bacterial groups associated with paediatric coeliac disease.* Journal of clinical pathology. 2009; 62(3): 264-9.
http://dx.doi.org/10.1136/jcp.2008.061366
PMid:18996905

10. De Palma G, Nadal I, Collado MC, Sanz Y. *Effects of a gluten-free diet on gut microbiota and immune function in healthy adult human subjects.* The British journal of nutrition. 2009; 102(8): 1154-60.
http://dx.doi.org/10.1017/S0007114509371767
PMid:19445821

11. Koenig JE, Spor A, Scalfone N, Fricker AD, Stombaugh J, Knight R et al. *Succession of microbial consortia in the developing infant gut microbiome.* Proceedings of the National Academy of Sciences of the United States of America. 2011; 108 Suppl 1: 4578-85.
http://dx.doi.org/10.1073/pnas.1000081107
PMid:20668239 PMCid:PMC3063592

12. Palmer C, Bik EM, DiGiulio DB, Relman DA, Brown PO. *Development of the human infant intestinal microbiota.* PLoS biology. 2007; 5(7): e177.
http://dx.doi.org/10.1371/journal.pbio.0050177
PMid:17594176 PMCid:PMC1896187

13. Madan JC, Salari RC, Saxena D, Davidson L, O'Toole GA, Moore JH et al. *Gut microbial colonisation in premature neonates predicts neonatal sepsis.* Archives of disease in childhood Fetal and neonatal edition. 2012; 97(6): F456-62.
http://dx.doi.org/10.1136/fetalneonatal-2011-301373

14. Pozo-Rubio T, de Palma G, Mujico JR, Olivares M, Marcos A, Acuna MD et al. *Influence of early environmental factors on lymphocyte subsets and gut microbiota in infants at risk of celiac disease; the PROFICEL study.* Nutricion hospitalaria. 2013; 28(2): 464-73.
PMid:23822699

15. Decker E, Engelmann G, Findeisen A, Gerner P, Laass M, Ney D et al. *Cesarean delivery is associated with celiac disease but not inflammatory bowel disease in children.* Pediatrics. 2010; 125(6): e1433-40.
http://dx.doi.org/10.1542/peds.2009-2260
PMid:20478942

16. Biasucci G, Benenati B, Morelli L, Bessi E, Boehm G. *Cesarean delivery may affect the early biodiversity of intestinal bacteria.* The Journal of nutrition. 2008; 138(9): 1796S-800S.
PMid:18716189

17. Akobeng AK, Ramanan AV, Buchan I, Heller RF. *Effect of breast feeding on risk of coeliac disease: a systematic review and meta-analysis of observational studies.* Archives of disease in childhood. 2006; 91(1): 39-43.
http://dx.doi.org/10.1136/adc.2005.082016
PMid:16287899 PMCid:PMC2083075

18. Ivarsson A, Myleus A, Norstrom F, van der Pals M, Rosen A, Hogberg L et al. *Prevalence of childhood celiac disease and changes in infant feeding.* Pediatrics. 2013; 131(3): e687-94.
http://dx.doi.org/10.1542/peds.2012-1015
PMid:23420914

19. Norris JM, Barriga K, Hoffenberg EJ, Taki I, Miao D, Haas JE et al. *Risk of celiac disease autoimmunity and timing of gluten introduction in the diet of infants at increased risk of disease.* JAMA: the journal of the American Medical Association. 2005; 293(19): 2343-51.
http://dx.doi.org/10.1001/jama.293.19.2343
PMid:15900004

20. Welander A, Tjernberg AR, Montgomery SM, Ludvigsson J, Ludvigsson JF. *Infectious disease and risk of later celiac disease in childhood.* Pediatrics. 2010; 125(3): e530-6.
http://dx.doi.org/10.1542/peds.2009-1200
PMid:20176673

21. Martin R, Jimenez E, Heilig H, Fernandez L, Marin ML, Zoetendal EG et al. *Isolation of bifidobacteria from breast milk and assessment of the bifidobacterial population by PCR-denaturing gradient gel electrophoresis and quantitative real-time PCR.* Applied and environmental microbiology. 2009; 75(4): 965-9.
http://dx.doi.org/10.1128/AEM.02063-08
PMid:19088308 PMCid:PMC2643565

22. Gronlund MM, Gueimonde M, Laitinen K, Kociubinski G, Gronroos T, Salminen S et al. *Maternal breast-milk and intestinal bifidobacteria guide the compositional development of the Bifidobacterium microbiota in infants at risk of allergic disease.* Clinical and experimental allergy: journal of the British Society for Allergy and Clinical Immunology. 2007; 37(12): 1764-72.
http://dx.doi.org/10.1111/j.1365-2222.2007.02849.x
PMid:17941914

23. Fallani M, Young D, Scott J, Norin E, Amarri S, Adam R et al. *Intestinal microbiota of 6-week-old infants across Europe: geographic influence beyond delivery mode, breast-feeding, and antibiotics.* Journal of pediatric gastroenterology and nutrition. 2010; 51(1): 77-84.
http://dx.doi.org/10.1097/MPG.0b013e3181d1b11e
PMid:20479681

24. Tannock GW, Lawley B, Munro K, Gowri Pathmanathan S, Zhou SJ, Makrides M et al. *Comparison of the compositions of the stool microbiotas of infants fed goat milk formula, cow milk-based formula, or breast milk.* Applied and environmental microbiology. 2013; 79(9): 3040-8.
http://dx.doi.org/10.1128/AEM.03910-12
PMid:23455335 PMCid:PMC3623157

25. Garrido D, Barile D, Mills DA. *A molecular basis for bifidobacterial enrichment in the infant gastrointestinal tract.* Advances in nutrition. 2012; 3(3): 415S-21S.
http://dx.doi.org/10.3945/an.111.001586
PMid:22585920 PMCid:PMC3649478

26. Di Gioia D, Aloisio I, Mazzola G, Biavati B. *Bifidobacteria: their impact on gut microbiota composition and their applications as probiotics in infants.* Applied microbiology and biotechnology. 2014; 98(2): 563-77.
http://dx.doi.org/10.1007/s00253-013-5405-9
PMid:24287935

27. Schwiertz A, Jacobi M, Frick JS, Richter M, Rusch K, Kohler H. *Microbiota in pediatric inflammatory bowel disease.* The Journal of pediatrics. 2010; 157(2): 240-51.
http://dx.doi.org/10.1016/j.jpeds.2010.02.046
PMid:20400104

28. Murri M, Leiva I, Gomez-Zumaquero JM, Tinahones FJ, Cardona F, Soriguer F et al. *Gut microbiota in children with type 1 diabetes differs from that in healthy children: a case-control study.* BMC medicine. 2013; 11: 46.
http://dx.doi.org/10.1186/1741-7015-11-46
PMid:23433344 PMCid:PMC3621820

29. Kirjavainen PV, Apostolou E, Arvola T, Salminen SJ, Gibson GR, Isolauri E. *Characterizing the composition of intestinal microflora as a prospective treatment target in infant allergic disease.* FEMS immunology and medical microbiology. 2001; 32(1): 1-7.
http://dx.doi.org/10.1111/j.1574-695X.2001.tb00526.x
PMid:11750215

30. Garofalo R. *Cytokines in human milk.* The Journal of pediatrics. 2010; 156: S36-40.
http://dx.doi.org/10.1016/j.jpeds.2009.11.019
PMid:20105664

31. Peroni DG, Pescollderungg L, Piacentini GL, Rigotti E, Maselli M, Watschinger K et al. *Immune regulatory cytokines in the milk of lactating women from farming and urban environments.* Pediatric allergy and immunology: official publication of the European Society of Pediatric Allergy and Immunology. 2010; 21(6): 977-82.
http://dx.doi.org/10.1111/j.1399-3038.2010.00995.x
PMid:20718928

32. Olivares M, Albrecht S, De Palma G, Ferrer MD, Castillejo G, Schols HA et al. *Human milk composition differs in healthy mothers and mothers with celiac disease.* European Journal of Nutrition. 2015; 54(1): 119-28.
http://dx.doi.org/10.1007/s00394-014-0692-1

33. Tomicic S, Johansson G, Voor T, Bjorksten B, Bottcher MF, Jenmalm MC. *Breast milk cytokine and IgA composition differ in Estonian and Swedish mothers-relationship to microbial pressure and infant allergy.* Pediatric research. 2010; 68(4): 330-4.
http://dx.doi.org/10.1203/PDR.0b013e3181ee049d
PMid:20581738

34. Laiho K, Lampi AM, Hamalainen M, Moilanen E, Piironen V, Arvola T et al. *Breast milk fatty acids, eicosanoids, and cytokines in mothers with and without allergic disease.* Pediatric research. 2003; 53(4): 642-7.
http://dx.doi.org/10.1203/01.PDR.0000055778.58807.C8
PMid:12612204

35. Troncone R, Scarcella A, Donatiello A, Cannataro P, Tarabuso A, Auricchio S. *Passage of gliadin into human breast milk.* Acta paediatrica Scandinavica. 1987; 76(3): 453-6.
http://dx.doi.org/10.1111/j.1651-2227.1987.tb10498.x
PMid:3300148

36. Ozkan T, Ozeke T, Meral A. *Gliadin-specific IgA antibodies in breast milk.* The Journal of international medical research. 2000; 28(5): 234-40.
http://dx.doi.org/10.1177/147323000002800506
PMid:11092234

37. Toivanen P, Vaahtovuo J, Eerola E. *Influence of major histocompatibility complex on bacterial composition of fecal flora.* Infection and immunity. 2001; 69(4): 2372-7.
http://dx.doi.org/10.1128/IAI.69.4.2372-2377.2001
PMid:11254595 PMCid:PMC98167

38. Bolnick DI, Snowberg LK, Caporaso JG, Lauber C, Knight R, Stutz WE. *Major Histocompatibility Complex class IIb polymorphism influences gut microbiota composition and diversity.* Mol Ecol. 2014; 23(19): 4831-45.
http://dx.doi.org/10.1111/mec.12846
PMid:24975397

39. Van de Merwe JP, Stegeman JH, Hazenberg MP. *The resident faecal flora is determined by genetic characteristics of the host. Implications for Crohn's disease?* Antonie van Leeuwenhoek. 1983; 49(2): 119-24.
http://dx.doi.org/10.1007/BF00393669
PMid:6684413

40. Zoetendal EG, Akkermans ADL, Akkermans-van Vliet WM, de Visser JAGM, de Vos WM. *The host genotype affects the bacterial community in the human gastrointestinal tract.* Microb Ecol Health Dis. 2001; 13: 129-34.
http://dx.doi.org/10.1080/08910600175046266941. Stewart JA, Chadwick VS, Murray A. *Investigations into the influence of host genetics on the predominant eubacteria in the faecal microflora of children.* Journal of medical microbiology. 2005; 54(Pt 12): 1239-42.
http://dx.doi.org/10.1099/jmm.0.46189-0
PMid:16278440
42. Goodrich JK, Waters JL, Poole AC, Sutter JL, Koren O, Blekhman R et al. *Human genetics shape the gut microbiome.* Cell. 2014; 159(4): 789-99.
http://dx.doi.org/10.1016/j.cell.2014.09.053
PMid:25417156
43. De Palma G, Capilla A, Nadal I, Nova E, Pozo T, Varea V et al. *Interplay between human leukocyte antigen genes and the microbial colonization process of the newborn intestine.* Current issues in molecular biology. 2010; 12(1): 1-10.
PMid:19478349
44. Palma GD, Capilla A, Nova E, Castillejo G, Varea V, Pozo T et al. *Influence of milk-feeding type and genetic risk of developing coeliac disease on intestinal microbiota of infants: the PROFICEL study.* PloS one. 2012; 7(2): e30791.
http://dx.doi.org/10.1371/journal.pone.0030791
PMid:22319588 PMCid:PMC3272021
45. Sanchez E, De Palma G, Capilla A, Nova E, Pozo T, Castillejo G et al. *Influence of environmental and genetic factors linked to celiac disease risk on infant gut colonization by Bacteroides species.* Applied and environmental microbiology. 2011; 77(15): 5316-23.
http://dx.doi.org/10.1128/AEM.00365-11
PMid:21642397 PMCid:PMC3147488
46. Olivares M, Neef A, Castillejo G, Palma GD, Varea V, Capilla A et al. *The HLA-DQ2 genotype selects for early intestinal microbiota composition in infants at high risk of developing coeliac disease.* Gut. 2014. http://dx.doi.org/10.1136/gutjnl-2014-306931
47. Li E, Hamm CM, Gulati AS, Sartor RB, Chen H, Wu X et al. *Inflammatory bowel diseases phenotype, C. difficile and NOD2 genotype are associated with shifts in human ileum associated microbial composition.* PloS one. 2012; 7(6): e26284.
http://dx.doi.org/10.1371/journal.pone.0026284
PMid:22719818 PMCid:PMC3374607

48. Kalliomaki M, Kirjavainen P, Eerola E, Kero P, Salminen S, Isolauri E. *Distinct patterns of neonatal gut microflora in infants in whom atopy was and was not developing.* The Journal of allergy and clinical immunology. 2001; 107(1): 129-34.
http://dx.doi.org/10.1067/mai.2001.111237
PMid:11150002

49. Fagarasan S, Honjo T. *Intestinal IgA synthesis: regulation of front-line body defences.* Nat Rev Immunol. 2003; 3(1): 63-72.
http://dx.doi.org/10.1038/nri982
PMid:12511876

50. Cebula A, Seweryn M, Rempala GA, Pabla SS, McIndoe RA, Denning TL et al. *Thymus-derived regulatory T cells contribute to tolerance to commensal microbiota.* Nature. 2013; 497(7448): 258-62.
http://dx.doi.org/10.1038/nature12079
PMid:23624374 PMCid:PMC3711137

51. Rajagopalan G, Polich G, Sen MM, Singh M, Epstein BE, Lytle AK et al. *Evaluating the role of HLA-DQ polymorphisms on immune response to bacterial superantigens using transgenic mice.* Tissue antigens. 2008; 71(2): 135-45.
http://dx.doi.org/10.1111/j.1399-0039.2007.00986.x
PMid:18086265

52. Di Sabatino A, Corazza GR. *Coeliac disease.* Lancet. 2009; 373(9673): 1480-93.
http://dx.doi.org/10.1016/S0140-6736(09)60254-3

53. Romanos J, Rosen A, Kumar V, Trynka G, Franke L, Szperl A et al. *Improving coeliac disease risk prediction by testing non-HLA variants additional to HLA variants.* Gut. 2014; 63(3): 415-22.
http://dx.doi.org/10.1136/gutjnl-2012-304110
PMid:23704318 PMCid:PMC3933173

54. Parmar AS, Alakulppi N, Paavola-Sakki P, Kurppa K, Halme L, Farkkila M et al. *Association study of FUT2 (rs601338) with celiac disease and inflammatory bowel disease in the Finnish population.* Tissue antigens. 2012; 80(6): 488-93.
http://dx.doi.org/10.1111/tan.12016
PMid:23075394

55. Wacklin P, Makivuokko H, Alakulppi N, Nikkila J, Tenkanen H, Rabina J et al. *Secretor genotype (FUT2 gene) is strongly associated with the composition of Bifidobacteria in the human intestine.* PloS one. 2011; 6(5): e20113.
http://dx.doi.org/10.1371/journal.pone.0020113
PMid:21625510 PMCid:PMC3098274

56. Shippa S, Iebba V, Barbaro M, Di Nardo G, Totino V, Checchi MP et al. *A distinctive "microbial signature" in celic pediatric patients.* BMC Microbiol. 2010; 10: 1757.

57. Di Cagno R, De Angelis M, De Pasquale I, Ndagijimana M, Vernocchi P, Ricciuti P et al. *Duodenal and faecal microbiota of celiac children: molecular, phonotype and metabolome characterization.* BMC Microbiol. 2011; 11: 219.
http://dx.doi.org/10.1186/1471-2180-11-219
PMid:21970810 PMCid:PMC3206437

58. Nistal E, Caminero A, Vivas S, Ruiz de Morales JM, Saenz de Miera LE, Rodriguez-Aparicio LB et al. *Differences in faecal bacteria populations and faecal bacteria metabolism in healthy adults and celiac disease patients.* Biochimie. 2012; 94(8): 1724-9.
http://dx.doi.org/10.1016/j.biochi.2012.03.025
PMid:22542995

59. Collado MC, Donat E, Ribes-Koninckx C, Calabuig M, Sanz Y. *Imbalances in faecal and duodenal Bifidobacterium species composition in active and non-active coelic disease.* BMC Microbilo. 2008; 8: 232.
http://dx.doi.org/10.1186/1471-2180-8-232
PMid:19102766 PMCid:PMC2635381

60. Golfetto L, Senna FD, Hermes J, Beserra BT, Franca Fda S, Martinello F. *Lower bifidobacteria counts in adult patients with celiac disease on a gluten-free diet.* Arq Gastroenterol. 2014; 51(2): 139-43.
http://dx.doi.org/10.1590/S0004-28032014000200013
PMid:25003267

61. Kalliomaki M, Satokari R, Lahteenoja H, Vahamiko S, Gronlund J, Routi T et al. *Expression of microbiota, Toll-like receptors, and their regulators in the small intestinal mucosa in celiac disease.* Journal of pediatric gastroenterology and nutrition. 2012; 54(6): 727-32.
http://dx.doi.org/10.1097/MPG.0b013e318241cfa8
PMid:22134550

62. Wacklin P, Kaukinen K, Tuovinen E, Collin P, Lindfors K, Partanen J et al. *The duodenal microbiota composition of adult celiac disease patients is associated with the clinical manifestation of the disease.* Inflammatory bowel diseases. 2013; 19(5): 934-41.
http://dx.doi.org/10.1097/MIB.0b013e31828029a9
PMid:23478804

63. Forsberg G, Fahlgren A, Horstedt P, Hammarstrom S, Hernell O, Hammarstrom ML. *Presence of bacteria and innate immunity of intestinal epithelium in childhood celiac disease.* The American journal of gastroenterology. 2004; 99(5): 894-904.
http://dx.doi.org/10.1111/j.1572-0241.2004.04157.x
PMid:15128357

64. Ou G, Hedberg M, Horstedt P, Baranov V, Forsberg G, Drobni M et al. *Proximal small intestinal microbiota and identification of rod-shaped bacteria associated with childhood celiac disease.* The American journal of gastroenterology. 2009; 104(12): 3058-67.
http://dx.doi.org/10.1038/ajg.2009.524
PMid:19755974

65. Sánchez E, Nadal I, Donat E, Ribes-Koninckx C, Calabuig M, Sanz Y. *Reduced diversity and increased virulence-gene carriage in intestinal enterobacteria of coeliac children.* BMC gastroenterology. 2008; 8: 50.
http://dx.doi.org/10.1186/1471-230X-8-50
PMid:18983674 PMCid:PMC2615025

66. Sánchez E, Laparra JM, Sanz Y. *Discerning the role of Bacteroides fragilis in celiac disease pathogenesis.* Applied and environmental microbiology. 2012; 78(18): 6507-15.
http://dx.doi.org/10.1128/AEM.00563-12
PMid:22773639 PMCid:PMC3426693

67. Sánchez E, Ribes-Koninckx C, Calabuig M, Sanz Y. *Intestinal Staphylococcus spp. and virulent features associated with coeliac disease.* Journal of clinical pathology. 2012; 65(9): 830-4.
http://dx.doi.org/10.1136/jclinpath-2012-200759
PMid:22718843

68. Fasano A, Shea-Donohue T. *Mechanisms of disease: the role of intestinal barrier function in the pathogenesis of gastrointestinal autoimmune diseases.* Nature clinical practice Gastroenterology & hepatology. 2005; 2(9): 416-22.
http://dx.doi.org/10.1038/ncpgasthep0259
PMid:16265432

69. Laparra JM, Sanz Y. *Bifidobacteria inhibit the inflammatory response induced by gliadins in intestinal epithelial cells via modifications of toxic peptide generation during digestion.* Journal of cellular biochemistry. 2010; 109(4): 801-7.
PMid:20052669

70. Koropatkin NM, Cameron EA, Martens EC. *How glycan metabolism shapes the human gut microbiota.* Nature reviews Microbiology. 2012; 10(5): 323-35.
http://dx.doi.org/10.1038/nrmicro2746

71. Cinova J, De Palma G, Stepankova R, Kofronova O, Kverka M, Sanz Y et al. *Role of intestinal bacteria in gliadin-induced changes in intestinal mucosa: study in germ-free rats.* PloS one. 2011; 6(1): e16169.
http://dx.doi.org/10.1371/journal.pone.0016169
PMid:21249146 PMCid:PMC3020961

72. Hüe S, Mention JJ, Monteiro RC, Zhang S, Cellier C, Schmitz J *et al. A direct role forNKG2D/MICA interaction in villous atrophy during celiac disease.* Immunity. 2004; 21(3): 367-77.
http://dx.doi.org/10.1016/j.immuni.2004.06.018
PMid:15357948

73. Clemente MG, De Virgiliis S, Kang JS, Macatagney R, Musu MP, Di Pierro MR et al. *Early effects of gliadin on enterocyte intracellular signalling involved in intestinal barrier function.* Gut. 2003; 52(2): 218-23.
http://dx.doi.org/10.1136/gut.52.2.218
PMid:12524403 PMCid:PMC1774976

74. Fernandez-Feo M, Wei G, Blumenkranz G, Dewhirst FE, Schuppan D, Oppenheim FG et al. *The cultivable human oral gluten-degrading microbiome and its potencial implications in coeliac disease and gluten sensitivity.* Clin Microbiol Infect. 2013; 19,E386-94.
http://dx.doi.org/10.1111/1469-0691.12249
PMid:23714165 PMCid:PMC3749263

75. Caminero A, Herrán AR, Nistal E, Pérez-Andrés J, Vaquero L, Vivas S et al. *Diversity of the cultivable human gut microbiome involved in gluten metabolism: isolation of microorganisms with potential interest for coeliac disease.* FEMS Microbiol Ecol. 2014; 88(2): 309-19.
http://dx.doi.org/10.1111/1574-6941.12295
PMid:24499426

76. Orlando A, Linsalata M, Notarnicola M, Tutino V, Russo F. *Lactobacillus GG restoration of the gliadin induced epithelial barrier disruption: the role of cellular polyamines.* BMC microbiology. 2014; 14: 19.
http://dx.doi.org/10.1186/1471-2180-14-19
PMid:24483336 PMCid:PMC3911798

77. Sjoberg V, Sandstrom O, Hedberg M, Hammarstrom S, Hernell O, Hammarstrom ML. *Intestinal T-cell responses in celiac disease - impact of celiac disease associated bacteria.* PloS one. 2013; 8(1): e53414.
http://dx.doi.org/10.1371/journal.pone.0053414
PMid:23326425 PMCid:PMC3541273

78. D'Arienzo R, Stefanile R, Maurano F, Mazzarella G, Ricca E, Troncone R et al. *Immunomodulatory effects of Lactobacillus casei administration in a mouse model of gliadin-sensitive enteropathy.* Scandinavian journal of immunology. 2011; 74(4): 335-41.
http://dx.doi.org/10.1111/j.1365-3083.2011.02582.x
PMid:21615450

79. Laparra JM, Olivares M, Gallina O, Sanz Y. *Bifidobacterium longum CECT 7347 modulates immune responses in a gliadin-induced enteropathy animal model.* PLoS One, 2012; 7(2): e30744.
http://dx.doi.org/10.1371/journal.pone.0030744
PMid:22348021 PMCid:PMC3277586

80. Medina M, De Palma G, Ribes-Koninckx C, Calabuig M, Sanz Y. *Bifidobacterium strains suppress in vitro the pro-inflammatory milieu triggered by the large intestinal microbiota of coeliac patients.* Inflamm (Lond). 2008: 5: 19.
http://dx.doi.org/10.1186/1476-9255-5-19
PMid:18980693 PMCid:PMC2640389

81. De Palma G, Kamanova J, Cinova J, Olivares M, Drasarova H, Tuckova L et al. *Modulation of phenotypic and functional maturation of dendritic cells by intestinal bacteria and gliadin: relevance for celiac disease.* J Leukoc Biol. 2012; 92(5): 1043-54.
http://dx.doi.org/10.1189/jlb.1111581
PMid:22891290

82. Miranda J, Lasa A, Bustamante MA, Churruca I, Simon E. *Nutritional differences between a gluten-free diet and a diet containing equivalent products with gluten.* Plant foods for human nutrition. 2014; 69(2): 182-7.
http://dx.doi.org/10.1007/s11130-014-0410-4
PMid:24578088

83. Olivares M, Castillejo G, Varea V, Sanz Y. *Double-blind, randomised, placebo-controlled intervention trial to evaluate the effects of Bifidobacterium longum CECT 7347 in children with newly diagnosed coeliac disease.* The British journal of nutrition. 2014; 112(1): 30-40.
http://dx.doi.org/10.1017/S0007114514000609
PMid:24774670

84. Marietta EV, Gomez AM, Yeoman C, Tilahun AY, Clark CR, Luckey DH et al. *Low incidence of spontaneous type 1 diabetes in non-obese diabetic mice raised on gluten-free diets is associated with changes in the intestinal microbiome.* PloS one. 2013; 8(11): e78687.
http://dx.doi.org/10.1371/journal.pone.0078687
PMid:24236037 PMCid:PMC3827256

85. Hanser CH, Krych L, Buschard K, Metzdorff SB, Nellemann C, Hansen LH et al. *A maternal gluten-free diet reduces inflammation and diabetes indicente in the offspring of NOD mice.* Diabetes, 2014; 63(8): 2821-32.
http://dx.doi.org/10.2337/db13-1612
PMid:24696449

86. Hansen CH, Krych L, Nielsen DS, Vogensen FK, Hansen LH, Sorensen SJ et al. *Early life treatment with vancomycin propagates Akkermansia muciniphila and reduces diabetes incidence in the NOD mouse.* Diabetologia. 2012; 55(8): 2285-94.

http://dx.doi.org/10.1007/s00125-012-2564-7
PMid:22572803

87. Smecuol E, Hwang HJ, Sugai E, Corso L, Chernavsky AC, Bellavite FP et al. *Exploratory, randomized, double-blind, placebo-controlled study on the effects of Bifidobacterium infantis natren life start strain super strain in active celiac disease.* Journal of clinical gastroenterology. 2013; 47(2): 139-47.

http://dx.doi.org/10.1097/MCG.0b013e31827759ac
PMid:23314670

Chapter 6

Celiac Treatments, Adjuvant Therapies and Alternatives to the Gluten-Free Diet

Justin L. McCarville, Alberto Caminero, Elena F. Verdu

Farncombe Family Digestive Health Research Institute; McMaster University; Hamilton, ON Canada.

mccarvjl@mcmaster.ca, acamine@mcmaster.ca, verdue@mcmaster.ca

Doi: http://dx.doi.org/10.3926/oms.254

How to cite this chapter

McCarville JL, Caminero A, Verdu EF. *Celiac Treatments, Adjuvant Therapies and Alternatives to the Gluten-Free Diet.* In Arranz E, Fernández-Bañares F, Rosell CM, Rodrigo L, Peña AS, editors. *Advances in the Understanding of Gluten Related Pathology and the Evolution of Gluten-Free Foods*. Barcelona, Spain: OmniaScience; 2015. p. 223-253.

Abstract

Celiac disease (CD) is a chronic enteropathy triggered by exposure to dietary gluten in genetically susceptible individuals. The only currently accepted therapy for CD is a lifetime gluten-free diet (GFD). Although a GFD has proven to be a safe and effective in most celiac patients, there are limitations that warrant new adjuvant therapies for the treatment of CD. The therapies in development for CD fall into the following categories 1) Gluten detoxification 2) Luminal therapies 3) Intestinal barrier enhancing therapies 4) Immune targeted therapies and 5) Experimental therapies. Gluten detoxification includes altering gluten proteins in foods before commercialization. Luminal therapies aim at neutralizing gluten in the lumen of the small intestine. These include enzymatic digestion therapy, probiotics and gluten binders. Barrier enhancing therapies decrease the leaky intestinal condition associated with the disease, which could enhance translocation of gluten peptides, or of other harmful antigens, into the lamina propria. Immune targeted therapies include TG2 blockers, HLA blockers, T cell targeted therapies, alteration of inflammatory mediators and vaccine therapy. Finally, experimental therapies comprise compounds or biological strategies in discovery phase. Of these, Elafin was recently proposed to play a role in CD and have potential therapeutic applications in an animal model. To date, none of the discussed therapies have been approved for clinical use and are at different stages of development. However, adjuvant therapies to the GFD will likely become a reality to the coming years and will increase the quality of life of patients living with gluten-related disorders.

Keywords

Therapies for CD, gluten free alternatives, celiac therapies, gluten detoxification, gluten proteolysis.

1. Introduction

Celiac disease (CD) is a chronic autoimmune enteropathy triggered by exposure to dietary gluten in genetically susceptible individuals. Patients with a diagnosis of CD need to adopt a strict gluten-free diet (GFD) for life[1]. A GFD leads to significant clinical and histological improvement in CD patients, although it often results in social burden. This diet is expensive, not readily available in many countries, and if not properly supervised, may lead to nutritional deficiencies, which can affect the patient's quality of life. A gluten-containing diet based on consumption of cereals such as wheat, rye and barley is an important source of iron, dietary fibre and vitamin B[2-4]. A major problem underlying compliance with the GFD resides in the difficulty of complete avoidance of gluten[5] since its presence in processed foods, as well as its use in cosmetics and pharmaceutical industries, is ubiquitous. Gluten may be present in non-starchy foodstuff such as soy sauce and beer, and thus CD patients can be exposed inadvertently to small amounts of gluten that generate inflammation[6]. Furthermore, studies have shown that mucosal recovery is not immediate upon the start of a GFD, and that a substantial proportion of CD patients exhibit long-lasting low-grade inflammatory changes in the small intestinal mucosa[7-10]. Therefore, although a GFD has proven to be a safe and effective therapy, the limitations described above warrant that new adjuvant therapies are needed in the treatment of CD. Based on the current understanding of the pathogenesis of CD, several potential therapeutic targets are being explored and many reviews have recently been written on this topic[11-13]. The aim of this chapter is to summarize the current approaches and discuss the recent progress in the development of potential adjuvant treatments for CD.

2. Gluten Detoxification

Wheat gluten and related proteins in barley and rye trigger CD in genetically susceptible people. The complete elimination of gluten proteins contained in cereals from the diet is key to CD management[14]. Currently,

novel techniques are being developed to generate cereal varieties with lower immunogenic or toxic capacity for CD patients. Selective breeding and genetic manipulation of the disease-activating grains have been proposed to reach this goal[15-17]. The use of genetic engineering to down-regulate gene expression by RNA interference is an attractive opportunity for reducing the immunotoxic components of gluten. This technology has been applied to down-regulate the expression of gliadins and low molecular weight glutenins in bread wheat. Results have shown the usefulness of RNAi to silence specific genes corresponding to gluten proteins, which are the known sources of immunogenic peptides[18-22]. Flour from these lines may be an important breakthrough in the development of new products for the celiac community. However, additional studies, such as clinical trials in patients with gluten-related disorders are needed in order to determine whether or not the product can be consumed by the general celiac population[18, 23].

An alternative approach to detoxify gluten is the digestion of immunogenic gluten peptides with peptidases during food processing and before administration to CD patients[24]. Unlike mammalian digestive proteases, proteolytic enzymes from plants, fungi and microorganisms can hydrolyze toxic peptides in foods to amino acids or non-toxic peptides[25]. Di cagno et al. (2010) have isolated *Lactobacillus* strains from sourdough bread that showed considerable hydrolysis of gliadin during wheat sourdough fermentation and investigated a novel bread making method for the production of safe sourdoughs[26]. Similarly, Rizello et al. (2007) showed that fermentation with a complex formula of sourdough *Lactobacillus* and fungal proteases decreased the concentration of gluten considerably. This wheat flour hydrolyzed during food processing was shown to be safe for consumption by CD patients in a clinical study[27, 28]. Although results with RNA interference and proteases in sourdough fermentation are promising, one important question is how baking quality will be affected and whether widespread consumption of these foods by CD patients will be safe[14, 15, 23].

3. Luminal Therapies

3.1. Enzymatic Therapy

Gluten proteins are poorly digested in the human intestine because they are relatively resistant to human proteolytic enzymes. As a consequence, the gastrointestinal digestion of gluten leads to the generation of toxic peptides which trigger inflammation in genetically susceptible individuals[29]. Thus, oral enzymatic therapy is focused on inactivating immunogenic gluten peptides in the human gastrointestinal tract[30,31]. The most commonly studied enzymes with the ability to carry out this process are proteases from the prolyl endopeptidase family (PEPs) which are not present in humans. PEPs from *Flavobacterium meningosepticum*, *Sphingomonas capsulata* and *Myxococcus xanthus* are able to cleave immunodominant proline-rich regions present in gluten proteins[32-34].

For these enzymes to be effective, they must be resistant to both the acidic environment and digestive proteases of the stomach. Also, the majority of the epitope hydrolysis should occur in the stomach, to avoid toxic peptides entering the small intestine and triggering immune responses. Although encapsulation of PEPs was proposed in order to protect them from gastric secretions, recent studies have shown that only high doses of PEPs are capable of eliminating immunogenic peptides in a daily gluten load[35,36]. AN-PEP is an enzyme derived from *Aspergillus niger* that is being developed by an alimentary company (DSM)[37]. *In vitro* studies have shown that AN-PEP is active at acidic pH, resists digestion by pepsin and degrades all tested gluten peptides with a half-life ranging between 2 and 6 minutes[34,38]. Based on these *in vitro* findings, a number of *in vivo* studies are underway in CD patients. Although AN-PEP appears to be well tolerated in CD patients, clinical improvements in these patients are not clear[39]. (ClinicalTrials.gov Identifier: NCT01335503). Another drug candidate, ALV003, is being developed as an orally administered mixture of two glutenases (ALV001 and ALV002)[40]. ALV001 is a glutamine-specific cysteine endoprotease derived from germinating barley seeds (EP-B2) and ALV002 is a PEP from *Sphingomonas*

capsulata[33,41]. Both enzymes are active in the acidic environment of the stomach, and a 1:1 (w/w) formulation (ALV003) maximizes their glutenasic activity[33]. Phase 1 and Phase 2a clinical trials have been performed in CD patients receiving ALV003. These studies demonstrated that ALV003 can attenuate gluten-induced small intestinal mucosal injury and decrease the immune response to gluten in CD patients, but ALV003 did not improve the clinical response (NCT00959114 and NCT01255696)[42,43]. A Phase 2b, randomized, double-blind, placebo-controlled dose-ranging study of the efficacy and safety of ALV003 treatment in symptomatic CD patients maintained on a GFD is currently underway (ClinicalTrials.gov Identifier: NCT01917630). A third protease mixture (STAN 1) has been tested in a Phase 2 clinical trial (ClinicalTrials.gov Identifier: NCT00962182). STAN1 is a cocktail of microbial enzymes commonly used in food supplements that showed modest gluten detoxification capacity[31,44]. The study evaluated the effect of STAN1 in CD patients ingesting 1 g of gluten per day for 12 weeks. No differences were found in serology between the placebo group and the patients treated with STAN1[44,45]. A common setback with oral enzyme therapy seems to be the need for sufficiently active enzyme delivery to allow interaction with immunogenic gluten peptides present in a daily gluten load. However, although these enzymes may not eliminate the need for a GFD, they may provide substantial flexibility and prevention of detrimental side effects from lower gluten exposures, reducing long term complications of delayed mucosal healing[12]. A live commensal or beneficial bacterium that produces gluten-specific proteolytic molecules *in situ* would be an attractive alternative.

3.2. Probiotic Therapy

"Probiotics are defined as live microorganisms that when administered in adequate amounts confer health benefits to the host"[46]. Probiotics show a variety of immuno-modulatory, barrier enhancing and even mood-modulating effects that may be attractive to CD patients[47-49]. The probiotic preparation VSL#3 has been shown to hydrolyze gliadin proteins *in vitro* and may

produce pre-digested gliadins during food processing[50]. Other studies with cell cultures and mouse models of gluten sensitivity have demonstrated that "particular probiotic bacteria such as *Bifidobacterium lactis* or *Lactobacillus casei* could be of potential use in CD"[51-53]. Administration of a specific *Bifidobacterium infantis* strain to patients with active CD (ClinicalTrials.gov identifier: NCT01257620) led to improvement in CD-associated symptoms accompanied by immunogenic changes, without a significant change in intestinal permeability[54].

Another suggested alternative to facilitate gluten degradation and immune modulation includes the use of whole cultured bacteria from the human gastrointestinal tract. A number of studies from different groups have described substantial differences in the intestinal microbiota of patients with CD[55-57]. *Bifidobacterium longum* CECT7347 is a probiotic bacterial strain isolated from a healthy breastfed child with anti-inflammatory effects and proteolytic activity toward gliadin peptides *in vitro*[58-60]. To date, a double-bind, randomized, placebo-controlled intervention trial to evaluate the effects of *Bifidobacterium longum* CECT7347 in children with newly diagnosed CD has been completed. The findings suggest that *B. longum* CECT 7347 could help improve the health status of CD patients who tend to show alterations in gut microbiota composition and a biased immune response even on a GFD[61]. Moreover, several studies have isolated commensal bacteria strains from the human oral cavity and large intestine with the ability to hydrolyze peptides rich in proline including immunogenic peptides from gliadin such as the 33-mer and 26-mer peptide. These bacteria are candidate probiotics of interest in the treatment of CD[62,63]. For example, some *Lactobacillus* and *Bifidobacterium* strains have shown beneficial effects *in vitro* due to immunomodulation and restoration of the gliadin-induced epithelial barrier disruption[60,64]. Additional pre-clinical and clinical data are necessary to support the use of specific probiotics in CD patients.

Due to the ability of PEPs to hydrolyze gluten, Alvarez-Sieiro et al. (2014) have engineered two food-grade *Lactobacillus casei* strains to deliver PEP in a small intestinal *in vitro* model. One strain secretes PEP into the

surrounding environment, whereas the other retains PEP intracellularly. The extracellular secreting strain is the most effective at degrading the 33-mer and is resistant to simulated gastrointestinal stress. Results suggest that in the future, a genetically engineered (GMO) food-grade lactic acid bacterium may be useful as a vector for *in situ* production of PEP in the upper small intestine of CD patients[65]. This may raise discussions on the public acceptability of GMO's, despite the fact these have been shown to be safe for administration to mice and humans[66, 67].

3.3. Gluten Binding Polymer: BL-7010

The gluten binding polymer, BL-7010 or copolymer poly(hydroxythyl methacrylate-*co*-styrene sulfonate (P(HEMA-*co*-*SS*) is a non-absorbable polymer that binds with high specificity to gliadin or gluten, intraluminally. Upon binding of the polymer to gliadin, digestive enzymes are unable to access cleavage sites on the protein, thereby avoiding the production of immunogenic peptides[68]. Further, these peptides are not absorbed by the small intestine and therefore do not seem to induce immune responses in the host. BL-7010 has shown preclinical *in vitro*[69] and *in vivo* beneficial effects using a humanized mouse model of gluten sensitivity (HLA-HCD4/DQ8 mice), BL-7010 decreased gluten associated pathology, including intraepithelial lymphocytosis, reduced villus-to-crypt ratios, and normalized altered barrier function[68]. This therapy has a high safety profile in animal models and Phase 1 clinical trials are currently underway (ClinicalTrials.gov Identifier: NTC01990885).

4. Barrier Enhancing Therapies

CD is associated with altered barrier[70] and disrupted tight junction (TJ) function[71,72]. The mechanisms for gluten peptide translocation in CD are controversial, and several pathways have been proposed[73,74]. One is related to increases in paracellular uptake and increases in the release of zonulin, an endogenous modulator of TJs[75]. Zonulin has been reported to be regulated by

the direct binding of gliadin to CXCR3 in intestinal epithelial cells, increasing its release and subsequent decrease in barrier function[74]. Larazotide acetate, or AT-1001, is being developed as a TJ modulator by Alba Therapeutics. This molecule is an octapeptide derived from cholera toxin, secreted by *Vibrio cholerae*[44]. *In vitro*, larazotide acetate was shown to promote actin rearrangement and prevent disassembly of tight junctions due to external stimuli[76], including gliadin[77], in cell culture. Additionally, this small peptide inhibited translocation of gliadin constituents (13-mer) across cell culture monolayers, *in vitro*[77]. In an *in vivo* animal model using HLA-HCD4/DQ8 mice, administration of larazotide acetate normalized TJ proteins and inhibited macrophage recruitment in the intestine induced by gliadin[77]. In human trials, AT-1001 tended to improve increased intestinal permeability in CD patients upon gluten challenge compared to CD individuals who received placebo, but this did not achieve statistical significance[78]. However, larazotide acetate decreased gluten-induced gastrointestinal symptoms[79], and decreased gluten-induced INF-γ levels[78]. A follow up study demonstrated that CD patients, when on a GFD receiving a daily challenge of 2.7 grams of gluten and larazotide acetate had lower TG2 IgA levels in comparison to patients on placebo receiving the same challenge[80]. Phase 2b trial results have recently been released by ALBA Therapeutics, GI and non-GI symptoms were reduced in individuals on a GFD for more than 12 months, while taking larazotide acetate in comparison to placebo. Larazotide acetate will now enter phase 3 trials[81].

5. Immune Targeted Therapies

There are several immune therapies under development for chronic gastrointestinal inflammation that could be applied to CD. Some target CD specific pathways, other target inflammatory mediators common in gastrointestinal inflammation. For instance, drugs for the treatment of inflammatory bowel disease (IBD) could be useful in CD[44]. On the other hand, immune specific therapies for CD include transglutaminase-2 (TG2) blockers,

human leukocyte antigen (HLA) blockers, anti-IL-15 monoclonal antibodies and vaccine approaches[25].

5.1. TG2 Blockers

TG2 plays a critical role in CD pathogenesis by unmasking gluten-derived T cell epitopes via demidation[82]. Therefore it is of great interest as a therapeutic target. There have been many TG2 blockers developed, as TG2 is associated with other diseases, such as Huntington's disease and certain cancers[83]. The different subsets of TG2 inhibitors include, competitive amine inhibitors, reversible inhibitors and irreversible inhibitors[84]. The blocking of TG2 *in viv*o in humans has not been demonstrated and *in vivo* models are scarce. Therefore most studies have concentrated on *in vitro* and *in situ* models. TG2 inhibitors are capable of reducing certain gliadin-induced effects *in vitro*[85]. Further, in organ culture from CD patient biopsies, blockers are capable of reducing $CD25^+$ and $IL\text{-}15^+$ cells induced by gluten[85] and it has been shown that CD biopsies incubated with gliadin and the TG2 inhibitor cystamine, led to a reduction in the proliferation of gliadin-specific T cells[83]. Similarly, it has been demonstrated that 2-[(2-oxopropyl)thio]imidazolium inhibitor L682777 is effective at blocking T cell activation in small intestinal CD biopsies when incubated with non-deamindated gliadin[83]. ERW1041E is the only TG2 inhibitor to date, that has been shown *in vivo* to be effective at blocking TG2[86]. TG2 inhibitors may not be capable of treating innate immune responses, as shortening of villus-to-crypt ratios induced by poly (I:C) is unaffected by inhibiting TG2[86]. The biological significance of TG2 inhibitors is unknown, as TG2s exact physiological function is still unclear, however, in *in situ* and *in vitro*, no side effects have been observed[83]. However this will need to be defined before clinical trials are planned. Gianfrani et al. (2007) have proposed an enzyme strategy to inactivate immunogenic peptides and, at the same time, preserve the integrity of the protein structure using transamidation of wheat flour with a food-grade enzyme and an appropriate amine donor. The authors treated wheat flour with one microbial

transglutaminase and lysine methyl ester generating modified gliadin peptides which decreased their affinity to HLA-DQ[87].

5.2. HLA Blockers

The genetic component of CD, the HLA-DQ2/8 molecules, are required for the development the disease, making them a desirable target for therapies. HLA blockers have been attempted as a therapy in other diseases, such as multiple sclerosis and rheumatoid arthritis[25]. The major drawback of these therapies was the inability of the HLA blocker to reach the diseased site. However, the rationale for treating CD with HLA blockers is the ease of accessibility to the site of disease (small intestine)[25]. Therefore, researchers are developing molecules with similar structure to gliadin that do not elicit an immune response because they are not recognized by gluten-specific T cells. Kaporerchan et al. (2013) developed a strategy in which the proline residues of gluten were replaced with azidoprolines. This molecule binds to HLA-DQ2 decreasing immune responses in T cells isolated from individuals with CD[88]. Similarly, Xia et al. (2007) developed cyclic and dimeric peptides with the capacity to bind DQ2, partially blocking T cell proliferation and antigen presentation[84]. However, these molecules do not fully block T cell activation[25].

5.3. T cell Targeted Therapies

T cells play a critical role in the pathology of CD, being responsible for the proinflammatory immune response and villus atrophy[89]. There are no current T cell mediated therapies that are being developed specifically for CD. Anti-CD3 monoclonal antibodies could potentially block pathogenic gluten-specific T cells[25] and are currently undergoing clinical trials for diabetes and ulcerative colitis. CCR9 is a chemokine receptor on T cells and antagonists of this receptor are currently being tested in clinical trials for CD[25] and Crohn's disease[90]. The drug CCX282-B, Vercirnon, or Traficet-EN could be effective in CD, by blocking the recruitment of T cells to the intestine. Traficet-EN is currently being investigated in a phase 2a clinical trial (ClinicalTrials.gov Identifier: NCT00540657)[44].

5.4. Alteration of Inflammatory Mediators

A proportion of patients with CD have increased levels of IL-15. IL-15 plays a critical role in IEL cell activation and is an important cytokine linking the innate and adaptive immune response in CD[91,92]. Therefore, blocking the actions of IL-15 in individuals with IL-15 driven CD is an attractive target. It has been shown that destruction of the small intestine can be reversed when blocking IL-15 with a monoclonal antibody in mice *in vivo*[93]. The humanized version of this antibody has been tested in humans for T cell large granular lymphocytic leukemia with success (ClinicalTrials.gov Identifier: NCT00076180)[94]. The antibody, Hu-Mik-β-1, targets IL-2/IL-15Rβ, blocking IL-15 transpresentation[95]. Recruitment for clinical trials for Hu-Mik-β-1 in CD is underway (ClinicalTrials.gov Identifier: NCT01893775). Similarly, tofacitnib, a Jak2/3 inhibitor that blocks IL-15 signaling, reversed CD-related damage in an IL-15 transgenic mouse model[96].

5.5. Vaccine Therapy

Vaccine therapy for CD is based on the concept that immune tolerance to an antigen can be induced by repetitive exposure to that same antigen. In the case of CD, immunization with gluten epitopes would induce the expansion of regulatory T cells[97], thereby restoring oral tolerance to gluten. NEXVAX2 is being developed by ImmunsanT for the treatment of CD, and comprises the use of three gluten epitopes. These peptides were chosen based on a study by Tye-Din et al. (2010), wherein they screened a library of 16,000 peptides within wheat, barley and rye for their ability to induce and stimulate T cells isolated from the serum of CD patients on a gluten containing diet. They identified three peptides responsible for the majority of the immune responses by isolated T cells, which have been incorporated into the vaccine[98]. The vaccine requires repetitive intradermal injections and is currently in phase 1b (ClinicalTrials.gov Identifier: NCT00879749)[44,99]. NEXVAX2 is only specific to HLA-DQ2 individuals (90% of the CD population)[25,99]. Of the therapies currently in development, the vaccine approach would be curative if proven efficacious.

6. Experimental Therapies

6.1 Necator americanus

The parasite *Necator americanus* is a human gastrointestinal nematode or hookworm believed to infect over 500 million people worldwide[100]. Infection with this hookworm has no major side effects, and is associated with normal mucosal appearance in duodenal biopsies[101]. However, the development of anemia may be of concern, as the parasite feeds on host blood (0.03-0.08 mL per day)[100]. Administration of *N. americanus* infective larvae to individuals with CD has shown to suppress increased $CD4^+CD25^+Foxp3^+$ cells in serum, which are associated with CD[102]. Duodenal biopsies from individuals with CD, infected with *N. americanus* and exposed to the gliadin constituent QE65[103] had decreased ability to produce IL-2, IFN-γ and IL-17A[102]. In a separate study, *Necator americanus* was shown to resist changes in villus-to-crypt ratios, increases in IELs, IgA production towards TG2, decrease IFNγ-producing IELs and lamina propria cells, as well as increase $CD3^+CD4^+Foxp3^+$ cells in IEL compartments in CD patients after gluten challenge[104]. *N. americanus* is currently in clinical trials phase 2a (ClinicalTrials.gov Identifier: NCT00671138)[44], however compared to other developing therapies, some side effects associated with this therapy may be anticipated[105]. Patient acceptance may also be an issue. It is unclear how the decrease in serum $CD4^+CD25^+Foxp3^+$ could be of advantage in CD, as these could include T regulatory cells important for inflammatory T cell suppression.

6.2. Elafin

Elafin, an anti-inflammatory serine protease inhibitor, is decreased in the colon of patients with inflammatory bowel disease, and delivery of elafin to mice alleviated chemical-induced colitis[106]. Recently, the decreased mucosal expression of elafin in the small intestine of patients with active CD was described[92]. Also, delivery of elafin to the small intestine via the food grade bacterium *Lactobacillus lactis*, ameliorated immune and pathological

responses to gluten in a mouse model (NOD-DQ8) that develops decreased villus-to-crypt ratios, anit-gliadin and anti-tissue TG2 antibodies upon sensitization[107]. Future research will need to determine the optimal delivery mode of this molecule to humans and its clinical efficacy.

7. Discussion and Conclusion

In summary, there are many therapies being developed for CD, which target different mechanisms of the disease process. Many of these therapies are already being tested in clinical trials, others are at discovery level of development. At the time this chapter was written, the most advanced therapy in clinical trial testing was the barrier enhancing therapy, AT-1001. However, it cannot be predicted that this will be the first drug to be approved for clinical use. Even when one or more drugs for CD are approved in the future years, further testing will be required to investigate whether combination therapies are more efficacious than single therapies. For example, the enzymatic therapy ALV003 or the gluten binder BL-7010 could be used in conjunction with most other therapies currently in the pipeline. However combinations of ALV003 and BL-7010 would not be advisable, since both therapies have opposite mechanisms of action. While ALV003 increases proteolytic digestion of gluten, BL-7010 reduces the action of the digestive enzymes on the gluten molecule, and the production of immunogenic peptides. Other possible combinations may include elafin therapy if further developed, with immunomodulatory or barrier enhancing probiotics. Finally, an issue of concern is whether availability of these therapies could encourage patients to abandon the gluten-free diet. Guidelines for the “adjunctive” use of these therapies with the GFD will need to be clearly established. These drugs may also prove effective in other gluten-related disorders, and this will require further research. We are approaching exciting years in the pharmacological management of gluten-related disorders. Availability of one or more of the described therapies will increase the quality of life of patients living with gluten-related disorders.

***Table 1**. Summary of current therapies in development for CD.*

Therapy	Product	Mode of Action	Stage in Development	Reference
Luminal Therapies				
Transgenic reduced-gliadin lines of Triticum	Bread wheat with low expression of harmful gliadins	Raw material for developing food products that can be safely tolerated by CD patients	Preclinical	[18,19]
Gluten-free sourdough wheat	Sourdough lactobacilli-derived peptidases	Digestion of immunogenic gluten peptides during food processing	Preclinical	[26]
	Sourdough lactobacilli-derived peptidases in combination with fungal proteases		2a	[28]
Transamidation of gliadin	wheat flour treated with TG and lysine methyl ester	Inactivates immunogenic epitopes via the transamidation of wheat flour with a food-grade enzyme and an appropriate amino donor	Preclinical	[87]
Oral enzymatic therapy	PEP from *S.capsulata, F. meningosepticum, M. xanthus*	Hydrolysis of proline-rich peptides of gliadin in the upper gastrointestinal tract	Preclinical	[32,35]
	AN-PEP		2a	[37,39]
	ALV003		2b	[42,43]
	STAN-1		2a	
Probiotic bacteria	VSL#3	Live microorganisms that confer health benefits on the host	Preclinical	[50]
	Bifidobacterium infantis		2a	[54]
	Bifidobacterium longum CECT7347		2a	[58,59,61]

Therapy	Product	Mode of Action	Stage in Development	Reference
Gluten binding therapy	p(HEMA-*co*-SS) or BL-7010	Binds to gluten in the intestinal lumen, avoiding gluten's translocation and immune induction	Preclinical	[68]
Barrier Enhancing Therapies				
Zonulin inhibitor	AT-1001	Lazazotide acetate inhibits zonulin activation, increasing associations between tight junctions and therefore decreases intestinal permeability	2b	[80,81]
Immune Targeted Therapies				
HLA blockers	Azidoprolines/cyclic and dimeric peptides	Binders of HLA-DQ2 that block T cell proliferation and activation towards natural gluten peptides	preclinical	[84,88]
IL-15 signaling blockers	Hu-Mik-β-1	Monoclonal antibody that targets IL-2/IL-15Rβ, blocking IL-15 transpresentation	1	[93,94]
	Tofacitinib	Jak2/3 inhibitor that blocks IL-15 signaling	3 for treatment of ulcerative colitis	[96]
CCR9 antagonist	Traficet-EN	Antagonizes CCR9 on T cells, blocking their recruitment and localization to the small intestine	2a	[90]
Vaccine	NEXVAX2	Intradermal injection of 3 gluten peptides to induce tolerance in individuals harboring HLA-DQ2	1b	[44]

Therapy	Product	Mode of Action	Stage in Development	Reference
Other Therapies				
Parasitic infection	Necator americanus	Suppresses induction of $CD4^+CD25^+Foxp3^+$ T cells in serum, increases $CD3^+CD4^+Foxp3^+$ cells in IEL compartments, decreases IL-2, IFN-γ and IL-17a from small intestinal biopsies	2a	[101,104]
Elafin	*L. lactis* secreting elafin	Decreases severity of gluten-induced pathologies	Discovery	[107]

References

1. Ludvigsson JF, Leffler DA, Bai JC, Biagi F, Fasano A, Green PH et al. *The Oslo definitions for coeliac disease and related terms.* Gut. 2012.
   http://dx.doi.org/10.1136/gutjnl-2011-301346
2. Kupper C. *Dietary guidelines and implementation for celiac disease.* Gastroenterology. 2005; 128(4 Suppl 1): S121-7.
   http://dx.doi.org/10.1053/j.gastro.2005.02.024
   PMid:15825119
3. Freeman HJ. *Non-dietary forms of treatment for adult celiac disease.* World J Gastrointest Pharmacol Ther. 2013; 4(4): 108-12.
   PMid:24199026 PMCid:PMC3817285
4. McAllister CS, Kagnoff MF. *The immunopathogenesis of celiac disease reveals possible therapies beyond the gluten-free diet.* Semin Immunopathol. 2012; 34(4): 581-600.
   http://dx.doi.org/10.1007/s00281-012-0318-8
   PMid:22674144
5. Hall NJ, Rubin GP, Charnock A. *Intentional and inadvertent non-adherence in adult coeliac disease. A cross-sectional survey.* Appetite. 2013; 68: 56-62.
   http://dx.doi.org/10.1016/j.appet.2013.04.016
   PMid:23623778
6. Collin P, Mäki M, Kaukinen K. *It is the compliance, not milligrams of gluten, that is essential in the treatment of celiac disease.* Nutr Rev. 2004; 62(12): 490; author reply 1.
   http://dx.doi.org/10.1111/j.1753-4887.2004.tb00022.x
   PMid:15648825
7. Rubio-Tapia A, Murray JA. *Classification and management of refractory coeliac disease.* Gut. 2010; 59(4): 547-57.
   http://dx.doi.org/10.1136/gut.2009.195131
   PMid:20332526 PMCid:PMC2861306
8. Rubio-Tapia A, Rahim MW, See JA, Lahr BD, Wu TT, Murray JA. *Mucosal recovery and mortality in adults with celiac disease after treatment with a gluten-free diet.* Am J Gastroenterol. 2010; 105(6): 1412-20.
   http://dx.doi.org/10.1038/ajg.2010.10
   PMid:20145607 PMCid:PMC2881171
9. Leffler DA, Dennis M, Hyett B, Kelly E, Schuppan D, Kelly CP. *Etiologies and predictors of diagnosis in nonresponsive celiac disease.* Clin Gastroenterol Hepatol. 2007; 5(4): 445-50.
   http://dx.doi.org/10.1016/j.cgh.2006.12.006
   PMid:17382600

10. Theethira TG, Dennis M, Leffler DA. *Nutritional consequences of celiac disease and the gluten-free diet.* Expert Rev Gastroenterol Hepatol. 2014; 8(2): 123-9.
http://dx.doi.org/10.1586/17474124.2014.876360
PMid:24417260

11. Pinier M, Fuhrmann G, Verdu EF, Leroux JC. *Prevention measures and exploratory pharmacological treatments of celiac disease.* Am J Gastroenterol. 2010; 105(12): 2551-61; quiz 62.
http://dx.doi.org/10.1038/ajg.2010.372
PMid:20877349

12. Mukherjee R, Kelly CP, Schuppan D. *Nondietary therapies for celiac disease.* Gastrointest Endosc Clin N Am. 2012; 22(4): 811-31.
http://dx.doi.org/10.1016/j.giec.2012.09.001
PMid:23083995

13. Kurppa K, Hietikko M, Sulic AM, Kaukinen K, Lindfors K. *Current status of drugs in development for celiac disease.* Expert Opin Investig Drugs. 2014; 7.
http://dx.doi.org/10.1517/13543784.2014.916274

14. Comino I, Moreno MeL, Real A, Rodríguez-Herrera A, Barro F, Sousa C. *The gluten-free diet: testing alternative cereals tolerated by celiac patients.* Nutrients. 2013; 5(10): 4250-68.
http://dx.doi.org/10.3390/nu5104250
PMid:24152755 PMCid:PMC3820072

15. van den Broeck HC, van Herpen TW, Schuit C, Salentijn EM, Dekking L, Bosch D et al. *Removing celiac disease-related gluten proteins from bread wheat while retaining technological properties: a study with Chinese Spring deletion lines.* BMC Plant Biol. 2009; 9: 41.
http://dx.doi.org/10.1186/1471-2229-9-41
PMid:19351412 PMCid:PMC2670835

16. Spaenij-Dekking L, Kooy-Winkelaar Y, van Veelen P, Drijfhout JW, Jonker H, van Soest L et al. *Natural variation in toxicity of wheat: potential for selection of nontoxic varieties for celiac disease patients.* Gastroenterology. 2005; 129(3): 797-806.
http://dx.doi.org/10.1053/j.gastro.2005.06.017
PMid:16143119

17. Mitea C, Salentijn EM, van Veelen P, Goryunova SV, van der Meer IM, van den Broeck HC et al. *A universal approach to eliminate antigenic properties of alpha-gliadin peptides in celiac disease.* PLoS One. 2010; 5(12): e15637.
http://dx.doi.org/10.1371/journal.pone.0015637
PMid:21179575 PMCid:PMC3002971

18. Gil-Humanes J, Pistón F, Altamirano-Fortoul R, Real A, Comino I, Sousa C et al. *Reduced-gliadin wheat bread: an alternative to the gluten-free diet for consumers suffering gluten-related pathologies.* PLoS One. 2014; 9(3): e90898.
http://dx.doi.org/10.1371/journal.pone.0090898
PMid:24621595 PMCid:PMC3951262

19. Gil-Humanes J, Pistón F, Barro F, Rosell CM. *The Shutdown of Celiac Disease-Related Gliadin Epitopes in Bread Wheat by RNAi Provides Flours with Increased Stability and Better Tolerance to Over-Mixing.* PLoS One. 2014; 9(3): e91931.
http://dx.doi.org/10.1371/journal.pone.0091931
PMid:24633046 PMCid:PMC3954839

20. Gil-Humanes J, Pistón F, Tollefsen S, Sollid LM, Barro F. *Effective shutdown in the expression of celiac disease-related wheat gliadin T-cell epitopes by RNA interference.* Proc Natl Acad Sci USA. 2010; 107(39): 17023-8.
http://dx.doi.org/10.1073/pnas.1007773107
PMid:20829492 PMCid:PMC2947919

21. Altenbach SB, Allen PV. *Transformation of the US bread wheat 'Butte 86' and silencing of omega-5 gliadin genes.* GM Crops. 2011; 2(1): 66-73.
http://dx.doi.org/10.4161/gmcr.2.1.15884
PMid:21844700

22. Wen S, Wen N, Pang J, Langen G, Brew-Appiah RA, Mejias JH et al. *Structural genes of wheat and barley 5-methylcytosine DNA glycosylases and their potential applications for human health.* Proc Natl Acad Sci USA. 2012; 109(50): 20543-8.
http://dx.doi.org/10.1073/pnas.1217927109
PMid:23184965 PMCid:PMC3528507

23. Gil-Humanes J, Pistón F, Giménez MJ, Martín A, Barro F. *The introgression of RNAi silencing of γ-gliadins into commercial lines of bread wheat changes the mixing and technological properties of the dough.* PLoS One. 2012; 7(9): e45937.
http://dx.doi.org/10.1371/journal.pone.0045937
PMid:23029328 PMCid:PMC3454332

24. Caputo I, Lepretti M, Martucciello S, Esposito C. *Enzymatic strategies to detoxify gluten: implications for celiac disease.* Enzyme Res. 2010; 2010: 174354.
http://dx.doi.org/10.4061/2010/174354
PMid:21048862 PMCid:PMC2963796

25. Sollid LM, Khosla C. *Novel therapies for coeliac disease.* J Intern Med. 2011; 269(6): 604-13.
http://dx.doi.org/10.1111/j.1365-2796.2011.02376.x
PMid:21401739 PMCid:PMC3101315

26. Di Cagno R, De Angelis M, Lavermicocca P, De Vincenzi M, Giovannini C, Faccia M et al. *Proteolysis by sourdough lactic acid bacteria: effects on wheat flour protein fractions and gliadin peptides involved in human cereal intolerance.* Appl Environ Microbiol. 2002; 68(2): 623-33.

http://dx.doi.org/10.1128/AEM.68.2.623-633.2002
PMid:11823200 PMCid:PMC126681

27. Rizzello CG, De Angelis M, Di Cagno R, Camarca A, Silano M, Losito I et al. *Highly efficient gluten degradation by lactobacilli and fungal proteases during food processing: new perspectives for celiac disease.* Appl Environ Microbiol. 2007; 73(14): 4499-507.

http://dx.doi.org/10.1128/AEM.00260-07
PMid:17513580 PMCid:PMC1932817

28. Di Cagno R, Barbato M, Di Camillo C, Rizzello CG, De Angelis M, Giuliani G et al. *Gluten-free sourdough wheat baked goods appear safe for young celiac patients: a pilot study.* J Pediatr Gastroenterol Nutr. 2010; 51(6): 777-83.

http://dx.doi.org/10.1097/MPG.0b013e3181f22ba4
PMid:20975578

29. Shan L, Molberg Ø, Parrot I, Hausch F, Filiz F, Gray GM et al. *Structural basis for gluten intolerance in celiac sprue.* Science. 2002; 297(5590): 2275-9.

http://dx.doi.org/10.1126/science.1074129
PMid:12351792

30. Gass J, Khosla C. *Prolyl endopeptidases.* Cell Mol Life Sci. 2007; 64(3): 345-55.

http://dx.doi.org/10.1007/s00018-006-6317-y
PMid:17160352

31. Bethune MT, Khosla C. *Oral enzyme therapy for celiac sprue.* Methods Enzymol. 2012; 502: 241-71.

http://dx.doi.org/10.1016/B978-0-12-416039-2.00013-6
PMid:22208988 PMCid:PMC3382113

32. Shan L, Marti T, Sollid LM, Gray GM, Khosla C. *Comparative biochemical analysis of three bacterial prolyl endopeptidases: implications for coeliac sprue.* Biochem J. 2004; 383(Pt 2): 311-8.

PMid:15245330 PMCid:PMC1134072

33. Gass J, Bethune MT, Siegel M, Spencer A, Khosla C. *Combination enzyme therapy for gastric digestion of dietary gluten in patients with celiac sprue.* Gastroenterology. 2007; 133(2): 472-80.

http://dx.doi.org/10.1053/j.gastro.2007.05.028
PMid:17681168

34. Stepniak D, Spaenij-Dekking L, Mitea C, Moester M, de Ru A, Baak-Pablo R et al. *Highly efficient gluten degradation with a newly identified prolyl endoprotease: implications for celiac disease.* Am J Physiol Gastrointest Liver Physiol. 2006; 291(4): G621-9.

http://dx.doi.org/10.1152/ajpgi.00034.2006
PMid:16690904

35. Gass J, Ehren J, Strohmeier G, Isaacs I, Khosla C. *Fermentation, purification, formulation, and pharmacological evaluation of a prolyl endopeptidase from Myxococcus xanthus: implications for Celiac Sprue therapy.* Biotechnol Bioeng. 2005; 92(6): 674-84.

http://dx.doi.org/10.1002/bit.20643
PMid:16136593

36. Marti T, Molberg O, Li Q, Gray GM, Khosla C, Sollid LM. *Prolyl endopeptidase-mediated destruction of T cell epitopes in whole gluten: chemical and immunological characterization.* J Pharmacol Exp Ther. 2005; 312(1): 19-26.

http://dx.doi.org/10.1124/jpet.104.073312
PMid:15358813

37. Tack G, van de Water J, Kooy-Winkelaar E, van Bergen J, Meijer G, vom Blomberg B et al. *Can Prolyl Endoprotease Enzyme Treatment Mitigate the Toxic Effect of Gluten in Coeliac Patients?* Gastroenterology. 2010; 138(5): S-54.

http://dx.doi.org/10.1016/S0016-5085(10)60247-8

38. Mitea C, Havenaar R, Drijfhout JW, Edens L, Dekking L, Koning F. *Efficient degradation of gluten by a prolyl endoprotease in a gastrointestinal model: implications for coeliac disease.* Gut. 2008; 57(1): 25-32.

http://dx.doi.org/10.1136/gut.2006.111609
PMid:17494108

39. Tack GJ, van de Water JM, Bruins MJ, Kooy-Winkelaar EM, van Bergen J, Bonnet P et al. *Consumption of gluten with gluten-degrading enzyme by celiac patients: a pilot-study.* World J Gastroenterol. 2013; 19(35): 5837-47.

http://dx.doi.org/10.3748/wjg.v19.i35.5837
PMid:24124328 PMCid:PMC3793137

40. Siegel M, Garber ME, Spencer AG, Botwick W, Kumar P, Williams RN et al. *Safety, tolerability, and activity of ALV003: results from two phase 1 single, escalating-dose clinical trials.* Dig Dis Sci. 2012; 57(2): 440-50.

http://dx.doi.org/10.1007/s10620-011-1906-5
PMid:21948339

41. Siegel M, Bethune MT, Gass J, Ehren J, Xia J, Johannsen A et al. *Rational design of combination enzyme therapy for celiac sprue.* Chem Biol. 2006; 13(6): 649-58.

http://dx.doi.org/10.1016/j.chembiol.2006.04.009
PMid:16793522

42. Tye-Din JA, Anderson RP, Ffrench RA, Brown GJ, Hodsman P, Siegel M et al. *The effects of ALV003 pre-digestion of gluten on immune response and symptoms in celiac disease in vivo.* Clin Immunol. 2010; 134(3): 289-95.
http://dx.doi.org/10.1016/j.clim.2009.11.001
PMid:19942485

43. Lähdeaho ML, Kaukinen K, Laurila K, Vuotikka P, Koivurova OP, Kärjä-Lahdensuu T et al. *The Glutenase ALV003 Attenuates Gluten-Induced Mucosal Injury in Patients with Celiac Disease.* Gastroenterology. 2014.
http://dx.doi.org/10.1053/j.gastro.2014.02.031
PMid:24583059

44. Crespo Pérez L, Castillejo de Villasante G, Cano Ruiz A, León F. *Non-dietary therapeutic clinical trials in coeliac disease.* European journal of internal medicine. 2012; 23(1): 9-14.
http://dx.doi.org/10.1016/j.ejim.2011.08.030
PMid:22153524

45. Stoven S, Murray JA, Marietta E. *Celiac disease: advances in treatment via gluten modification.* Clin Gastroenterol Hepatol. 2012; 10(8): 859-62.
http://dx.doi.org/10.1016/j.cgh.2012.06.005
PMid:22728383 PMCid:PMC3788609

46. Bertazzoni E, Donelli G, Midtvedt T, Nicoli J, Sanz Y. *Probiotics and clinical effects: is the number what counts?* J Chemother. 2013; 25(4): 193-212.
http://dx.doi.org/10.1179/1973947813Y.0000000078
PMid:23906073

47. Borchers AT, Selmi C, Meyers FJ, Keen CL, Gershwin ME. *Probiotics and immunity.* J Gastroenterol. 2009; 44(1): 26-46.
http://dx.doi.org/10.1007/s00535-008-2296-0
PMid:19159071

48. Saulnier DM, Ringel Y, Heyman MB, Foster JA, Bercik P, Shulman RJ et al. *The intestinal microbiome, probiotics and prebiotics in neurogastroenterology.* Gut Microbes. 2013; 4(1): 17-27.
http://dx.doi.org/10.4161/gmic.22973
PMid:23202796 PMCid:PMC3555881

49. Verdú EF, Bercik P, Collins SM. *Effect of probiotics on gastrointestinal function: evidence from animal models.* Therap Adv Gastroenterol. 2009; 2(4): 31-5.
http://dx.doi.org/10.1177/1756283X09337645
PMid:21180552 PMCid:PMC3002531

50. De Angelis M, Rizzello CG, Fasano A, Clemente MG, De Simone C, Silano M et al. *VSL#3 probiotic preparation has the capacity to hydrolyze gliadin polypeptides responsible for Celiac Sprue.* Biochim Biophys Acta. 2006; 1762(1): 80-93.

http://dx.doi.org/10.1016/j.bbadis.2005.09.008
PMid:16311022

51. Lindfors K, Blomqvist T, Juuti-Uusitalo K, Stenman S, Venäläinen J, Mäki M et al. *Live probiotic Bifidobacterium lactis bacteria inhibit the toxic effects induced by wheat gliadin in epithelial cell culture.* Clin Exp Immunol. 2008; 152(3): 552-8.

http://dx.doi.org/10.1111/j.1365-2249.2008.03635.x
PMid:18422736 PMCid:PMC2453197

52. D'Arienzo R, Maurano F, Luongo D, Mazzarella G, Stefanile R, Troncone R et al. *Adjuvant effect of Lactobacillus casei in a mouse model of gluten sensitivity.* Immunol Lett. 2008; 119(1-2): 78-83.

http://dx.doi.org/10.1016/j.imlet.2008.04.006
PMid:18547649

53. D'Arienzo R, Stefanile R, Maurano F, Mazzarella G, Ricca E, Troncone R et al. *Immunomodulatory effects of Lactobacillus casei administration in a mouse model of gliadin-sensitive enteropathy.* Scand J Immunol. 2011; 74(4): 335-41.

http://dx.doi.org/10.1111/j.1365-3083.2011.02582.x
PMid:21615450

54. Smecuol E, Hwang HJ, Sugai E, Corso L, Cherñavsky AC, Bellavite FP et al. *Exploratory, randomized, double-blind, placebo-controlled study on the effects of Bifidobacterium infantis natren life start strain super strain in active celiac disease.* J Clin Gastroenterol. 2013; 47(2): 139-47.

http://dx.doi.org/10.1097/MCG.0b013e31827759ac
PMid:23314670

55. Nadal I, Donat E, Donant E, Ribes-Koninckx C, Calabuig M, Sanz Y. *Imbalance in the composition of the duodenal microbiota of children with coeliac disease.* J Med Microbiol. 2007; 56(Pt 12): 1669-74.

http://dx.doi.org/10.1099/jmm.0.47410-0
PMid:18033837

56. De Palma G, Nadal I, Medina M, Donat E, Ribes-Koninckx C, Calabuig M et al. *Intestinal dysbiosis and reduced immunoglobulin-coated bacteria associated with coeliac disease in children.* BMC Microbiol. 2010; 10: 63.

http://dx.doi.org/10.1186/1471-2180-10-63
PMid:20181275 PMCid:PMC2843610

57. Nistal E, Caminero A, Vivas S, Ruiz de Morales JM, Sáenz de Miera LE, Rodríguez-Aparicio LB et al. *Differences in faecal bacteria populations and faecal bacteria metabolism in healthy adults and celiac disease patients.* Biochimie. 2012; 94(8): 1724-9.

http://dx.doi.org/10.1016/j.biochi.2012.03.025
PMid:22542995

58. Medina M, De Palma G, Ribes-Koninckx C, Calabuig M, Sanz Y. *Bifidobacterium strains suppress in vitro the pro-inflammatory milieu triggered by the large intestinal microbiota of coeliac patients.* J Inflamm (Lond). 2008; 5: 19.

http://dx.doi.org/10.1186/1476-9255-5-19
PMid:18980693 PMCid:PMC2640389

59. Laparra JM, Olivares M, Gallina O, Sanz Y. *Bifidobacterium longum CECT 7347 modulates immune responses in a gliadin-induced enteropathy animal model.* PLoS One. 2012; 7(2): e30744.

http://dx.doi.org/10.1371/journal.pone.0030744
PMid:22348021 PMCid:PMC3277586

60. Laparra JM, Sanz Y. *Bifidobacteria inhibit the inflammatory response induced by gliadins in intestinal epithelial cells via modifications of toxic peptide generation during digestion.* J Cell Biochem. 2010; 109(4): 801-7.

PMid:20052669

61. Olivares M, Castillejo G, Varea V, Sanz Y. *Double-blind, randomised, placebo- controlled intervention trial to evaluate the effects of Bifidobacterium longum CECT 7347 in children with newly diagnosed coeliac disease.* Br J Nutr. 2014; 28: 1-11.

http://dx.doi.org/10.1017/S0007114514000609

62. Fernandez-Feo M, Wei G, Blumenkranz G, Dewhirst FE, Schuppan D, Oppenheim FG et al. *The cultivable human oral gluten-degrading microbiome and its potential implications in coeliac disease and gluten sensitivity.* Clin Microbiol Infect. 2013.

http://dx.doi.org/10.1111/1469-0691.12249
PMid:23714165 PMCid:PMC3749263

63. Caminero A, Herrán AR, Nistal E, Pérez-Andrés J, Vaquero L, Vivas S et al. *Diversity of the cultivable human gut microbiome involved in gluten metabolism: Isolation of microorganisms with potential interest for coeliac disease.* FEMS Microbiol Ecol. 2014.

http://dx.doi.org/10.1111/1574-6941.12295
PMid:24499426

64. Orlando A, Linsalata M, Notarnicola M, Tutino V, Russo F. *Lactobacillus GG restoration of the gliadin induced epithelial barrier disruption: the role of cellular polyamines.* BMC Microbiol. 2014; 14: 19.

http://dx.doi.org/10.1186/1471-2180-14-19
PMid:24483336 PMCid:PMC3911798

65. Alvarez-Sieiro P, Martin MC, Redruello B, Del Rio B, Ladero V, Palanski BA et al. *Generation of food-grade recombinant Lactobacillus casei delivering Myxococcus xanthus prolyl endopeptidase.* Appl Microbiol Biotechnol. 2014.

http://dx.doi.org/10.1007/s00253-014-5730-7
PMid:24752841

66. Martin R, Chain F, Miquel S, Natividad JM, Sokol H, Verdu EF et al. *Effects in the use of a genetically engineered strain of Lactococcus lactis delivering in situ IL-10 as a therapy to treat low-grade colon inflammation.* Hum Vaccin Immunother. 2014; 14; 10(6).

67. Braat H, Rottiers P, Hommes DW, Huyghebaert N, Remaut E, Remon JP et al. *A phase I trial with transgenic bacteria expressing interleukin-10 in Crohn's disease.* Clin Gastroenterol Hepatol. 2006; 4(6): 754-9.

http://dx.doi.org/10.1016/j.cgh.2006.03.028
PMid:16716759

68. Pinier M, Fuhrmann G, Galipeau HJ, Rivard N, Murray JA, David CS et al. *The copolymer P(HEMA-co-SS) binds gluten and reduces immune response in gluten-sensitized mice and human tissues.* Gastroenterology. 2012; 142(2): 316-25 e1-12.

69. Pinier M, Verdu EF, Nasser-Eddine M, David CS, Vezina A, Rivard N et al. *Polymeric binders suppress gliadin-induced toxicity in the intestinal epithelium.* Gastroenterology. 2009; 136(1): 288-98.

http://dx.doi.org/10.1053/j.gastro.2008.09.016
PMid:18992747

70. Sapone A, Lammers KM, Casolaro V, Cammarota M, Giuliano MT, De Rosa M et al. *Divergence of gut permeability and mucosal immune gene expression in two gluten-associated conditions: celiac disease and gluten sensitivity.* BMC Med. 2011; 9: 23.

http://dx.doi.org/10.1186/1741-7015-9-23
PMid:21392369 PMCid:PMC3065425

71. Ciccocioppo R, Finamore A, Ara C, Di Sabatino A, Mengheri E, Corazza GR. *Altered expression, localization, and phosphorylation of epithelial junctional proteins in celiac disease.* Am J Clin Pathol. 2006; 125(4): 502-11.

http://dx.doi.org/10.1309/DTYRA91G8R0KTM8M
PMid:16627260

72. Schumann M, Gunzel D, Buergel N, Richter JF, Troeger H, May C et al. *Cell polarity-determining proteins Par-3 and PP-1 are involved in epithelial tight junction defects in coeliac disease.* Gut. 2012; 61(2): 220-8.

http://dx.doi.org/10.1136/gutjnl-2011-300123
PMid:21865402

73. Matysiak-Budnik T, Moura IC, Arcos-Fajardo M, Lebreton C, Menard S, Candalh C et al. *Secretory IgA mediates retrotranscytosis of intact gliadin peptides via the transferrin receptor in celiac disease.* J Exp Med. 2008; 205(1): 143-54.

http://dx.doi.org/10.1084/jem.20071204
PMid:18166587 PMCid:PMC2234361

74. Lammers KM, Lu R, Brownley J, Lu B, Gerard C, Thomas K et al. *Gliadin induces an increase in intestinal permeability and zonulin release by binding to the chemokine receptor CXCR3.* Gastroenterology. 2008; 135(1): 194-204 e3.

75. Fasano A, Not T, Wang W, Uzzau S, Berti I, Tommasini A et al. *Zonulin, a newly discovered modulator of intestinal permeability, and its expression in coeliac disease.* Lancet. 2000; 355(9214): 1518-9.

http://dx.doi.org/10.1016/S0140-6736(00)02169-3

76. Gopalakrishnan S, Tripathi A, Tamiz AP, Alkan SS, Pandey NB. *Larazotide acetate promotes tight junction assembly in epithelial cells.* Peptides. 2012; 35(1): 95-101.

http://dx.doi.org/10.1016/j.peptides.2012.02.016
PMid:22401910

77. Gopalakrishnan S, Durai M, Kitchens K, Tamiz AP, Somerville R, Ginski M et al. *Larazotide acetate regulates epithelial tight junctions in vitro and in vivo.* Peptides. 2012; 35(1): 86-94.

http://dx.doi.org/10.1016/j.peptides.2012.02.015
PMid:22401908

78. Paterson BM, Lammers KM, Arrieta MC, Fasano A, Meddings JB. *The safety, tolerance, pharmacokinetic and pharmacodynamic effects of single doses of AT-1001 in coeliac disease subjects: a proof of concept study.* Aliment Pharmacol Ther. 2007; 26(5): 757-66.

http://dx.doi.org/10.1111/j.1365-2036.2007.03413.x
PMid:17697209

79. Leffler DA, Kelly CP, Abdallah H, Colatrella A, Harris L, Leon F et al. *A randomized, double-blind study of larazotide acetate to prevent the activation of celiac disease during gluten challenge.* The American journal of gastroenterology. 2012.

http://dx.doi.org/10.1038/ajg.2012.211
PMCid:PMC3463856

80. Kelly CP, Green PHR, Murray JA, DiMarino A, Colatrella A, Leffler DA et al. *Larazotide acetate in patients with coeliac disease undergoing a gluten challenge: a randomised placebo-controlled study.* Aliment Pharm Ther. 2013; 37(2): 252-62.

http://dx.doi.org/10.1111/apt.12147
PMid:23163616

81. Wang C, Rasmussen H, Perrow W, Ciaran KP, Le7ffler D, Green P et al. *Larazotide acetate, a First In-Class, Novel Tight Junction Regulator, Meets Primary Endpoint and Significantly Reduces Signs and Symptoms of Celiac Disease in Patients on a Gluten-Free Diet: Results of a Multicentre, Randomized, Placebo Controlled Trial.* Digestive Disease Week; Chicago (United States). 2014.

82. Arentz-Hansen H, Korner R, Molberg O, Quarsten H, Vader W, Kooy YM et al. *The intestinal T cell response to alpha-gliadin in adult celiac disease is focused on a single deamidated glutamine targeted by tissue transglutaminase.* J Exp Med. 2000; 191(4): 603-12.

http://dx.doi.org/10.1084/jem.191.4.603
PMid:10684852 PMCid:PMC2195837

83. Siegel M, Khosla C. *Transglutaminase 2 inhibitors and their therapeutic role in disease states.* Pharmacol Ther. 2007; 115(2): 232-45.

http://dx.doi.org/10.1016/j.pharmthera.2007.05.003
PMid:17582505 PMCid:PMC1975782

84. Xia J, Bergseng E, Fleckenstein B, Siegel M, Kim CY, Khosla C et al. *Cyclic and dimeric gluten peptide analogues inhibiting DQ2-mediated antigen presentation in celiac disease.* Bioorg Med Chem. 2007; 15(20): 6565-73.

http://dx.doi.org/10.1016/j.bmc.2007.07.001
PMid:17681795 PMCid:PMC2034199

85. Rauhavirta T, Oittinen M, Kivisto R, Mannisto PT, Garcia-Horsman JA, Wang Z et al. *Are transglutaminase 2 inhibitors able to reduce gliadin-induced toxicity related to celiac disease? A proof-of-concept study.* J Clin Immunol. 2013; 33(1): 134-42.

http://dx.doi.org/10.1007/s10875-012-9745-5
PMid:22878839

86. Dafik L, Albertelli M, Stamnaes J, Sollid LM, Khosla C. *Activation and inhibition of transglutaminase 2 in mice.* PLoS One. 2012; 7(2): e30642.

http://dx.doi.org/10.1371/journal.pone.0030642
PMid:22319575 PMCid:PMC3271093

87. Gianfrani C, Siciliano RA, Facchiano AM, Camarca A, Mazzeo MF, Costantini S et al. *Transamidation of wheat flour inhibits the response to gliadin of intestinal T cells in celiac disease.* Gastroenterology. 2007; 133(3): 780-9.

http://dx.doi.org/10.1053/j.gastro.2007.06.023
PMid:17678925

88. Kapoerchan VV, Wiesner M, Overhand M, van der Marel GA, Koning F, Overkleeft HS. *Design of azidoproline containing gluten peptides to suppress CD4+ T-cell responses associated with celiac disease.* Bioorg Med Chem. 2008; 16(4): 2053-62.

http://dx.doi.org/10.1016/j.bmc.2007.10.091
PMid:18037302

89. Meresse B, Malamut G, Cerf-Bensussan N. *Celiac disease: an immunological jigsaw.* Immunity. 2012; 36(6): 907-19.

http://dx.doi.org/10.1016/j.immuni.2012.06.006
PMid:22749351

90. Keshav S, Vanasek T, Niv Y, Petryka R, Howaldt S, Bafutto M et al. *A randomized controlled trial of the efficacy and safety of CCX282-B, an orally-administered blocker of chemokine receptor CCR9, for patients with Crohn's disease.* PLoS One. 2013; 8(3): e60094.

http://dx.doi.org/10.1371/journal.pone.0060094
PMid:23527300 PMCid:PMC3603920

91. Maiuri L, Ciacci C, Auricchio S, Brown V, Quaratino S, Londei M. *Interleukin 15 mediates epithelial changes in celiac disease.* Gastroenterology. 2000; 119(4): 996-1006.

http://dx.doi.org/10.1053/gast.2000.18149
PMid:11040186

92. Di Sabatino A, Ciccocioppo R, Cupelli F, Cinque B, Millimaggi D, Clarkson MM et al. *Epithelium derived interleukin 15 regulates intraepithelial lymphocyte Th1 cytokine production, cytotoxicity, and survival in coeliac disease.* Gut. 2006; 55(4): 469-77.

http://dx.doi.org/10.1136/gut.2005.068684
PMid:16105889 PMCid:PMC1856172

93. Yokoyama S, Watanabe N, Sato N, Perera PY, Filkoski L, Tanaka T et al. *Antibody-mediated blockade of IL-15 reverses the autoimmune intestinal damage in transgenic mice that overexpress IL-15 in enterocytes.* Proc Natl Acad Sci USA. 2009; 106(37): 15849-54.

http://dx.doi.org/10.1073/pnas.0908834106
PMid:19805228 PMCid:PMC2736142

94. Waldmann TA, Conlon KC, Stewart DM, Worthy TA, Janik JE, Fleisher TA et al. *Phase 1 trial of IL-15 trans presentation blockade using humanized Mikbeta1 mAb in patients with T-cell large granular lymphocytic leukemia.* Blood. 2013; 121(3): 476-84.

http://dx.doi.org/10.1182/blood-2012-08-450585
PMid:23212516 PMCid:PMC3548167

95. Waldmann TA. *The biology of IL-15: implications for cancer therapy and the treatment of autoimmune disorders.* J Investig Dermatol Symp Proc. 2013; 16(1): S28-30.

http://dx.doi.org/10.1038/jidsymp.2013.8
PMid:24326545

96. Yokoyama S, Perera PY, Waldmann TA, Hiroi T, Perera LP. *Tofacitinib, a janus kinase inhibitor demonstrates efficacy in an IL-15 transgenic mouse model that recapitulates pathologic manifestations of celiac disease.* J Clin Immunol. 2013; 33(3): 586-94.

http://dx.doi.org/10.1007/s10875-012-9849-y
PMid:23269601 PMCid:PMC3594487

97. Verhoef A, Alexander C, Kay AB, Larche M. *T cell epitope immunotherapy induces a CD4+ T cell population with regulatory activity.* PLoS Med. 2005; 2(3): e78.

http://dx.doi.org/10.1371/journal.pmed.0020078
PMid:15783262 PMCid:PMC1069669

98. Tye-Din JA, Stewart JA, Dromey JA, Beissbarth T, van Heel DA, Tatham A et al. *Comprehensive, quantitative mapping of T cell epitopes in gluten in celiac disease.* Sci Transl Med. 2010; 2(41): 41ra51.

http://dx.doi.org/10.1126/scitranslmed.3001012
PMid:20650871

99. Riedmann EM. *Human vaccines: news.* Hum Vaccin Immunother. 2012; 8(11): 1550-3.

http://dx.doi.org/10.4161/hv.22753
PMid:23151443 PMCid:PMC3601129

100. Croese J, Gaze ST, Loukas A. *Changed gluten immunity in celiac disease by Necator americanus provides new insights into autoimmunity.* Int J Parasitol. 2013; 43(3-4): 275-82.

http://dx.doi.org/10.1016/j.ijpara.2012.12.005
PMid:23291460

101. Daveson AJ, Jones DM, Gaze S, McSorley H, Clouston A, Pascoe A et al. *Effect of hookworm infection on wheat challenge in celiac disease-a randomised double-blinded placebo controlled trial.* PLoS One. 2011; 6(3): e17366.

http://dx.doi.org/10.1371/journal.pone.0017366
PMid:21408161 PMCid:PMC3050888

102. McSorley HJ, Gaze S, Daveson J, Jones D, Anderson RP, Clouston A et al. *Suppression of inflammatory immune responses in celiac disease by experimental hookworm infection.* PLoS One. 2011; 6(9): e24092.

http://dx.doi.org/10.1371/journal.pone.0024092
PMid:21949691 PMCid:PMC3174943

103. Anderson RP, van Heel DA, Tye-Din JA, Jewell DP, Hill AV. *Antagonists and non-toxic variants of the dominant wheat gliadin T cell epitope in coeliac disease.* Gut. 2006; 55(4): 485-91.

http://dx.doi.org/10.1136/gut.2005.064550
PMid:16299041 PMCid:PMC1856168

104. Croese J, Giacomin P, Navarro S, Clouston A, McCann L, Dougall A et al. *Experimental hookworm infection and gluten microchallenge promote tolerance in celiac disease.* J Allergy Clin Immunol. 2014.

PMid:25248819

105. Tennyson CA, Lewis SK, Green PH. *New and developing therapies for celiac disease.* Therap Adv Gastroenterol. 2009; 2(5): 303-9.

http://dx.doi.org/10.1177/1756283X09342759
PMid:21180558 PMCid:PMC3002532

106. Motta JP, Bermudez-Humaran LG, Deraison C, Martin L, Rolland C, Rousset P et al. *Food-grade bacteria expressing elafin protect against inflammation and restore colon homeostasis.* Sci Transl Med. 2012; 4(158): 158ra44.

http://dx.doi.org/10.1126/scitranslmed.3004212
PMid:23115353

107. Galipeau HJ, Wiepjes M, Motta JP, Schulz JD, Jury J, Natividad JM et al. *Novel Role of the Serine Protease Inhibitor Elafin in Gluten-Related Disorders.* Am J Gastroenterol. 2014.

http://dx.doi.org/10.1038/ajg.2014.48
PMid:24710505

SECTION II: Diagnosis, Serology, Pathology and Clinical entities and syndromes

Preface Section II

Fernando Fernández-Bañares, MD, PhD

Chapters 7 to 14

7. New Tools for the Diagnosis of Celiac Disease. Fernando Fernández Bañares, Carme Farré, Anna Carrasco, Meritxell Mariné, Maria Esteve.

8. Intestinal Biopsy in the Diagnosis of Celiac Disease: Is it still the Gold Standards? Juan P. Palazzo.

9. Clinical Manifestations of Celiac Disease and Diagnostic Criteria: Differences among Children, Adolescents and Adults. Maria Luisa Mearin, Miguel Montoro Huguet, Isabel Polanco, Carmen Ribes Köninckx, Santos Santolaria.

10. Extraintestinal Manifestations of Celiac Disease and Associated Disorders. Alfredo J. Lucendo, Luis Rodrigo, A. Salvador Peña.

11. Follow-up of Celiac Disease Patient: Is Mucosal Recovery a Goal Therapy? Santiago Vivas, Laura Arias, Luis Vaquero.

12. Quality of Life and Psychological Distress in the Patient with Celiac Disease. Claudia Herrera-de Guise, Francesc Casellas.

Go to TOC

13. Non-Celiac Gluten Sensitivity. Javier Molina-Infante, Santos Santolaria, Fernando Fernández Bañares.

14. Wheat as an allergen: Baker's asthma, food and wheat polen allergy. Alicia Armentia, Eduardo Arranz, José A. Garrote, Javier Santos.

The scope of **Section II** is to provide a comprehensive review about both diagnostic and clinical aspects of celiac disease, with particular emphasis on new aspects have appeared in recent years.

Chapter 7 describes new tools for diagnosing celiac disease, which may be of help in at least three frequent clinical situations: 1) HLA-DQ2/8+ individuals on a self-prescribed gluten-free diet; 2) Patients with seronegative villous atrophy; and 3) HLA-DQ2/8+ patients with lymphocytic enteritis and either positive (often with low/borderline titers increasing the risk of false positives) or negative celiac serology. In this sense, the role of γδ+ IEL count, the detection of subepithelial tissue transglutaminase antibodies, the whole blood cytokine release assays (ELISPOT), and the tetramer test are discussed.

Chapter 8 reviews how important is and what role the biopsy of the small bowel plays in the diagnosis of celiac disease. The histological differential diagnosis of the abnormal small bowel and the work-up of suspected refractory sprue are reviewed.

Chapter 9 discusses the clinical manifestations of celiac disease with specific emphasis in the differences between child and adult celiac disease. The present diagnostic criteria of celiac disease in children and adult are discussed. Likewise, present rules for clinical practice of how

to diagnose celiac disease are provided. The diagnosis of celiac disease in special situations is also discussed.

Chapter 10 extensively reviews the extraintestinal manifestations and associated disorders of celiac disease. First, extraintestinal manifestations such as oral manifestations, hematological disorders, and osteoporosis. Second, gluten-related associated diseases with genetic links, such as dermatitis herpetiformis and gluten ataxia. Finally, associated diseases such as type-1 diabetes mellitus, thyroid diseases, and malignancy.

Chapter 11 deals with the follow-up of the celiac disease patient and discusses on the basis of present acknowledge if mucosal recovery is a goal of therapy. The importance of a strict gluten-free diet compliance and how monitoring the diet adherence are reviewed.

Chapter 12 reviews health-related quality of life measurements in celiac disease and their usefulness for healthcare providers and patients. The effect of gluten-free diet on quality of life is also evaluated.

Chapter 13 deals with non-celiac gluten sensitivity. This chapter updates evidence on epidemiology, pathophysiology, diagnosis and dietary interventions in NCGS, stressing the need of thorough screening for celiac disease before a diagnosis of NCGS is given, considering that natural history and dietary restriction for both entities are radically different.

Chapter 14 reviews the allergenic power among wheat proteins and the relationship between cereals in diet and allergic digestive symptoms. Also the changes in allergenic properties of wheat induced by heat and industrial processing and the allergenic cross-reactivity between cereals, pollens and other vegetal foods are discussed.

This Associate Editor would like to thank all contributors for their excellent and critical revision, and also would like to thank Dr. Luis Rodrigo and Amado Salvador Peña, the Book Editors, for their invitation to edit this book section.

Chapter 7

New Tools for the Diagnosis of Celiac Disease

Fernando Fernández-Bañares[1], Carme Farré[2], Anna Carrasco[1], Meritxell Mariné[1], Maria Esteve[1]

[1] Department of Gastroenterology, Hospital Universitari Mútua Terrassa, University of Barcelona, CIBERehd, Terrassa, Barcelona, Spain.

[1] Department of Biochemistry, Hospital Universitari Sant Joan de Déu, University of Barcelona, Esplugues de Llobregat, Barcelona, Spain.

ffbanares@mutuaterrassa.es, farre@hsjdbcn.org, acarrasco@mutuaterrassa.es, mmarine@mutuaterrassa.cat, mariaesteve@mutuaterrassa.es

Doi: http://dx.doi.org/10.3926/oms.255

How to cite this chapter

Fernández-Bañares F, Farré C, Carrasco A, Mariné M, Esteve M. *New Tools for the Diagnosis of Celiac Disease.* In Arranz E, Fernández-Bañares F, Rosell CM, Rodrigo L, Peña AS, editors. *Advances in the Understanding of Gluten Related Pathology and the Evolution of Gluten-Free Foods.* Barcelona, Spain: OmniaScience; 2015. p. 259-276.

Abstract

New tools for celiac disease (CD) diagnosis may be of help in at least three frequent clinical situations: 1) HLA-DQ2/8+ individuals on a self-prescribed gluten-free diet; 2) Patients with seronegative villous atrophy; and 3) HLA-DQ2/8+ patients with lymphocytic enteritis and either positive (often with low/borderline titers increasing the risk of false positives) or negative celiac serology.

The $\gamma\delta$+ IEL count, assessed by either immunohistochemistry or flow cytometry, may help to identify CD patients when serology and clinical data are not conclusive, or when the histological diagnosis remains equivocal. The detection of subepithelial tissue transglutaminase antibodies seems to be very sensitive and specific in diagnosing CD in patients with potential CD or seronegative villous atrophy. The presence of these autoantibodies reinforces the CD diagnosis in borderline cases. EmA or anti-tTG2 assay of the culture medium of intestinal biopsy specimens in patients with negative serology, but with symptoms suggestive of CD and the HLA-DQ2 and/or HLA-DQ8+, seems to be a good option to help confirm the diagnosis of CD. It also may be useful in suspected cases showing conflicting laboratory and histological data. The whole blood cytokine release assays (ELISPOT) seems to be both sensitive and specific for detection of gluten-reactive T cells in CD; further clinical studies addressing the utility of these tests in patients with an uncertain diagnosis of CD is warranted. The tetramer test may be of help to confirm the diagnosis of CD after a short 3-days gluten challenge. However, the results seem comparable to the ELISPOT test; for that

reason, and also taking into account that the tetramer test is technically difficult, widespread use of the test is almost not expected.

Keywords

Celiac disease, 'celiac-lite' disease, potential celiac disease, $\gamma\delta$+ cells, subepithelial tissue transglutaminase antibodies, tTG2 in culture of intestinal biopsy, ELISPOT test, tetramer test.

1. Introduction

Celiac disease (CD) is an enteropathy caused by an immune reaction triggered by dietary gluten, a protein found in wheat, rye, barley, and some varieties of oats, which manifests in genetically predisposed individuals. Since the first morphological lesion description by John Paulley in 1954, CD diagnosis was based precisely on the demonstration of the characteristic, gluten-dependent small intestinal lesion. This basic general concept is still valid. However, in recent decades, the discovery of accurate diagnostic methods (serological and genetic), through mass screening techniques or evaluating at-risk groups, has allowed the identification of large numbers of patients with silent or paucisymptomatic forms. This has afforded the knowledge that CD is not a rare disease, that its spectrum of clinical manifestations, both in type and severity, is very wide, and that there is not always a correlation between the severity of the histological lesion and intensity of the clinical manifestations. In this regard, an important change in CD diagnostic criteria has been the gradual acceptance that histological mild enteropathy forms (type 1 Marsh lesions, also called lymphocytic enteritis, lymphocytic enteropathy or lymphocytic duodenosis) are also part of the CD spectrum and must to be treated as such, when they produce clinically relevant symptoms or signs[1].

Tissue transglutaminase IgA class autoantibodies (anti-tTG2) are the serological markers of choice for the detection of CD as recommended by the ESPGHAN. The anti-tTG2 are equivalent to the classic endomysial IgA autoantibodies (EmA). After the identification of transglutaminase as the autoantigen by itself, anti-tTG2 are determined by a quantitative and automated immunoassay, overcoming the technical drawbacks of indirect immunofluorescence used to determine EmA. This remains a manual, subjective and qualitative technique. The recommendations on how, when and to whom perform serum anti-tTG2 have been recently reviewed[2].

It is well known that celiac serology may be negative in the milder forms of CD[2]. In this context, gluten challenge has been performed in order to

determine if it worsens the histological lesion or if antibodies become positive, which would lead to CD diagnosis[3,4]. Though, this requires repeated endoscopies, before and after gluten challenge, that together with symptom relapse are often intolerable for patients, precluding achieving a definite diagnosis.

In addition, the overlap between patients with non-celiac gluten sensitivity and celiac disease patients with type I Marsh lesion becomes evident and differential diagnosis quite difficult often clinicians are confronted with the challenge of patients who choose to live without gluten, even without a proper diagnosis of CD. This is particularly so as both the serology and small intestine histology normalize in CD patients on a gluten-free diet. In those circumstances, HLA genotyping is of value, since CD is extremely improbable in those patients who are HLA-DQ2/8 negative, but it is not enough in HLA-DQ2/8 positive patients, since 30-40% of the healthy population are also positive.

Thus, new tools for CD diagnosis may be of help in at least three frequent clinical situations: 1) HLA-DQ2/8+ individuals on a self-prescribed gluten-free diet; 2) Patients with seronegative villous atrophy; and 3) HLA-DQ2/8+ patients with lymphocytic enteritis and either positive (often with low/borderline titers increasing the risk of false positives) or negative celiac serology. Also it would be interesting for monitoring gluten reactivity in latent or potential CD, as well as in first-degree relatives with the highest risk of developing the disease.

2. When Does Celiac Serology Fail in the Diagnosis of Celiac Disease?

It is well known that celiac serology is often negative in the milder forms of CD: in 30% of the patients with partial villous atrophy and up to 80% of those with Marsh 1 lesions[5]. Since histological damage is worse at clinical presentation in children than in adults[6], seronegative CD is more frequent in adult patients.

Prospective studies have shown that the diagnostic accuracy of serology is not as high as described, since there is around 10-20% seronegative CD patients[7,8]. We agree with the opinion of Catassi and Fasano who affirmed that 'Seronegative CD is likely to be underestimated due to the tendency to perform small-intestinal biopsy only in patients with positive-CD serum markers (so-called self-fulfilling prophecy)[9].

Although there are other etiologies of villous atrophy, it is important to take in mind that the most frequent etiology of villous atrophy in a patient with negative CD serology is CD[10]. Finally, we should not misinterpret as negative the IgA serology results obtained in patients with IgA deficiency, in children under two years of age, in patients on immunosuppressive treatment, or in patients on a gluten-poor or gluten-free diet since a few weeks without gluten can give a negative serological result.

False positive anti-tTG results have been described in adult patients with autoimmune diseases[11], acute coronary disease[12], primary biliary cirrhosis[13], psoriasis[14], chronic inflamed ileal pouches[15], and children with common infections[16]. Low titers or borderline values are more often associated to false positive results.

3. Usefulness of Intraepithelial $\gamma\delta$+ Determination

The TCR$\gamma\delta$+ intraepithelial lymphocyte (IEL) determination is considered useful in doubtful or difficult CD cases[17]. In CD patients these $\gamma\delta$+ T cells are increased in all stages of the disease, both in untreated CD and under the gluten-free diet[17]. It has also been observed that they are increased both in potential and latent CD[18,19]. The $\gamma\delta$ IEL increase is not totally specific to CD, since it has occasionally been found in other conditions such as cow's milk intolerance, food allergy, cryptosporidiosis, giardiasis, Sjögren syndrome, and IgA deficiency[17]. However, the increase in $\gamma\delta$ IEL in a minority of patients with these conditions tends to be mild and transient[17]. It has been stated that CD is the only disease in which $\gamma\delta$ IEL, are increased systematically, permanently, and intensely[17,20-22].

Assessment of the density of $\gamma\delta$ IEL is in general performed with immunohistochemistry techniques. Noteworthy, Järvinen et al. reported that $\gamma\delta$+ T cells had a positive predictive value of 95% and a negative predictive value of 85%, in the detection of CD[23]. An increase in this type of cells has also been detected in most patients with CD mild enteropathy[24]. Identification and count of $\gamma\delta$+ T cells are usually performed on cryosectioned snap-frozen biopsy, which have limited its use to the research setting and has rarely been adopted for routine clinical practice. Recently, a new anti-TCRγ antibody, suitable on formalin-fixed paraffin-embedded samples, has been described, and its feasibility to count $\gamma\delta$+ T cells together with CD3 cells in patients with lymphocytic enteritis has been demonstrated[25].

Lymphogram on IEL isolated by flow cytometry has been proposed, as an initial screening for CD. Using this technique, an IEL pattern typical of CD (CD IEL cytometric pattern) was defined, consisting of both an increase in $\gamma\delta$+ IEL and a decrease in CD3- IEL (reviewed by Leon F)[17]. The concomitant decrease in CD3- IEL provides increased specificity for the diagnosis of CD[26]. A description of this CD3- IEL population has been made, showing a CD3- CD7+ CD103+ CD45+ phenotype[18,26,27].

Flow cytometry is a powerful analytical tool for the study of IEL, compared to immunohistochemistry. It allows the analysis of a greater number of cells and yields a computerized record of the results. It gives fast, sensitive, reproducible and objective semi-quantitative results. Since an increase of CD3+TCR$\gamma\delta$+ and a decrease in CD3- IEL has been previously described as a characteristic flow cytometric pattern of CD with atrophy[17,18,28], a recent study[29] assessed the usefulness of this technique for diagnosing lymphocytic enteritis due to CD. In this study, 205 patients who underwent duodenal biopsy for clinical suspicion of CD and positive HLA-DQ2 and/or HLA-DQ8 were evaluated. Fifty patients had villous atrophy, 70 patients lymphocytic enteritis, and 85 had normal histology. Duodenal biopsies were obtained to assess two typical flow cytometric patterns: complete CD flow cytometric pattern was defined when TCR$\gamma\delta$+ was increased and CD3- decreased, and incomplete CD flow cytometric pattern was defined when an isolated TCR$\gamma\delta$+

increase was detected. Anti-TG2 IgA subepithelial deposits were also assessed. Sensitivity of anti-TG2 intestinal deposits, and complete and incomplete cytometric patterns for CD diagnosis in patients with positive serology (Marsh 1+3) was 92%, 85% and 97% respectively, but only the complete cytometric pattern had 100% specificity. Taking into account these definitions and the response to a gluten-free diet, we studied HLA-DQ2/8+ patients with lymphocytic enteritis and negative serology to either confirm or ruled out CD. CD cytometric pattern showed a better diagnostic performance than anti-TG2 intestinal deposits to detect CD in the initial diagnostic biopsy of these patients. This methodology allowed to establish the diagnosis of CD in more than twice the number of patients with lymphocytic enteritis diagnosed on the basis of serological results alone.

In conclusion, the $\gamma\delta$+ IEL count, assessed by either immunohistochemistry or flow cytometry, may help to identify CD patients when serology and clinical data are not conclusive, or when the histological diagnosis remains equivocal.

4. Diagnostic Utility of Tissue IgA Transglutaminase Subepithelial Deposits

It has been shown that the production of CD autoantibodies, takes place locally in the small intestinal mucosa, and subsequently circulate into to the bloodstream. However, besides being detectable in the bloodstream, these autoantibodies remain sequestered in the place where they have been produced. In untreated CD it is possible to detect IgA tTG deposits in the intestinal mucosa subepithelially and around blood vessels of the lamina propria[30]. Interestingly, these deposits can be detected in patients with positive EmA and without villous atrophy[24, 30-32] and even in patients with negative serology and Marsh type 1 to 3 lesions[33-35]. In a recent study on untreated CD patients, it was demonstrated that 100% of 261 patients with villous atrophy had subepithelial IgA tTG deposits (9% had negative serum EmA), 90% had moderate to strong intensity. In contrast, 18% of the controls

had deposits of minor intensity. After a gluten-free diet, there was a gradual decrease in the intensity of these deposits, which remained positive, in the long term, in 56% of the patients. The sensitivity and specificity of these deposits for CD diagnosis was of 100% and 82%; however, serology sensitivity and specificity were of 91% and 100%, respectively[35]. In a study on children with positive EmA or tTG and positive genetics (HLA-DQ2 or DQ8) but without villous atrophy, IgA tTG deposits were detected in 85% of 39 patients. Similarly, a study on another group of children revealed negative serology and Marsh type I lesions, with increased TCR$\gamma\delta$+ intraepithelial lymphocytes, allowing the detection of IgA tTG deposits in 66% of 18 patients. These deposits were detected in 9% of 34 children with normal intestinal mucosa and absence of gluten sensitivity markers[34]. Another recent study showed that IgA tTG deposits were detected in 12 of 20 (60%) adult patients with Marsh type I lesions diagnosed with CD on the basis of the "4 of 5" rule by Catassi and Fasano[9]; four of these 12 positive patients were seronegative[29].

In conclusion, the detection of subepithelial tissue transglutaminase antibodies seems to be very sensitive and specific in diagnosing CD in patients with potential CD or seronegative villous atrophy. The presence of these autoantibodies reinforces the CD diagnosis in borderline cases.

5. Anti-tTG2 and EmA Assays in the Culture Medium of Biopsy Samples

The assay of the culture medium of intestinal biopsy specimens for EmA or anti-tTG2 antibodies can help to identify as CD either the infiltrative/hyperplastic (Marsh 1-2) or the partial villous atrophy (Marsh 3a) lesions often associated with negative serology[8,36,37]. In a study[37], EmA and anti-tTG assayed in the culture medium had 98% sensitivity, 100% specificity, and 98% diagnostic accuracy. These assays were positive in 24 out of 29 seronegative CD patients (77% with partial villous atrophy, and 23% with lymphocytic enteritis). In another study by the same group[8], EmA assay in

the culture medium had a higher sensitivity (98 vs. 80%) and specificity (99 vs. 95%) than serum EmA and/or anti-tTG assay. In that study, 32 adults and 39 children had a seronegative CD (17% of 418 CD patients).

In addition, combined serum and supernatants of cultured intestinal duodenal biopsy anti-tTG assessment increased CD serological sensitivity from 19% to 30% in Marsh I patients carrying the risk haplotypes HLA-DQ2 and/or HLA-DQ8[38]. It was concluded that supernatants of duodenal biopsies anti-tTG detection improves serological determination sensitivity in Marsh I patients, providing diagnostic value and therapeutic impact.

The diagnostic yield of the anti-tTG2 assay of the culture medium of biopsy seems to be similar, or perhaps better, that the diagnostic accuracy of IgA tTG subepithelial deposits. However, a recent study comparing the two techniques suggests that the measurement of antibodies secreted into culture supernatant is the best method for detecting intestinal anti-tTG2 antibodies[39].

In conclusion, EmA or anti-tTG2 assay of the culture medium of intestinal biopsy specimens in patients with negative serology, but with symptoms suggestive of CD and the HLA-DQ2 and/or HLA-DQ8+, seems to be a good option to help confirm the diagnosis of CD. It also may be useful in suspected cases showing conflicting laboratory and histological data.

6. IFN-γ ELISPOT

The histological features of the small intestine of celiac disease probably result from an increased Th1-deviated immune response. Gluten appears to induce a non-proliferative activation of CD4+ lamina propria T-cells, especially activated Th1-like cells secreting IFN-gamma[40]. However, one year after the introduction of a gluten-free diet, the transcription of IFN-gamma is downregulated[41].

Enzyme Linked Immuno-spot (ELISPOT) is a technique by which immune markers, e.g., cytokine and chemokine secretion, can be detected at the single-cell level, since secreted cytokines are captured and accumulated in the ELISPOT plate[42].

In children with untreated CD, the number of IFN-gamma-producing cells, detected by ELISPOT, is shown to be increased and actually, after gluten challenge, the numbers of IFN-gamma-producing cells still remain high[43].

It has also been shown that an *in vivo* gluten challenge is a simple and safe method that allows gliadin-specific T-cells to be analyzed and quantified in peripheral blood by ELISPOT[44]. This technique could differentiate patients with CD from other patients who have adopted a gluten-free diet. No T cell assay could distinguish between CD patients and controls prior to gluten challenge, but after gluten challenge the IFN-γ ELISPOT was 85% sensitive and 100% specific for CD patients[45].

As an added benefit over current diagnostic tests being performed on patients already following a gluten-free diet, the mobilization of gluten-reactive T cells specific for CD into the bloodstream requires oral gluten challenge for only 3 days, instead of the weeks or months required for diagnosis based on abnormal small bowel histology. Oral gluten challenge consists of four slices (4 x 50 g) of white bread daily for three days[44]. Blood for cytokine release assays is drawn immediately before and on day 6 after starting with the gluten challenge, or prior to begin a gluten-free diet in untreated CD patients.

In conclusion, the whole blood cytokine release assays seems to be both sensitive and specific for detection of gluten-reactive T cells in CD; further clinical studies addressing the utility of these tests in patients with an uncertain diagnosis of CD is warranted.

7. HLA-DQ2-Gliadin Tetramer Assay

Brottveit et al. recently assessed the potential of a fluorescence-activated cell sorter (FACS)-based assay utilizing MHC class II-peptide tetramers detecting DQ2 · 5-glia-α1a and DQ2 · 5-glia-α2 epitope-specific T cells in blood, after 3-days gluten challenge, for the diagnosis of CD in patients following a gluten-free diet[46]. This tetramer assay was 85% sensitive and 100%

specific for HLA-DQ2.5+ CD[46]. Recently, these findings using MHC tetramers have also been replicated in CD patients from the United States[47].

This test, as the ELISPOT assay, may be a superior method to diagnose CD in individuals currently on a gluten-free diet. Available tests, including antibody levels and intestinal biopsy results, can be completely normal in CD patients on a gluten-free diet. These individuals are often asked to reintroduce gluten-containing foods for 2-4 weeks prior re-testing for an accurate diagnosis. This clinical practice may be intolerable in some patients precluding the definite diagnosis. In contrast, a short-term gluten exposure is, in general, well tolerated.

In conclusion, the tetramer test may be of help to confirm the diagnosis of CD after a short 3-days gluten challenge. However, the results seem comparable to the ELISPOT test; for that reason, and also taking into account that the tetramer test is technically difficult, quite laborious and the tetramer reagents have limited stability, widespread use of the test is almost not expected.

References

1. Fernández Bañares F, Mariné M, Rosinach M, Carrasco A, Esteve M. *Type 1 Marsh Celiac Disease: Diagnosis and Response.* In Rodrigo L and Pena AS, editors. Celiac Disease and Non-Celiac Gluten Sensitivity. Barcelona, Spain: OmniaScience. 2014; 289-302.

   http://dx.doi.org/10.3926/oms.223

2. Farré C. *The Role of Serology in Celiac Disease Screening, Diagnosis and Follow-up.* In Rodrigo L and Pena AS, editors. Celiac Disease and Non-Celiac Gluten Sensitivity. Barcelona, Spain: OmniaScience. 2014; 151-169.

   http://dx.doi.org/10.3926/oms.234

3. Aziz I, Evans KE, Hopper AD, Smillie DM, Sanders DS. *A prospective study into the etiology of lymphocytic duodenosis.* Aliment Pharmacol Ther. 2010; 32: 1392-7.

   http://dx.doi.org/10.1111/j.1365-2036.2010.04477.x
   PMid:21050242

4. Wahab PJ, Meijer JWR, Goerres MS, Mulder CJJ. *Coeliac disease: Changing views on gluten-sensitive enteropathy.* Scand J Gastroenterol. 2002; 37 Suppl 236: 60-5.

   http://dx.doi.org/10.1080/003655202320621472

5. Rostami K, Kerckhaert J, Tiemessen R, von Blomberg BM, Meijer JW, Mulder CJ. *Sensitivity of antiendomysium and antigliadin antibodies in untreated celiac disease: disappointing in clinical practice.* Am J Gastroenterol. 1999; 94: 888-94.

   http://dx.doi.org/10.1111/j.1572-0241.1999.983_f.x
   PMid:10201452

6. Vivas S, Ruiz de Morales JM, Fernandez M, Hernando M, Herrero B, Casqueiro J et al. *Age-related clinical, serological, and histopathological features of celiac disease.* Am J Gastroenterol. 2008; 103: 2360-5.

   http://dx.doi.org/10.1111/j.1572-0241.2008.01977.x
   PMid:18702652

7. Hopper AD, Cross SS, Hurlstone DP, McAlindon ME, Lobo AJ, Hadjivassiliou M et al. *Pre-endoscopy serological testing for celiac disease: evaluation of a clinical decision tool.* BMJ. 2007; 334: 729-33.

   http://dx.doi.org/10.1136/bmj.39133.668681.BE
   PMid:17383983 PMCid:PMC1847864

8. Carroccio A, Iacono G, Di Prima L, Pirrone G, Cavataio F, Ambrosiano G et al. *Antiendomysium antibodies assay in the culture medium of intestinal mucosa: an accurate method for celiac disease diagnosis.* Eur J Gastroenterol Hepatol. 2011; 23: 1018-23.

   http://dx.doi.org/10.1097/MEG.0b013e328349b8a5

9. Catassi C, Fasano A. *Celiac disease diagnosis: simple rules are better than complicated algorithms.* Am J Med. 2010; 123: 691-3.
http://dx.doi.org/10.1016/j.amjmed.2010.02.019
PMid:20670718

10. DeGaetani M, Tennyson CA, Lebwohl B, Lewis SK, Abu Daya H, Arguelles-Grande C et al. *Villous atrophy and negative celiac serology: a diagnostic and therapeutic dilemma.* Am J Gastroenterol. 2013; 108: 647-53.
http://dx.doi.org/10.1038/ajg.2013.45
PMid:23644957

11. Sárdy M, Csikós M, Geisen C, Preisz K, Kornseé Z, Tomsits E et al. *Tissue transglutaminase ELISA positivity in autoimmune disease independent of gluten-sensitive disease.* Clin Chim Acta. 2007; 376: 126-35.
http://dx.doi.org/10.1016/j.cca.2006.08.006
PMid:16987503

12. Di Tola M, Barillà F, Trappolini M, Palumbo HF, Gaudio C, Picarelli A. *Antitissue transglutaminase antibodies in acute coronary syndrome: an alert signal of myocardial tissue lesion?* J Intern Med. 2008; 263: 43-51.
http://dx.doi.org/10.1111/j.1365-2796.2007.01881.x
PMid:18088251

13. Bizzaro N, Tampoia M, Villalta D, Platzgummer S, Liguori M, Tozzoli R et al. *Low specificity of anti-tissue transglutaminase antibodies in patients with primary biliary cirrhosis.* J Clin Lab Anal. 2006; 20: 184-9.
http://dx.doi.org/10.1002/jcla.20130
PMid:16960894

14. Damasiewicz-Bodzek A, Wielkoszyński T. *Serologic markers of celiac disease in psoriatic patients.* J Eur Acad Dermatol Venereol. 2008; 22: 1055-61.
http://dx.doi.org/10.1111/j.1468-3083.2008.02713.x
PMid:18384553

15. Lian L, Remzi FH, Kiran RP, Fazio VW, Shen B. *Clinical implication of false-positive celiac serology in patients with ileal pouch.* Dis Colon Rectum. 2010; 53: 1446-51.
http://dx.doi.org/10.1007/DCR.0b013e3181eba46c
PMid:20847628

16. Ferrara F, Quaglia S, Caputo I, Esposito C, Lepretti M, Pastore S et al. *Anti-transglutaminase antibodies in non-coeliac children suffering from infectious diseases.* Clin Exp Immunol. 2010; 159: 217-23.
http://dx.doi.org/10.1111/j.1365-2249.2009.04054.x
PMid:19912255 PMCid:PMC2810390

17. Leon F. *Flow cytometry of intestinal intraepithelial lymphocytes in celiac disease.* J Immunol Meth. 2011; 363: 177-86.
http://dx.doi.org/10.1016/j.jim.2010.09.002
PMid:20833175

18. Camarero C, Eiras P, Asensio A, Leon F, Olivares F, Escobar H et al. *Intraepithelial lymphocytes and celiac disease: permanent changes in CD3⁻/CD7⁻ and T cell receptor γδ subsets studied by flow cytometry.* Act Paediatr. 2000; 89: 285-90.

http://dx.doi.org/10.1080/080352500750028410

19. Arranz E, Ferguson A. *Intestinal antibody pattern of celiac disease: occurrence in patients with normal jejunal biopsy histology.* Gastroenterology. 1993; 104: 1263.

PMid:8482440

20. Kutlu T, Brousse N, Rambaud C, Le Deist F, Schmitz J et al. *Numbers of T cell receptor (TCR) alpha beta+ but not of TcR gamma delta+ intraepithelial lymphocytes correlate with the grade of villous atrophy in coeliac patients on a long term normal diet.* Gut. 1993; 34: 208-214.

http://dx.doi.org/10.1136/gut.34.2.208
PMid:8432475 PMCid:PMC1373972

21. Savilahti E, Arato A, Verkasalo M. *Intestinal gamma/delta receptor-bearing T lymphocytes in celiac disease and inflammatory bowel diseases in children. Constant increase in celiac disease.* Pediatr Res. 1990; 28: 579-581.

http://dx.doi.org/10.1203/00006450-199012000-00005
PMid:2149449

22. Halstensen TS, Scott H, Brandtzaeg P. *Intraepithelial T cells of the TcR gamma/delta+ CD8- and V delta 1/J delta 1+ phenotypes are increased in coeliac disease.* Scand J Immunol. 1989; 30: 665-672.

http://dx.doi.org/10.1111/j.1365-3083.1989.tb02474.x
PMid:2481336

23. Järvinen TT, Kaukinen K, Laurila K, Kyronpalo S, Rasmussen M, Maki M et al. *Intraepithelial lymphocytes in celiac disease.* Am J Gastroenterol. 2003; 98: 1332-7.

http://dx.doi.org/10.1111/j.1572-0241.2003.07456.x
PMid:12818278

24. Salmi TT, Collin P, Reunala T, Mäki M, Kaukinen K. *Diagnostic methods beyond conventional histology in celiac disease diagnosis.* Dig Liver Dis. 2010; 42: 28-32.

http://dx.doi.org/10.1016/j.dld.2009.04.004
PMid:19473894

25. Lonardi S, Villanacci V, Lorenzi L, Lanzini A, Lanzarotto F, Carabellese N et al. *Anti-TCR gamma antibody in celiac disease: the value of count on formalin-fixed paraffin-embedded biopsies.* Virchows Arch. 2013; 463: 409-13.

http://dx.doi.org/10.1007/s00428-013-1448-7
PMid:23860877

26. Eiras P, Roldán E, Camarero C, Olivares F, Bootello A et al. *Flow cytometry description of a novel CD3-/CD7+ intraepithelial lymphocyte subset in human duodenal biopsies: potential diagnostic value in coeliac disease.* Cytometry. 1998; 34: 95-102.

http://dx.doi.org/10.1002/(SICI)1097-0320(19980415)34:2<95::AID-CYTO6>3.0.CO;2-B

27. León F, Roldán E, Sanchez L, Camarero C, Bootello A, Roy G. Human small-intestinal epithelium contains functional natural killer lymphocytes. Gastroenterology. 2003; 125: 345-356.

http://dx.doi.org/10.1016/S0016-5085(03)00886-2

28. Calleja S, Vivas S, Santiuste M, Arias L, Hernando M, Nistal E et al. *Dynamics of non-conventional intraepithelial lymphocytes-NK, NKT, and γδ T-in celiac disease: relationship with age, diet, and histopathology.* Dig Dis Sci. 2011; 56: 2042-9.

http://dx.doi.org/10.1007/s10620-010-1534-5
PMid:21221796

29. Fernández-Bañares F, Carrasco A, García-Puig R, Rosinach M, González C, Alsina M et al. *Intestinal intraepithelial lymphocyte cytometric pattern is more accurate than subepithelial deposits of anti-tissue transglutaminase IgA for the diagnosis of celiac disease in lymphocytic enteritis.* Plos One. 2014; 9: e101249.

http://dx.doi.org/10.1371/journal.pone.0101249
PMid:25010214 PMCid:PMC4091865

30. Korponay-Szabó IR, Halttunen T, Szalai Z, Király R, Kovács JB, Fésüs L et al. *In vivo targeting of intestinal and extraintestinal transglutaminase 2 by celiac autoantobodies.* Gut. 2004; 53: 641-8.

http://dx.doi.org/10.1136/gut.2003.024836
PMid:15082580 PMCid:PMC1774023

31. Kurppa K, Ashorn M, Iltanen S, Koskinen LLE, Saavalainen P, Koskinen O et al. *Celiac disease without villous atrophy in children: A prospective study.* J Pediatr. 2010; 157: 373-80.

http://dx.doi.org/10.1016/j.jpeds.2010.02.070
PMid:20400102

32. Salmi TT, Collin P, Korponay-Szabó IR, Laurila K, Partanen J, Huhtala H et al. *Endomysial antibody-negative celiac disease: clinical characteristics and intestinal autoantibody deposits.* Gut. 2006; 55: 1746-53.

http://dx.doi.org/10.1136/gut.2005.071514
PMid:16571636 PMCid:PMC1856451

33. Salmi TT, Collin P, Järvinen O, Haimila K, Partanen J, Laurila K et al. *Immunoglubulin A autoantibodies against transglutaminase 2 in the small intestinal mucosa predict forthcoming celiac disease.* Aliment Pharmacol Ther 2006; 24: 541-52.

http://dx.doi.org/10.1111/j.1365-2036.2006.02997.x
PMid:16886921

34. Tosco A, Maglio M, Paparo F, Rapacciuolo L, Sannino A, Miele E et al. *Immunoglubulin A anti-tissue transglutaminase antibody deposits in the small intestinal mucosa of children with no villous atrophy.* J Pediatr Gastroenterol Nutr. 2008; 47: 293-8.

http://dx.doi.org/10.1097/MPG.0b013e3181677067
PMid:18728524

35. Koskinen O, Collin P, Lindfords K, Laurila K, Mäki M, Kaukinen K. *Usefulness of small bowel mucosal transglutaminase-2 specific autoantibody deposits in the diagnosis and follow-up of celiac disease.* J Clin Gastroenterol. 2010; 44: 483-8.

PMid:19779364

36. Carroccio A, Iacono G, D'Amico D, Cavataio F, Teresi S, Caruso C et al. *Production of anti-endomysial antibodies in cultured duodenal mucosa: usefulness in celiac disease diagnosis.* Scand J Gastroenterol. 2002; 37: 32-8.

http://dx.doi.org/10.1080/003655202753387329
PMid:11843032

37. Carroccio A, Di Prima L, Pirrone G, Scalici C, Florena AM, Gasparin M et al. *Anti-transglutaminase antibody assay of the culture medium of intestinal biopsy specimens can improve the accuracy of celiac diagnosis.* Clin Chem. 2006; 52: 1175-80.

http://dx.doi.org/10.1373/clinchem.2005.061366
PMid:16574764

38. Santaolalla R, Fernández-Bañares F, Rodríguez R, Alsina M, Rosinach M, Mariné M et al. *Diagnostic value of duodenal antitissue transglutaminase antibodies in gluten-sensitive enteropathy.* Aliment Pharmacol Ther. 2008; 27: 820-9.

http://dx.doi.org/10.1111/j.1365-2036.2008.03652.x
PMid:18284655

39. Tosco A, Aitoro R, Auricchio R, Ponticelli D, Miele E, Paparo F et al. *Intestinal anti-tissue transglutaminase antibodies in potential coeliac disease.* Clin Exp Immunol. 2013; 171: 69-75.

http://dx.doi.org/10.1111/j.1365-2249.2012.04673.x
PMid:23199325 PMCid:PMC3530097

40. Nilsen EM, Jahnsen FL, Lundin KEA, Johansen FE, Fausa O, Sollid LM et al. *Gluten induces an intestinal cytokine response strongly dominated by interferon-gamma in patients with celiac disease.* Gastroenterology. 1998; 115: 551-63.

http://dx.doi.org/10.1016/S0016-5085(98)70134-9

41. Lahdenperä A, Ludvigsson J, Fälth-Magnusson K, Högberg L, Vaarala O. *The effect of gluten-free diet on Th1-Th2-Th3-associated intestinal immune responses in celiac disease.* Scand J Gastroent. 2011; 46: 538-49.

http://dx.doi.org/10.3109/00365521.2011.551888
PMid:21288140

42. Faresjö M. *Enzyme Linked Immuno-Spot: a Useful Tool in the Search for Elusive Immune Markers in Common Pediatric Immunological Diseases.* Cells. 2012; 1: 141-52.

http://dx.doi.org/10.3390/cells1020141
PMid:24710420 PMCid:PMC3901087

43. Hansson T, Dannaeus A, Klareskog L. *Cytokine-producing cells in peripheral blood of children with coeliac disease secrete cytokines with a type 1 profile.* Clin Exp Immunol. 1999; 116: 246-50.

http://dx.doi.org/10.1046/j.1365-2249.1999.00882.x
PMid:10337014 PMCid:PMC1905277

44. Anderson RP, van Heel DA, Tye-Din JA, Barnardo M, Salio M, Jewell DP et al. *T cells in peripheral blood after gluten challenge in coeliac disease.* Gut. 2005; 54: 1217-23.

http://dx.doi.org/10.1136/gut.2004.059998
PMid:16099789 PMCid:PMC1774637

45. Ontiveros N, Tye-Din JA, Hardy MY, Anderson RP. *Ex-vivo whole blood secretion of interferon (IFN)-γ and IFN-γ-inducible protein-10 measured by enzyme-linked immunosorbent assay are as sensitive as IFN-γ enzyme-linked immunospot for the detection of gluten-reactive T cells in human leucocyte antigen (HLA)-DQ2.5+ -associated coeliac disease.* Clin Exp Immunol. 2013; 175: 305-15.

http://dx.doi.org/10.1111/cei.12232
PMid:24192268 PMCid:PMC3892421

46. Brottveit M, Raki M, Bergseng E, Fallang LE, Simonsen B, Løvik A et al. *Assessing possible celiac disease by an HLA-DQ2-gliadin tetramer test.* Am J Gastroenterol. 2011; 106: 1318–24.

http://dx.doi.org/10.1038/ajg.2011.23
PMid:21364548

47. Han A, Newell EW, Glanville J, Fernandez-Becker N, Khosla C, Chien YH et al. *Dietary gluten triggers concomitant activation of CD4+ and CD8+ alphabeta T cells and gammadelta T cells in celiac disease.* Proc Natl Acad Sci USA. 2013; 110: 13073-8.

http://dx.doi.org/10.1073/pnas.1311861110
PMid:23878218 PMCid:PMC3740842

Chapter 8

Intestinal Biopsy in the Diagnosis of Celiac Disease: Is it Still the Gold Standard?

Juan P. Palazzo

Department of Pathology and Laboratory Medicine.

Sidney Kimmel Medical College at Thomas Jefferson University. Philadelphia, United States.

Juan.Palazzo@jefferson.edu

Doi: http://dx.doi.org/10.3926/oms.256

How to cite this chapter

Palazzo JP. *Intestinal Biopsy in the Diagnosis of Celiac Disease: Is it Still the Gold Standard?* In Arranz E, Fernández-Bañares F, Rosell CM, Rodrigo L, Peña AS, editors. *Advances in the Understanding of Gluten Related Pathology and the Evolution of Gluten-Free Foods*. Barcelona, Spain: OmniaScience; 2015. p. 277-290.

Abstract

Pathology plays a crucial role in the diagnosis of celiac disease (CD). The pathologist's role is to confirm the diagnosis of CD; to exclude other diseases that share morphologic features with CD and to diagnose complications in patients with CD. Therefore, the significance of the small bowel biopsy includes confirming the diagnosis but also reassuring the clinician that other etiologies are excluded. Some of these diseases share many similarities with CD, such as villous atrophy and intraepithelial lymphocytosis, and the small bowel biopsy helps with this distinction.

The use of standarized pathology reporting including the appropriate classification system is highly recommended in order to facilitate the interpretation of the pathology report and the communication between pathologists and clinicians. Despite the need for a small bowel biopsy in the initial work up of CD, some patients and particularly children, may be spared a small bowel biopsy if certain clinical and laboratory findings are present in order to confirm the diagnosis without a biopsy. It is important to emphasize that the pathologic findings need to be correlated with the clinical, endoscopic and serologic findings in all the patients suspected of CD.

Keywords

Celiac disease, pathology, differential diagnosis, villous atrophy.

1. Introduction

Celiac disease is known to affect people of all ages with an increasing recognition in older individuals as well as children. The increased awareness has led to more individuals being suspected of having celiac disease (CD) and as such pathologists have encountered in their practice an increasing number of small bowel biopsies. The diagnosis of CD includes clinical, laboratory, endoscopic and pathologic features[1-5]. The question that has emerged in recent years is how important and what role the biopsy of the small bowel plays in the diagnosis of CD[2,6]. Should all the patients suspected of having CD be biopsied? Also, the endoscopic procedure to biopsy the small bowel is not exempt of risk, can be expensive and time consuming.

The role of the pathologist in the study of patients with celiac disease is three fold. If the biopsy is done initially to confirm the diagnosis, the pathologist will be able to identify the changes seen in CD such as villous blunting, intraepithelial lymphocytosis (IELs) and crypt hyperplasia[4,7,8]. If the biopsy is normal, the possibility of CD cannot be excluded. In order to increase the possibility of finding abnormal features multiple small bowel biopsies are recommended including from the duodenal bulb. When the patient carries the diagnosis of CD and is rebiobsied, the pathologist can evaluate the response to therapy and render a report regarding the changes seen in the small bowel compared to the initial biopsy. The third situation is when the patient has either an atypical presentation or a suspected complication of CD[4,9]. In these cases, the pathologist plays a key role in confirming the diagnosis of CD, excluding other diseases that may show similar changes to CD, or diagnosing a complication of CD such as lymphoma, adenocarcinoma or collagenous sprue.

According to recent guidelines published by the European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPHGAN) children and adolescents can be spared a small bowel biopsy as long as the classic symptoms of celiac disease are present and the antibody titers are high (TTG-IgA levels >10 times ULN) and positive HLA-DQ2 or DQ8 subtyping[6].

These patients are thought to have enough evidence to support the diagnosis of CD, without histologic confirmation, so that they can be treated without biopsy confirmation[6]. Additional data with follow ups and comparison with patients who have small bowel biopsies in the workup are necessary to confirm the current recommendations advanced by ESPHGAN.

In the adult population suspected of having CD, most authors agree that small bowel biopsies should be an integral part of the work up of all patients[2,5]. Even in patients with negative serology but clinical findings suspicious of CD, a biopsy is frequently recommended[2]. A problem that could potentially arise if no small bowel biopsy is done in the initial work up of CD is that follow-up biopsies performed for lack of improvement, doubts about the diagnosis or a complication may not be easy to interpret to confirm or exclude the diagnosis of CD. The lack of improvement of the pathologic features of the small bowel biopsy has been associated with progression to refractory sprue[1]. Therefore, the lack of a baseline biopsy from the small bowel can potentially hampered the interpretation after the patient has been on a diet and treatment.

In CD, an early microscopic finding may include only IELs with or without evidence of villous blunting[7,8]. Both of these changes are non-specific and other conditions may show these features, only villous blunting or IELs. This is one of the main reasons proponents of performing a small bowel biopsy in all patients suspected of CD is justified in order to confirm the diagnosis.

The classic findings in small bowel biopsies in CD include: villous blunting that can range from minimal to severe flat mucosa, IELs and crypt hyperplasia (Figure 1). The villous: crypt ratio is variable and ranges from 1:1 to 3:1. In addition to these changes, there is an increased number of intraepithelial lymphocytes of over 25 lymphocytes per 100 enterocytes. The typical distribution in celiac disease is for the lymphocytes to be seen along the entire length of the villi. The presence of increased lymphocytes at the tip is more common in CD than in other conditions but it is not a specific finding[7,8]. The use of immunohistochemistry in the evaluation of intraepithelial lymphocytes is not recommended for routine use, however, there are

pathology laboratories that used the markers in all small bowel biopsies. These markers of T lymphocytes, CD3 and CD8, can be useful when there is doubts as to whether the intraepithelial lymphocytes are increased and in cases suspected of refractory sprue (RS). When they are used, the immuhistochemical stains should be interpreted with caution in order not to diagnose IELs and then consider that the patient may have CD. The number of IEL's should be increased to 30 per 100 enterocytes.

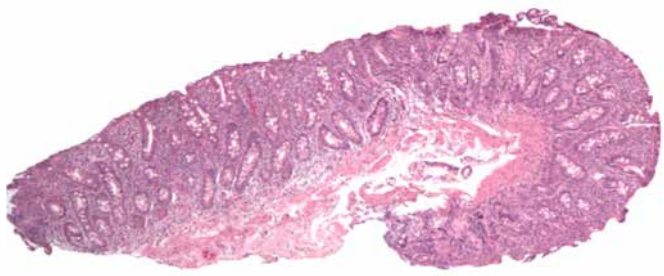


Figure 1. Small bowel mucosa showing severe villous blunting in a patient with untreated celiac disease.

In order to standardize reporting the interpretation of small bowel biopsies in patients with CD, two classification systems have been proposed[5,10]. They are the Marsh modified Oberhuber and the Corazza classifications[5,10]. The Marsh/Oberhuber system takes into consideration increased intraepithelial lymphocytes, crypt hyperplasia and the degree of villous atrophy. The Marsh classification uses a five tier system ranging from type 0 (normal) to type 3c (where the three parameters are abnormal with severe villous blunting). The Corazza/Villanacci system is a simplified version with only three categories: Grade A, that shows only increased intraepithelial lymphocytes, B1, with partial villous atrophy and B2 with total villous atrophy in addition to intraepithelial lymphocytes and crypt hyperplasia. Currently, the most widely used classification is the Marsh/Oberhuber system, however, the Corazza/Villanacci system includes only three groups and it is easier to apply and helps decreased the interobserver variability in the reporting of CD. The use of one of these systems is encouraged to facilitate the interpretation of the biopsy and the communication between gastroenterologist and pathologist.

2. The Differential Diagnosis of the Abnormal Small Bowel Biopsy

In addition to confirming the diagnosis of CD, the importance of the microscopic examination of the small bowel lies in identifying possible mimickers of CD which otherwise are difficult to recognize clinically.

The technical handling of the biopsy for a correct orientation of the tissue is crucial for the accurate interpretation. Whether there is villous atrophy or IELs or both, a biopsy that is not properly oriented will make the interpretation of the changes more difficult and may lead to the incorrect diagnosis. The right orientation will avoid artifact and misinterpretation of the biopsy as representing CD and this fact needs to be emphasize when handling small bowel biopsies.

The finding of villous blunting in small bowel biopsies is a non-specific finding and there are other conditions that show abnormal villi and do not represent CD[1,7,11,12]. Recognizing the possibility of other conditions and their microscopic features is one of the primary roles of pathologists when interpreting small bowel biopsies. No single pathologic feature of the small bowel biopsy is considered specific for the diagnosis of CD.

The conditions in the small bowel that can show villous atrophy excluding celiac disease are summarized in Table 1.

Table 1. Non-celiac causes of villous atrophy in the duodenum.

Tropical sprue
Small-bowel bacterial overgrowth
Autoimmune enteropathy
Drug-associated enteropathy
Whipple disease
Collagenous sprue
Crohn's disease
Infectious enteritis (tuberculosis; giardiasis)
Graft versus host disease
Malnutrition
Peptic duodenitis

These conditions include the following: tropical sprue, Crohn's disease, collagenous sprue, intestinal lymphoma, medications, infections, bacterial overgrowth, autoimmune enteropathy and common variable immunodeficiency (CVID). In addition to abnormal villi, these conditions can show IELs making the differential diagnosis with CD even more challenging.

The serologies in all of them are negative and before diagnosing CD the above entities should be excluded. For the pathologist, the presence of abnormal villi in patients that do not have other features of CD poses a significant challenge and is important to be aware of these mimmickers. A brief description of these conditions and their most important pathologic findings are presented below.

Medications that have been associated with villous blunting are olmesartan, mycophenolate mofetil, methotrexate and azathioprine[11,13-16]. For patients suspected of medication effect, the discontinuation of the medication leads to clinical and pathological improvements. An example of a small bowel biopsy showing villous blunting and a thickened basement membrane secondary to Olmesartan is illustrated in Figure 2.

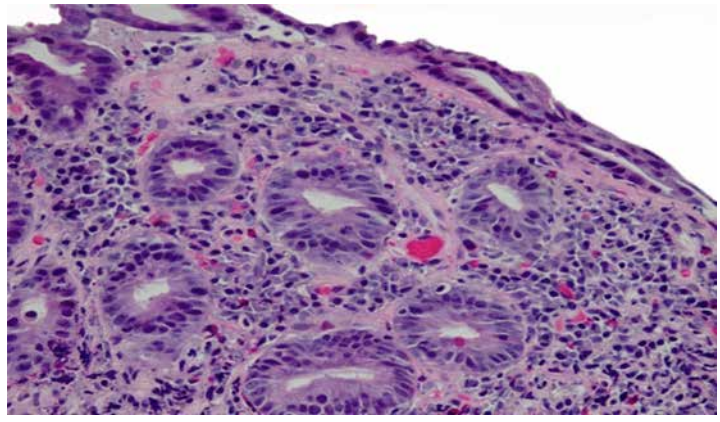


Figure 2. Small bowel mucosa of a 74 male with nausea, vomiting, abdominal pain, diarrhea and weight loss. The patient was taken Olmesartan.There is severe villous blunting and a thickened basement membrane.

CVID shows small bowel mucosa with decreased or absent plasma cells in the lamina propria and decreased serum levels of immunoglobulins.

Collagenous sprue is characterized microscopically by a diffusely thickened basement membrane and villous blunting. Collagenous sprue can be seen as an independent disease unrelated to CD or as a complication of CD[4,17]. Tropical sprue is seen in patients with a history of travel and who respond to antibiotic therapy.

Bacterial overgrowth develops in patients with motility disorders or anatomic abnormalities of the small bowel that promote colonization by gram negative flora from the colon. These patients have a positive breath test and they respond to antibiotic therapy. The small bowel biopsy can show mild to moderate villous blunting (in up to 25% of the patients) and less commonly IELs[18].

The SB biopsies may show abnormal villous architecture and acute inflammation involving the lamina propria and the crypts. CD can show mucosal acute inflammation in up to 50% of cases, and its presence should not preclude the diagnosis of CD. However, crypt abscesses and mucosal erosions are uncommon in CD[7]. When the biopsy shows acute inflammation, the possibility of other etiologies should be excluded[1,7]. Peptic duodenitis (injury) is a common diagnostic pitfall and represents the damage seen in the small bowel mucosa, frequently more prominent in the duodenal bulb, secondary to medication effect or gastric acid. Peptic injury shows acute inflammation in the lamina propria and foveolar metaplasia. Upper gastrointestinal Crohn's disease also shows acute inflammation, crypt abscesses and occasionally granulomas which are more common to see in the stomach than in the duodenum (Figure 3). Autoimmune enteropathy can affect children and adults. In the affected patients, the small bowel biopsy shows acute inflammation in the form of acute cryptitis absent goblet and parietal cells, apoptosis with villous blunting[19].

The SB biopsies that show only IELs with preserved villous architecture are a frequent pathologic finding in daily pathology practice. The minority of these patients have CD and it is estimated that between 5 to 15% of patients with IELs have celiac disease[20,21]. Other conditions that can be associated with IELs are medications (anti-inflammatory drugs), food allergies, H. pylori

gastritis, diabetes, inflammatory bowel disease, morbid obesity and autoimmune disorders[20-22].

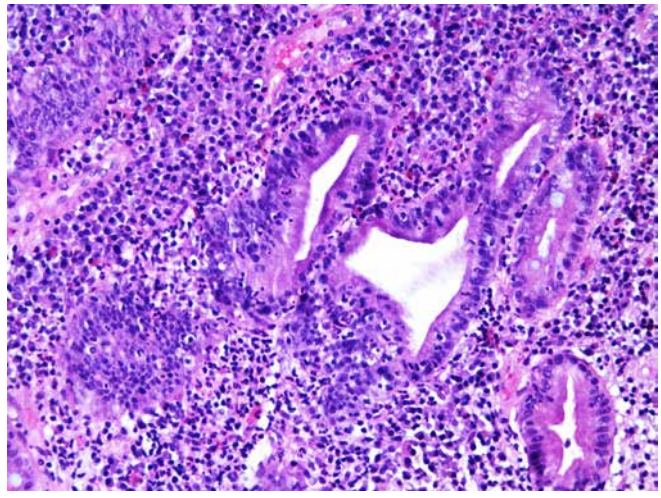


Figure 3. Small bowel biopsy from a patient with Crohn's disease showing acute inflammation involving the lamina propria and crypts.

Interestingly, some patients may have biopsies from either the stomach or large bowel that show increased IELs or a thickened basement membrane (collagenous gastritis and colitis), preceding the lymphocytosis of the small bowel[23]. If a small bowel biopsy is not available for review, the clinician needs to be alerted as to the possibility of celiac disease in these cases that show diffuse lymphocytosis throughout the gastrointestinal tract[1,23].

2.1. Refractory Sprue

The role of the pathologist is not confined to the initial diagnosis of CD and to the differential diagnosis with other conditions, but also in the workup of patients suspected of having refractory sprue (RS).

Refractory sprue is a complication of CD that develops in 1-2% of patients[5,9,24]. Patients are suspected to have RS when despite being in a gluten free diet their malabsorption symptoms persist. RS is an important diagnosis to make since the progression rates to T cell lymphoma and mortality secondary to infections are considerably higher in patients with RF type II. The lymphocytic phenotype of RS type I is similar to that seen in untreated CD[24].

The first step the pathologist should do when ask to evaluate a biopsy of a patient suspected of RS is to review the previous small bowel biopsy to confirm the diagnosis of CD. In the process of reviewing the biopsies the pathologist can exclude other diseases that present with increased intraepithelial lymphocytes or villous atrophy and that can simulate CD. If other diagnostic possibilities are excluded the use of immunohistochemical stains to characterize the presence of an aberrant clonal lymphocytic population can be done. Specifically, CD3 and CD8 are T cell markers that are analyzed in paraffin embedded material. If both of these markers are positive the differential diagnosis includes untreated CD or refractory sprue type I assuming that the patient has CD and other conditions have been excluded. If the biopsy shows an abnormal phenotype (lack of CD8 immunohistochemical staining) the possibility of refractory sprue type II should be considered. Type II refractory sprue is a more aggressive disease with a larger number of cases progressing to ulcerative jejunitis and small bowel lymphoma (Figure 4). The presence of an abnormal lymphocytic phenotype is a predictive factor but not a precondition to develop overt lymphoma[24]. In order to confirm the diagnosis of lymphoma of the small bowel, the use of molecular techniques to search for T cell receptor gamma gene rearrangement can be useful. Molecular analysis may reveal a monoclonal T-cell expansion of the lymphocytes in the small bowel mucosa.

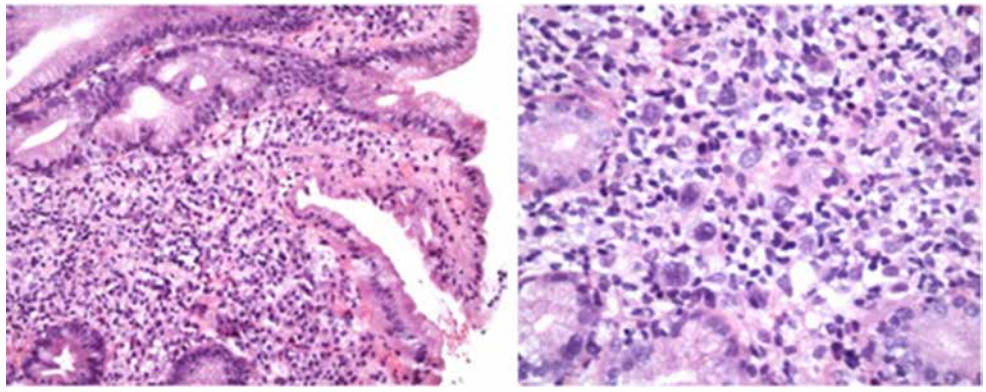


Figure 4. T-cell lymphoma of the small bowel in a patient with long standing celiac disease. Notice the atypical lymphocytes expanding the lamina propria and infiltrating crypts.

3. Conclusion

Pathology plays a crucial role in the diagnosis of celiac disease and in the interpretation of small bowel biopsies to confirm or exclude CD. The spectrum of changes in the biopsies of patients suspected of CD has broadened and the diagnosis can be subtle with minimal histopathologic changes. In order to confirm the diagnosis of CD, the pathologic features should be correlated with the clinical, endoscopic, serological findings and HLA haplotypes.

The small bowel biopsy should be considered an important diagnostic component in the workup for the diagnosis in all the patients suspected of having CD. It is crucial to be aware that other conditions share similar pathologic features with celiac disease. The clinician will decide in each individual case how important it is to biopsy the small bowel in order to confirm or exclude the possibility of celiac disease.

References

1. Walker MM, Murray JA. *An update in the diagnosis of coelic disease.* Histopathology. 2011; 59: 166-179.
   PMid:21054494
2. Rubio-Tapia A, Hill ID, Kelly CP, Calderwood AH, Murray JA. *ACG clinical guidelines: Diagnosis and management of Celiac Disease.* Am J Gastroenterol. 2013; 108(5): 656-676.
   http://dx.doi.org/10.1038/ajg.2013.79
   PMid:23609613 PMCid:PMC3706994
3. Fasano A, Catassi C. *Celiac Disease.* N Engl J Med. 2012; 367(25): 2419-2426.
   http://dx.doi.org/10.1056/NEJMcp1113994
   PMid:23252527
4. Owens SR, Greenson JK. *The pathology of malabsorption: current concepts.* Histopathology. 2007; 50: 64-82.
   http://dx.doi.org/10.1111/j.1365-2559.2006.02547.x
   PMid:17204022
5. Sapone A, Bai JC, Ciacci C, Dolinsek J, Green PH, Hadjivassiliou M et al. *Spectrum of gluten-related disorders: consensus on new nomenclature and classification.* BMC Med. 2012; 10: 13.
   http://dx.doi.org/10.1186/1741-7015-10-13
   PMid:22313950 PMCid:PMC3292448
6. Husby S, Koletzk S, Korponay-Szabo IR, Mearin ML, Phillips A, Shamir R et al. *The ESPHAN working group on celiac disease diagnosis, on behalf of the ESPGHAN gastroenterology committee: European Society of pediatric gastroenterology, hepatology, and nutrition guidelines for the diagnosis of coelic disease.* JPGN. 2012; 54: 136-160.
   PMid:22197856
7. Brown IS, Smith J, Rosty C. *Gastrointestinal pathology in celiac disease. A case series of 150 consecutive newly diagnosed patients.* Am J Clin Pathol. 2012; 138(1): 42-49.
   http://dx.doi.org/10.1309/AJCPE89ZPVJTSPWL
   PMid:22706856
8. Goldstein NS, Underhill J. *Morphologic features suggestive of gluten sensitivity in architecturally normal duodenal biopsy specimens.* Am J Clin Pathol. 2001; 116(1): 63-71.
   http://dx.doi.org/10.1309/5PRJ-CM0U-6KLD-6KCM
   PMid:11447753

9. Volta U, Villanacci V. *Celiac disease: diagnostic criteria in progress.* Cellular & Molecular Immunology. 2011; 8(2): 96-102.
http://dx.doi.org/10.1038/cmi.2010.64
PMid:21278763 PMCid:PMC4003134

10. Corazza GR, Villanacci V. *Coeliac disease.* J Clin Pathol. 2005; 58(6): 573-574.
http://dx.doi.org/10.1136/jcp.2004.023978
PMid:15917404 PMCid:PMC1770677

11. DeGaetani M, Tennyson CA, Lebwohl B, Lewis SK, Daya HA, Arguelles-Grande C et al. *Villous atrophy and negative celiac serology: a diagnostic and therapeutic dilemma.* Am J Gastroenterol. 2013; 108(5): 647-653.
http://dx.doi.org/10.1038/ajg.2013.45
PMid:23644957

12. Pallav K, Leffler DA, Tariq S, Kabbani T, Hansen J, Peer A et al. *Noncoeliac enteropathy: the differential diagnosis of villous atrophy in contemporary clinical practice.* Aliment Pharmacol Ther. 2012; 35(3): 380-390.
http://dx.doi.org/10.1111/j.1365-2036.2011.04938.x
PMid:22145590

13. Rubio-Tapia A, Herman ML, Ludvigsson JF, Kelly DG, Mangan TF, Wu TT et al. *Severe spruelike enteropathy associated with olmesartan.* Mayo Clin Proc. 2012; 87(8), 732-738.
http://dx.doi.org/10.1016/j.mayocp.2012.06.003
PMid:22728033 PMCid:PMC3538487

14. Kamar N, Faure P, Dupuis E, Cointault O, Joseph-Hein K, Durand D et al. *Villous atrophy induced by mycophenolate mofetil in renal –transplant patients.* Transpl Int. 2004; 17(8): 463-467.
http://dx.doi.org/10.1111/j.1432-2277.2004.tb00471.x
PMid:15322747

15. Bosca MM, Anon R, Mayordomo E, Villagrasa R, Balza N, Amoros C et al. *Methotrexate induced sprue-like syndrome.* World J Gastroenterol. 2008; 14(45): 7009-7011.
http://dx.doi.org/10.3748/wjg.14.7009
PMid:19058340 PMCid:PMC2773868

16. Ziegler TR, Fernandez-Estivariz C, Gu LH, Fried MW, Leader LM. *Severe villus atrohy and chronic malabsorption induced by azathioprine.* Gastroenterology. 2003; 124(7): 1950-1957.
http://dx.doi.org/10.1016/S0016-5085(03)00405-0

17. Vakiani E, Arguelles-Grande C, Mansukhani MM, Lewis SK, Rotterdam H, Green PH et al. *Collagenous sprue is not always associated with dismal outcomes: a clinicopathological study of 19 patients.* Mod Pathol. 2010; 23: 12-26.
http://dx.doi.org/10.1038/modpathol.2009.151
PMid:19855376

18. Lappinga PJ, Abraham SC, Murray JA, Vetter EA, Patel R, Wu TT. *Small intestinal bacterial overgrowth. Histopathologic features and clinical correlates in an underrecognized entity.* Arch Pathol Lab Med. 2010; 134(2): 264-270.
PMid:20121616

19. Singhi AD, Goyal A, Davison JM, Regueiro MD, Roche RL, Ranganathan S. *Pediatric autoimmune enteropathy: an entity frequently associated with immunodeficiency disorders.* Mod Pathol. 2014; 27: 543-553.
http://dx.doi.org/10.1038/modpathol.2013.150
PMid:24051695

20. Brown I, Mino-Kenudson M, Deshpande V, Lauwers GY. *Intraepithelial lymphocytosis in architecturally preserved proximal small intestinal mucosa: an increase diagnostic problem with a wide differential diagnosis.* Arch Pathol Lab Med. 2006; 130: 1020-1025.
PMid:16831028

21. Vande Voort JL, Murray JA, Lahr BD, Van Dyke CT, Kroning CM, Moore SB, Wu TT. *Lymphocytic duodenitis and the spectrum of celiac disease.* Am J Gastroenterol. 2009; 104(1): 142-148.
http://dx.doi.org/10.1038/ajg.2008.7
PMid:19098862 PMCid:PMC2608723

22. Memeo L, Jhang J, Hibshoosh H, Green PH, Rotterdam H, Bhagat G. *Duodenal intraepithelial lymphocytosis with normal villous architecture: common occurrence in H. Pylori gastritis.* Mod Pathol. 2005; 18(8): 1134-1144.
http://dx.doi.org/10.1038/modpathol.3800404
PMid:15803187

23. Feeley KM, Heneghan MA, Stevens FM, McCarthy CF. *Lymphocytic gastritis and coelic disease: evidence of a positive association.* J Clin Pathol. 1998; 51: 207-210.
http://dx.doi.org/10.1136/jcp.51.3.207
PMid:9659261 PMCid:PMC500640

24. Malamut G, Afchain P, Verkarre V, Lecomte T, Amiot A, Damotte D et al. *Presentation and long-term follow-up of refractory celiac disease: comparison of type I with type II.* Gastroenterology. 2009; 136: 81-90.
http://dx.doi.org/10.1053/j.gastro.2008.09.069
PMid:19014942

Go to TOC

Go to Section II

Chapter 9

Clinical Manifestations of Celiac Disease and Diagnostic Criteria: Differences Among Children, Adolescents and Adults

Maria Luisa Mearin[1], Miguel Montoro-Huguet[2], Isabel Polanco[3], Carmen Ribes-Köninckx[4], Santos Santolaria[5]

[1] Head of the Unit of Pediatric Gastroenterology. Department of Pediatrics. Leiden University Medical Center (LUMC). Leiden, The Netherlands.

[2] Department of Medicine. Universidad de Zaragoza. Head of Gastroenterology and Hepatology Unit. Hospital San Jorge. Huesca, Spain.

[3] Faculty of Medicine. Universidad Autonóma. Head of Gastroenterology and Pediatric Nutrition. Hospital Infantil Universitario La Paz. Madrid, Spain.

[4] Head of Pediatric Gastroenterology. Hospital Universitario y Politécnico La Fe. Valencia, Spain.

[5] Gastroenterology and Hepatology Unit. Hospital San Jorge. Huesca, Spain.

L.Mearin@LUMC.nl, maimontoro@gmail.com,
ipolanco@telefonica.net, ribes_car@gva.es,
ssantolariap@gmail.com

Doi: http://dx.doi.org/10.3926/oms.257

How to cite this chapter

Mearin ML, Montoro-Huguet M, Polanco I, Ribes-Köninckx C, Santolaria S. *Clinical Manifestations of Celiac Disease and Diagnostic Criteria: Differences Among Children, Adolescents and Adults.* In Arranz E, Fernández-Bañares F, Rosell CM, Rodrigo L, Peña AS, editors. *Advances in the Understanding of Gluten Related Pathology and the Evolution of Gluten-Free Foods.* Barcelona, Spain: OmniaScience; 2015. p. 291-339.

Abstract

Celiac disease (CD) was originally considered a pediatric disorder characterized by malabsorption and steathorrhea. Subsequently it was recognized that CD could affect adults at any age. Currently, in some centers, the greatest number of diagnosis of CD is performed in adults between 30 and 50 years. An overall decrease in the prevalence of diarrheal presentations over the past 2 decades, accompanied by an increase in "non-classical" manifestations of the disease, has been well described in both children and adults. Among children, clinical presentation is affected especially by the age. Very young children (< 3 years old) present more often with diarrhea, abdominal distension, and failure to thrive, whereas older children and adolescents are more likely to present with other gastrointestinal symptoms (recurrent abdominal pain, vomiting, or constipation) or extraintestinal symptoms. In adults, the major mode of presentation is diarrhea, although this presentation occurs in fewer than 50% of patients, and non-specific gastrointestinal symptoms, which bear a large degree of overlap with functional dyspepsia, irritable bowel syndrome or functional diarrhea. Extraintestinal symptoms such as iron-deficiency anemia, osteoporosis, dermatitis herpetiformis, recurrent apthous stomatitis, hipertransaminasemia, or neuropsychiatric manifestations are not infrequent. With the objective of improve the recognition and diagnosis of CD several guides to clinical practice have been published in both children and adults. In general, these guidelines recommend offering serologic testing for CD in patients with symptoms or conditions associated with CD. The confirmation of a diagnosis of CD should be based on a combination of findings from the clinical presentation, CD-specific antibodies, duodenal biopsies, HLA-DQ2/DQ8 genotyping, and

the response to a gluten free diet. Duodenal biopsies may not be mandatory for CD diagnosis in HLA-DQ2 and/or -DQ8 symptomatic patients with anti-transglutaminase antibodies over 10 times the upper limit of normal and positive endomysial antibodies.

Keywords

Celiac disease, diarrhea, gastrointestinal symptoms, extraintestinal symptoms, anti-transglutaminase antibodies, HLA-DQ2/DQ8, duodenal biopsies, gluten free diet.

1. Introduction

Celiac disease (CD) is an immune-mediated systemic disorder elicited by gluten and related prolamines in genetically susceptible individuals and characterized by the presence of a variable combination of gluten-dependent clinical manifestations, CD-specific antibodies, HLA-DQ2 or -DQ8 haplotypes, and enteropathy[1]. In genetically predisposed individuals, CD is precipitated by the ingestion of gluten, which are storage proteins in wheat (gliadin), rye (secalin) and barley (hordein). CD is a chronic, multi-organ disease in which small intestinal mucosal damage may lead to malabsorption of nutrients. The treatment of CD, adherence to a gluten free diet, was discovered by the Dutch pediatrician Willem-Karel Dicke (1905-1962)[2].

Genetic, immunology and environmental factors are important in the development of CD. The disease has a strong genetic component and the principal determinants are the class II HLA-DQ2 and -DQ8 genes[3]. CD is primarily a T cell–mediated immune disorder and in the small intestinal mucosa of individuals with CD, CD4+ T cells recognize gluten peptides selectively in the context of HLA-DQ2 or -DQ8 molecules[4]. The enzyme transglutaminase 2 (TG2) deaminates the positive charged gluten peptides, enhancing their binding to HLA-DQ2 and -DQ8 molecules. Both gluten-specific CD4+ T cells and cytotoxic intraepithelial T lymphocytes (IELs) play a key role in the development of CD, as defined by the presence of anti-TG2 antibodies and villous atrophy. The most important environmental factor related to CD is gluten, but other factors, such as infections, dysbiosis and drug exposure have been implicated[5,6].

CD is a common but frequently unrecognized disease, in part because of its variable clinical presentation and symptoms[7]. Screening studies have shown that CD is severely underdiagnosed, with of about 1%-3% among the European population, both in adults as in children[8-11]. Because CD can be very effectively treated with a gluten-free diet (GFD) it is important to identify people with the undiagnosed disease so as to provide satisfactory individual treatment. To improve the recognition of CD and to increase the

number of people diagnosed with the condition, a significant number of diagnosis clinical guidelines has been published over the last years[1,12-15]. Besides, the Oslo and London Consensuses recommendations tried to reached agreement on the definition of terms related to CD and/or gluten sensitivity to improve communication among researchers, clinicians and the general public (Table 1)[16,17].

Table 1. Classification of the main modes of clinical presentation according to the Oslo definitions for CD and related terms[17] and to ESPGHAN guideline[1].

OSLO Consensus	ESPGHAN guideline
Asymptomatic CD Absence of symptoms even in response to direct questioning at initial diagnosis. These patients are often diagnosed through testing of populations enrolled in screening programmes	**Silent CD** Presence of positive CD-specific antibodies, HLA, and small-bowel biopsy findings that are compatible with CD but without sufficient symptoms and signs to warrant clinical suspicion of CD.
Classical CD Presents with signs and symptoms of malabsorption. Diarrhea, steathorrhea, weight loss or growth failure is required	**Gastrointestinal symptoms and signs** Because atypical symptoms may be considerably more common than classic symptoms, the ESPGHAN working group decided to use the following nomenclature: gastrointestinal symptoms and signs (eg, chronic diarrhea)
Non-classical CD Presents without signs and symptoms of malabsorption. Patients with monosymptomatic disease (other than diarrhea or steathorrhea) usually have non-classical CD	**Extraintestinal symptoms and signs** eg, anemia, neuropathy, decreased bone density, increased risk of fractures
Subclinical CD Disease that is below the threshold of clinical detection without signs or symptoms sufficient to trigger CD testing in routine practice.	Not used. See Silent

OSLO Consensus	ESPGHAN guideline
Symptomatic CD Characterized by clinically evident gastrointestinal and/or extraintestinal symptoms attributable to gluten intake	See above gastrointestinal and extraintestinal symptoms
Potential CD Relates to people with a normal small intestinal mucosa who are at increased risk of developing CD as indicated by positive CD serology	Presence of CD-specific antibodies and compatible HLA but without histological abnormalities in duodenal biopsies. The patient may or may not have symptoms and signs and may or may not develop a gluten-dependent enteropathy later.
Not used	**Latent CD** Presence of compatible HLA but without enteropathy in a patient who has had a gluten-dependent enteropathy at some point in his or her life. The patient may or may not have symptoms and may or may not have CD-specific antibodies.
Refractory CD Persistent or recurrent malabsorptive symptoms and signs with villous atrophy despite a strict GFD for more than 12 months	
CD autoimmunity Relates to increased anti-TG2 or EMA on at least two occasions when status of the biopsy is not known. If the biopsy is positive, then this is CD, if the biopsy is negative than this is potential CD	
Genetically at risk of CD Family members of patients with CD that test positive for HLA-DQ2/DQ8	
Non-celiac gluten sensitivity Relates to one or more of a variety of immunological, morphological or symptomatic manifestations that are precipitated by the ingestion of gluten in people in whom CD has been excluded	

OSLO Consensus	ESPGHAN guideline
Gluten ataxia Idiopathic sporadic ataxia and positive serum antigliadin antibodies even in the absence of duodenal enteropathy	
Dermatitis herpetiformis Cutaneous manifestation of small intestinal immune-mediated enteropathy precipitated by exposure to dietary gluten. It is characterized by herpetiform clusters of pruritic urticated papules and vesicles on the skin, especially on the elbows, buttocks and knees, and IgA deposits in the dermal papillae. DH responds to a GFD	
Terms to avoid Typical CD; Atypical CD; Silent CD; Overt CD; Latent CD	Typical CD; Atypical CD; Classical CD; non-classical CD

2. Clinical Manifestations

CD was originally considered a pediatric disorder characterized by malabsorption and steathorrhea. Subsequently it was recognized that CD could affect adults at any age. Currently, in some centers, the greatest number of diagnoses of CD is performed in adults between 30 and 50 years[18]. Most children and adults with CD diagnosed before 1980 presented with diarrhea. With the advent of serologic tests in the 1980s, the wide spectrum of clinical manifestations became apparent. An overall decrease in the prevalence of diarrheal presentations over the past 2 decades, accompanied by an increase in "non-classical" manifestations of the disease, has been well described in both children and adults[7,19,20]. Table 2 summarizes clinical signs, symptoms and types of presentation or conditions associated with CD in both children and adults.

Table 2. Signs, symptoms, and associated conditions, that should prompt consideration of celiac disease in children and adults, according to the NICE guideline[13].

Signs and symptoms
• Chronic or intermittent diarrhea • Persistent or unexplained gastrointestinal symptoms including nausea and vomiting • Recurrent abdominal pain, cramping or distension • Growth failure or short stature • Prolonged fatigue (“tired all the time”) • Sudden or unexpected weight loss • Unexplained iron-deficiency anemia, or other unspecified anemia • Premature reduced bone mineral density • Elevated serum aminotransferase levels when no other etiology is found • Oral apthous ulcers or dental enamel defects
Conditions
• Dermatitis herpetiformis • Irritable bowel syndrome • Autoimmune thyroid disease • Type 1 diabetes • Autoimmune liver conditions • Ataxia • Peripheral neuropathy • Down’s, William’s and Turner’s syndromes. • First-degree relatives (parents, siblings or children) with celiac disease
Other signs, symptoms and conditions to consider offering serological testing
• Other gastrointestinal disorders: – Persistent or unexplained constipation – Microscopic colitis – Lymphocytic gastritis • Neuropsychiatric manifestations: – Depression or bipolar disorder; irritability; dysthymia – Headache – Epilepsy • Gynecological: – Amenorrhea – Recurrent miscarriage – Unexplained infertility

- Immunological/autoimmune disease:
  - IgA deficiency
  - IgA nepropathy
  - Addison's disease
  - Chronic thrombocytopenia purpura
  - Autoinmune myocarditis
  - Sarcoidosis
  - Sjogren syndrome
  - Rheumatoid arthritis
  - Systemic lupus erythematosus
- Malignancy
  - Lymphoma
  - Small bowel adenocarcinoma

2.1. Children

Among children, CD has a varied clinical presentation, and is affected especially by the age at presentation. Very young children (< 3 years old) present more often with "classic" CD, characterized by diarrhea, abdominal distension, and failure to thrive, whereas older children and adolescents are more likely to present with other gastrointestinal symptoms such as recurrent abdominal pain, vomiting, or constipation. In addition, extraintestinal symptoms such as arthritis, neurologic symptoms and anemia are not infrequent, as are asymptomatic cases[21]. A Canadian study[20] evaluated the incidence and clinical presentation of CD in patients <18 years and compared the results according to the time of diagnosis, before (pretesting group) or after (testing group) the introduction of serological testing. The frequency of classic CD presentations decreased from 67% (pretesting group) to 19% (testing group). The frequency of Marsh 3c lesions decreased from 64% (pretesting group) to 44% (testing group). In the testing group, classic CD remained predominant (67%) in young children (<3 years), whereas atypical gastrointestinal and silent presentations predominated in older children. The primary symptoms, signs or associated conditions that led to intestinal biopsy are presented in Table 3.

Table 3. Age and primary symptoms, signs, or indication leading to intestinal biopsy to diagnose CD in children, according to the time of diagnosis before (pretesting) or after (testing) the introduction of serological testing[20].

	Pretesting (n = 36)(%)	**Testing (n = 199)(%)**	**p**
Age at diagnosis, median (95% CI)	2 (2-4)	9 (8-10)	<0.01
Classic presentation	24 (67)	39 (19)	<0.01
Gastrointestinal symptoms	7 (19)	76 (38)	0.048
Abdominal pain plus other symptoms	5 (14)	34 (17)	
Abdominal pain only	0	18 (9)	
Endoscopy for other reason	0	8 (4)	
Chronic diarrhea	1 (2.7)	7 (3.5)	
Constipation	0	5 (2.5)	
Vomiting	1 (2.7)	2 (1)	
Food allergy	0	1 (0.5)	
Abdominal distention	0	1 (0.5)	
Extraintestinal symptoms	5 (14)	29 (15)	0.9
Failure to thrive			
Iron deficiency, with or without	2 (5.5)	13 (6.5)	
anemia	2 (5.5)	6 (3)	
Short stature	0	6 (3)	
Dermatitis herpetiformis	0	2 (1)	
Elevated transaminase levels	0	1 (0.5)	
Dental enamel defects	0	1 (0.5)	
Hypoalbuminemia	1 (2.7)	0	
Silent	0	55 (28)	<0.01
Family history	0	35 (17.6)	
Type 1 diabetes mellitus	0	14 (7)	
Trisomy 21	0	5 (2.5)	
Hypothyroidism	0	1 (0.5)	

A recent study from the Netherlands revealed that CD was more frequently represented in a cohort of children with chronic constipation fulfilling Rome III criteria for irritable bowel syndrome (IBS)[22]. Overweight and obese children and adolescents with CD are now frequently identified. A North American study in children showed that nearly 19% of patients had an elevated body mass index at diagnosis (12.6% overweight, 6% obese) and 74.5% presented with a normal body mass index[23]. Conditions associated with CD apart from type 1 diabetes mellitus are autoimmune liver disease (13.5%), Williams syndrome (9.5%), Turner syndrome (6.5%), Down syndrome (5.5%), immunoglobulin A (IgA) nephropathy (4%), IgA deficiency (3%), autoimmune thyroid disease (3%) and juvenile chronic arthritis (2.5%)(Table 2)[1].

In the last years, several studies have suggested a protective role of breast feeding and/or the timing and quantity of gluten introduction in the subsequent development of CD in children[24]. Especially, the data from the Swedish epidemic of symptomatic CD during the mid-1980s suggested that prolonged breast feeding during the introduction of gluten-containing feeding was associated with a reduced risk of developing CD in infancy[25]. However, recently two multicenter, randomized, double-blind, placebo-controlled dietary-intervention studies have reported that neither the delayed introduction of gluten nor breast-feeding modified the risk of celiac disease among at-risk infants[26,27].

2.2. Adults

In adults, the mean age of CD presentation is 44 years (range 1-81 years), with a clear female predominance (1: 3), that has also been shown in young children[26]. Approximately 15-25% of cases are diagnosed at an age equal to or greater than 65 years[18]. In some cases, a history of growth failure or other symptoms suggestive of unrecognized childhood CD is discovered. The classic presentation of the disease with malabsorption, diarrhea, weight loss and abdominal distension is less common than in children[19]. The major mode of presentation is diarrhea, although this presentation occurs in fewer than 50% of patients, and non-specific gastrointestinal symptoms, which bear a large

degree of overlap with functional dyspepsia, irritable bowel syndrome (IBS) or functional diarrhea[28,29].

Dyspepsia is a common symptom in CD patients, which may be present in 40-60% of the cases at the time of diagnosis[30,31]. The prevalence of CD in patients with dyspepsia is also increased. A meta-analysis and systematic review of these studies also shows a higher frequency of positive celiac serology (7.9% vs 3.9%) as well as of CD diagnosed by duodenal biopsy (3.2% vs 1.3%) in dyspepsia patients compared to the control population, although these differences were not statistically significant[32]. If we consider the whole spectrum of histological CD lesions, including forms of mild enteropathy, this prevalence could be even higher. A retrospective study in Spain in patients with dysmotility-like dyspepsia (postprandial distress) and normal upper endoscopy showed that 19.7% of these patients had enteropathy and gluten-dependent symptoms[33].

CD can frequently present with symptoms that are also characteristic of IBS, including abdominal pain (77%), bloating (73%), diarrhea (52%), constipation (7%) and/or an alternating bowel pattern (24%)[31,34]. This means that IBS often constitutes the initial diagnosis in many patients before the discovery of CD many years later. A systematic review and meta-analysis including 2278 patients with IBS diagnostic criteria, showed in these patients a higher prevalence of IgA anti-gliadin antibodies (AGA) (4%; CI 95% 1.7-7.2), endomysial antibodies (EMA) or anti-TG2 antibodies (1.6%, CI 95% 0.7-3) as well as CD demonstrated by duodenal biopsy (4.1%, CI 95% 1.9-7)[35]. A prospective Spanish study in patients with chronic watery diarrhea and Rome II criteria for functional diarrhea or IBS-diarrhea diagnosis showed that 16.1% of these patients had enteropathy and gluten-sensitive diarrhea[36].

The presence of gastroesophageal reflux disease-related symptoms (GERD-rs) refractory to antisecretory drugs should encourage considering CD in the differential diagnosis. An Argentinean study which evaluated GERD-rs at diagnosis of CD in adults' patients found a significantly higher reflux symptom mean score than healthy controls. At baseline, 30.1% of CD patients had moderate to severe GERD-rs compared with 5.7% of controls[37]. A case

control study in patients with CD and GERD-rs showed that gluten free diet improved symptoms and it was a useful approach in the prevention of recurrence[38].

The prevalence of extraintestinal manifestations is very high among adult patients, especially if a specific search is performed. Anemia, mainly caused by iron deficiency, osteoporosis, dermatitis herpetiformis, recurrent apthous stomatitis, hipertransaminasemia, as well as a variety of neuropsychiatric conditions, can be a common mode of presentation of CD in adults (Table 2)[13,39].

Finally, serologic screening of high-risk groups, especially relatives of patients with CD, has increased detection of the disease both in children as in adults, some of whom are asymptomatic or present with mild and unspecific symptoms[21].

3. Diagnostic Criteria

3.1. Children

Strict criteria for CD diagnosis in children were first established by the European Society of Pediatric Gastroenterology and Nutrition (ESPGAN) in 1969[40]. The so-called 3 biopsies rule recommended performing at least three small bowel biopsies (SBB): the first one at clinical suspicion and while the child was on a gluten containing diet, the second after a period of gluten-free diet, and the third after gluten reintroduction, i.e. after performing a gluten challenge (GC). Characteristic histological lesions in the first SBB lead to CD suspicion, but a definite diagnosis of CD was finally confirmed only after in the 3rd biopsy histological relapse related to GC was verified. This strict diagnostic protocol aimed at demonstrating that gluten sensitivity was a permanent condition and to avoid misdiagnosis of transient gluten intolerance associated to other conditions especially in young infants[40].

After 20 years experience in large series of children it was shown that GC could probably be avoided in 95% of the cases[41]; accordingly diagnostic criteria were modified in 1990 and GC was restricted to infants younger than 2 years at the first biopsy to exclude other causes of enteropathy or whenever the initial diagnosis is uncertain; this latter covers different special circumstances such as gluten exclusion prior to or without a biopsy or uncharacteristic histological lesions for CD at diagnosis[42]. Moreover, the new criteria had for the first time a disease marker to rely on, i.e. the antigliadin antibodies (AGA), who had recently been found to be associated to active CD[43-45]; so it was considered that the presence of AGA in serum at disease onset, followed by antibody vanishing after gluten withdrawal, added support to the diagnosis[42]. However, further development of antiendomysial antibodies (EMA) in the late 80's[45-48], followed by TG2 being recognized as the autoantigen of CD in the 90´s, represented a true revolution in the field of CD diagnosis[49]. It was indeed shown that both EMA and anti-TG2 recognize the same autoantigen and overall display a sensitivity and specificity for CD diagnosis higher than 95%[49-51]. A new serological tests for antibodies against deaminated gliadin peptides (DGP)[52] has more recently turned out to display a higher sensitivity and specificity than conventional AGA, thus replacing the later ones for diagnostic purposes.

Although pediatric series are shorter as compared to adults´ ones, correlation between duodenal histopathology and anti-TG2 levels in pediatric patients with CD has been reported, higher levels being associated with villous atrophy[53-55]. Thus, it has recently been suggested that strongly positive anti-TG2 antibodies levels might be considered sufficient for CD diagnosis in children and replace the SBB in the diagnostic work up[53].

Moreover the strong association of CD with genetic markers HLA-DQ2 and -DQ8, which combined reach a sensitivity of 96%, implies a negative result of HLA-DQ2 and/or -DQ8 renders CD diagnosis unlikely[56-58].

Additionally, a very high relapse rate after GC in children younger than 2 years with positive EMA and villous atrophy at diagnosis has been

demonstrated, supporting the view that routine GC should not be mandatory in these cases[59].

Not surprising a recent survey conducted among ESPGHAN members revealed that about 90 % of responders requested a revision and modification of the 1990 diagnostic criteria; 44% wanted to omit the first SBB in specific circumstances, the majority of them declaring that no first biopsy should be required for symptomatic cases with positive IgA anti-TG2 or EMA in HLA-DQ2/DQ8 positive individuals. Additionally about half of the respondents believed that GC should not be mandatory for all children diagnosed (1st biopsy) before the age of 2[60].

Thus within ESPGHAN a working group performed a revision of scientific and technical developments in an evidence-based approach, producing a detailed evidence report on antibody testing in CD[61] which served as the basis for new guidelines for CD diagnosis recently published[1]. Additionally the working group developed a new and broader definition of CD as a systemic disorder with different degrees of mucosal lesions not restricted to villous atrophy therefore the diagnosis cannot rely on one single parameter, but on a combination of clinical symptoms, CD-specific antibodies, histology and genetics[1]. In summary, the new guidelines state that SBB may not be mandatory for CD diagnosis in HLA-DQ2 and/or -DQ8 symptomatic patients with anti-TG2 over 10 times the upper limit of normal (ULN) and positive EMA. As for GC they establish that GC is no longer obligatory in all cases that underwent SBB before the age of 2, but only in unclear cases. These guidelines have been validated by a recently published retrospective study[62] and a prospective international multicenter one (PROCEDE, www.procede2011.jimdo.com) is currently on-going.

3.1.1. Who to Test for CD?

According to the new 2012 ESPGHAN guidelines for CD diagnosis in children and adolescents, beside patients with the classic clinical picture, i.e. malabsorption syndrome with chronic diarrhea, weight loss, abdominal distension and anorexia, children with a wide spectrum of other

gastrointestinal and extraintestinal symptoms - most of them also reported in adults - should be tested for CD; these are shown in Table 4. Failure to thrive, short stature and pubertal delay are CD features specific for the pediatric age range and should thus prompt serological testing as well. Also asymptomatic individuals pertaining to the so called high risk groups and specially those with a first degree relative with confirmed CD should be screened for CD (Table 4)[1].

Table 4. Who should be tested for CD according to the new 2012 ESPGHAN guidelines for CD Diagnosis in children and adolescents[1].

Children and adolescents with the otherwise unexplained symptoms and signs of:
• Chronic or intermittent diarrhea • Failure to thrive, weight loss, stunted growth • Delayed puberty, amenorrhea • Iron-deficiency anemia • Nausea or vomiting • Chronic abdominal pain, cramping or distension • Chronic constipation • Chronic fatigue, recurrent apthous stomatitis (mouth ulcers) • Dermatitis herpetiformis–like rash • Fracture with inadequate traumas/osteopenia/osteoporosis • Abnormal liver biochemistry
Asymptomatic children and adolescents with an increased risk for CD such as:
• Type 1 diabetes mellitus (T1DM) • Down syndrome • Autoimmune thyroid disease • Turner syndrome • Williams syndrome • Selective immunoglobulin A (IgA) deficiency • Autoimmune liver disease • First-degree relatives with CD

3.1.2. How to Test for CD?

CD specific antibodies detection in serum, EMA by immunofluorescence or anti-TG2 by various immunoassays (enzyme-linked immunosorbent assay, radioimmunoassay, or others) is the preferred initial approach to find CD[1]. Immunofluorescent tests such as EMA are subjected to interobserver variability. Despite these limitations, the specificity of EMA is 98% to 100% in expert laboratories[51,63] and is thus considered the reference standard for CD-specific antibody.

The performance of a particular antibody test depends on patient characteristics (age, genetic predisposition, IgA deficiency), on pretest probability, on the used commercial kit and last but not least the expertise of the laboratory is also relevant[51].

However in children serological tests display a much higher efficiency as compared to adults , partially because usually more severe histological lesion are found in the pediatric age range. So in the 2012 ESPGHAN guidelines it is stated that in absence of CD specific antibodies (anti-TG2 and EMA) the diagnosis of CD is unlikely[1].

According to the ESPGHAN evidence report on CD serology[61], EMA display the best positive and negative likelihood ratios, followed by anti-TG2. Furthermore, EMA results were more homogeneous than results obtained with other CD antibody tests and had a high diagnostic odds ratio (OR = 553.6). Thus CD is likely if the EMA test is positive. Moreover EMA positivity also is associated with the later development of villous atrophy in the few reported cases who initially had normal small-intestinal architecture[64,65].

High concentrations of anti-TG2 in serum predict villous atrophy better than low or borderline values[54,55,66] and these studies suggest that high anti-TG2 antibody levels can be defined as those exceeding 10 times the upper limit of normal (ULN) depending on the cut off of each test (concentration-dependent antibody tests based on calibration curves)[55,66,67].

Anti-DGP antibodies performed favorably and much better than antibodies against native gliadin, however their performance is inferior compared with

anti-TG2 or EMA assays[55,61]; In addition, their role in the diagnosis of children younger than 2 to 3 years requires further assessment.

Anti-TG2 antibody detection also can be done from the blood at the point of contact using rapid test kits (POC test)[68,69], but although they may achieve a high accuracy for CD diagnosis (pooled sensitivity of 96.4%, pooled specificity of 97.7%)[61], performance of these tests needs to be confirmed not only in high prevalence populations as current published studies, but also in less selected populations and/or when handled by laypeople or untrained medical staff. Also anti -DGP based POC have lately been made available, although only very few studies have been reported up to now, effectiveness seems to be similar to the previous ones (personal observation).

3.1.3. Diagnostic Confirmation

In the last few years the leading role of histology for the diagnosis of CD has been questioned[53,55,63,65]. One of the mean reasons is that histological findings are not specific for CD, especially low grade lesions; these can be found in other entities, such as cow's milk or soy protein hypersensitivity, intractable diarrhea of infancy, infestation with *Giardia lamblia*, immunodeficiencies, tropical sprue, and bacterial overgrowth (Table 5). Another issue is that lesions may be patchy[70], they can occur in the duodenal bulb only[71], but the most important matter of concern is that interpretation depends on preparation of the mucosa sample and above all that a high interobserver variability has been acknowledged[72].

Not withstanding current evidence recommend that histological assessment should be omitted only in very specific situation, namely in symptomatic patients who have high IgA anti-TG2 levels 10 times above ULN, verified by EMA positivity, and are HLA-DQ2 and/or -DQ8 heterodimer positive. In all other circumstances histological evaluation is mandatory for a definite diagnosis[1]. This is mainly due to the fact that high levels of anti-TG2 (10 times ULN) correlates better with lesion severity than low values; borderline

or low levels may be found in non-CD conditions, specially autoimmune diseases and are not related to histological lesion[55,67,73].

Table 5. Other causes of enteropathy.

Villous atrophy	Lymphocytic enteropathy
• Tropical sprue • Small-bowell bacterial overgrowth • Autoinmune enteropathy • Hypogammaglobulinemic sprue • Drug-associated enteropathy (e.g., olmesartan) • Whipple disease • Collagenous sprue • Crohn's disease • Eosinophilic enteritis • Intestinal lymphoma • Intestinal tuberculosis • Infectious enteritis (e.g. giardasis) • Graft versus host disease • Malnutrition • Adquired immune deficiency syndrome enteropathy	• *H.pylori* infection • Small-bowell bacterial overgrowth • Drugs (e.g., NSAIDs) • Intolerance to non-gluten proteins (e.g., Cow's milk, eggs) • Infectious enteritis (e.g. giardasis) • IgA deficiency • Common variable immunodeficiency • Eosinophilic enteritis • Crohn's disease

The histological features of the small-intestine enteropathy in CD have a variable severity. The spectrum of histological findings ranges from lymphocytic infiltration of the epithelium (Marsh 1) to villous atrophy (Marsh 3)[74]. The description of the lesions according to Marsh -Obberhuber classification are described in Table 6[75,76]. Marsh 2-3 lesions are considered consistent with CD[1]. If histology is normal (Marsh 0) or only increased IELs counts are observed (Marsh 1), the diagnosis of CD can not be firmly established. Further work up is necessary at the mucosal level specially immunohistochemical analysis of biopsies looking for high $\gamma\gamma\delta\delta$ cells count or $\gamma\delta$/CD3 ratio[77] or the presence of IgA anti-TG2 deposits in the mucosa[78,79]. These deposits in the mucosa seem to be specific for CD and to predict the

evolution to more severe histological patterns[80]. Counting villous tip IELs also increases the specificity for CD[81].

Table 6. Histological classifications used for celiac disease[88].

Marsh modified (Oberhuber)	**Histologic criterion**			**Corazza**
	Increased IELs*	Crypt hyperplasia	Villous atrophy	
Type 0	No	No	No	None
Type 1	Yes	No	No	Grade A
Type 2	Yes	Yes	No	
Type 3a	Yes	Yes	Yes (partial)	Grade B1
Type 3b	Yes	Yes	Yes (subtotal)	
Type 3c	Yes	Yes	Yes (total)	Grade B2

*IELs: Intraepithelial lymphocytes per 100 enterocytes; > 40 for Marsh modified; > 25 for Corazza.

3.1.4. Role of HLA-DQ2/DQ8 Genotyping in Celiac Disease

HLA-DQ2 and -DQ8 testing is valuable because CD is unlikely if both haplotypes are negative[1,57,58]. Thus its main utility is to discard patients at risk for CD and accordingly HLA testing is useful to select asymptomatic persons with CD-associated conditions or pertaining to high risk groups for further CD-specific antibody testing[1]. In clinical practice it is noteworthy to stress the relevance of HLA typing of siblings or the offspring of CD patients as it will establish those at risk in which periodic testing for CD markers may be recommended, especially during the pediatric age range.

Moreover HLA testing should be performed when the diagnosis of CD is unclear, for example, in patients with negative CD-specific antibodies and

mild histological lesion. In children with a strong clinical suspicion of CD and high specific CD antibodies, if no SBB is going to be performed HLA-DQ2/DQ8 typing is strongly recommended to add strength to the diagnosis[1].

3.1.5. Special Situations

In subjects with humoral IgA deficiency, corresponding IgG class CD-specific antibodies should be measured, preferably IgG anti-TG2, but alternatively EMA-IgG, IgG anti-DGP or blended kits for both IgA and IgG antibodies[1,48]. Thus it is important to exclude IgA deficiency by measuring serum total IgA levels moreover considering IgA deficiency is more prevalent in CD as compared to the general population.

Children, mainly infants, presenting with a severe malabsorption syndrome and malnutrition, may exceptionally been started on a GFD while awaiting the results of HLA and EMA testing[1]. If the findings do not allow a definite diagnosis and due to a poor clinical condition the SBB has to be postpone, additional workup such as looking for IgA anti-TG2 deposits in the mucosa may be helpful. Due to persistence of anti-TG2 deposits for months after a GFD has been initiated, the presence of deposits can be used as a high specific test for CD whenever the patient has started dietary restrictions before a definite diagnosis has been achieved[79,80]. Patients with associated autoimmune conditions may display false positive anti-TG2 or fluctuating results, usually at low levels[65,73]; however in type 1 diabetes, especially at the initial stages of the disease, higher levels of EMA and anti-TG2 can be detected, decreasing to below ULN on follow up.

3.1.6. Celiac Disease Diagnostic Approach in Clinical Practice

The new 2012 ESPGHAN guidelines include 2 practical algorithms for CD diagnosis, one to be applied to symptomatic cases (Figure 1) and another for asymptomatic individuals pertaining to high-risk groups (Figure 2). Neither of them are meant for mass screening or for fortuitously detected CD antibody

positivity[1]. It should be stressed that initial evaluation has to be performed while the child is on a gluten containing diet, thus before dietary restrictions are recommended.

In children and adolescents with otherwise unexplained signs and symptoms suggestive of CD it is recommended to start the diagnostic approach by IgA anti-TG2, together with total serum IgA to rule out IgA deficiency; in this situation IgG anti-TG2 testing is recommended (Figure 1).

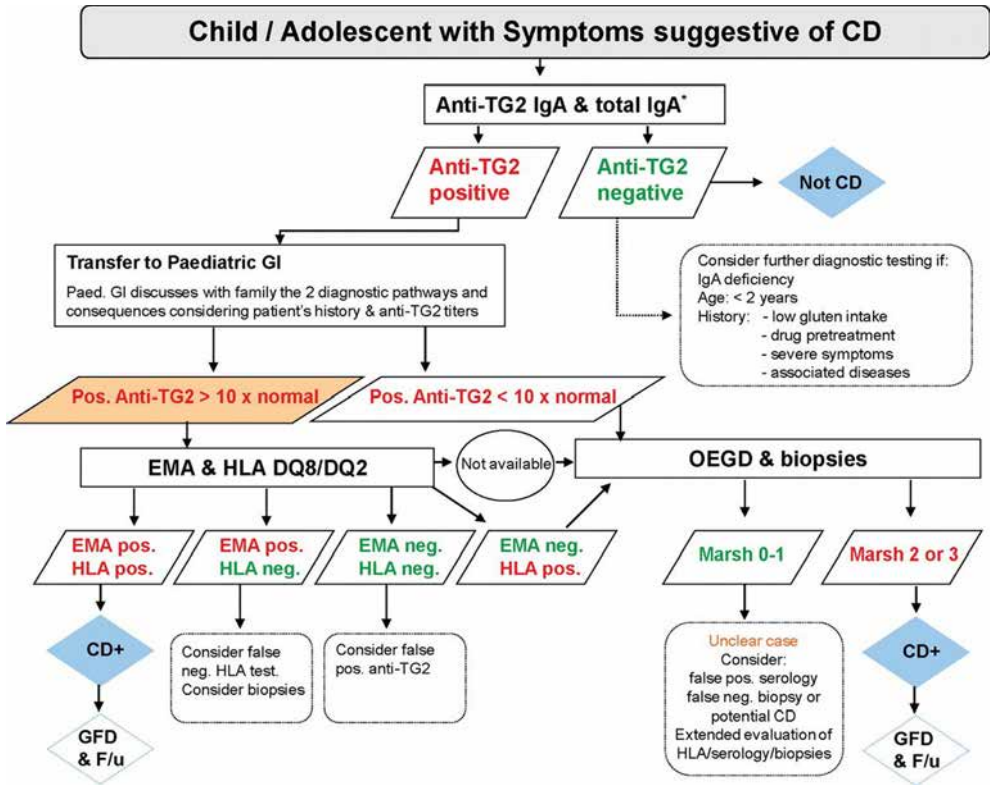


Figure 1. Diagnostic algorithm for children or adolescents with symptoms suggestive of CD. CD: celiac disease; EMA: endomysial antibodies; F/u: follow-up; GFD: gluten-free diet; GI: gastroenterologist; HLA: human leukocyte antigen; IgA: immunoglobulin A; IgG: immunoglobulin G; OEGD: oesophagogastroduodenoscopy; TG2: transglutaminase type 2. Adapted with permission from Lippincott Williams and Wilkins/Wolters Kluwer Health: Journal of Pediatric Gastroenterology & Nutrition, Husby S et al, ESPGHAN Guidelines for Diagnosis of Coeliac Disease[1], 2012.

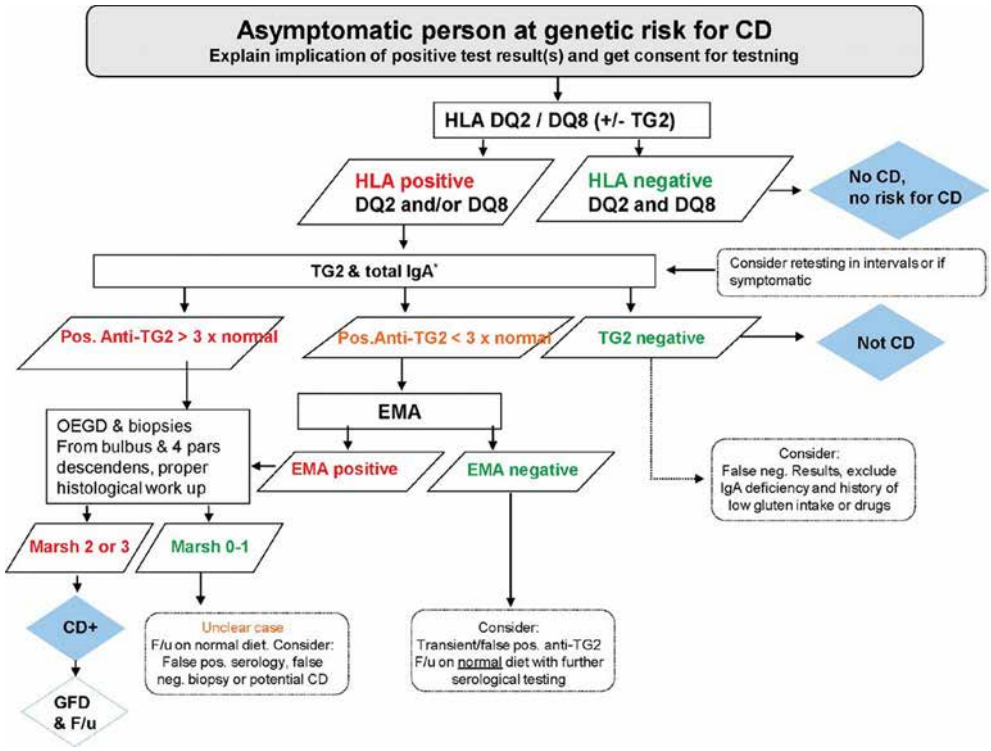


Figure 2. Diagnostic Algorithm for asymptomatic children and adolescents at genetic risk for CD (1st degree relatives or other at high-risk groups). See Fig. 1 for definitions. Adapted with permission from Lippincott Williams and Wilkins/Wolters Kluwer Health: Journal of Pediatric Gastroenterology & Nutrition, Husby S et al, ESPGHAN Guidelines for Diagnosis of Coeliac Disease[1], 2012.

In IgA sufficient patients, If **IgA anti-TG2 are negative** CD is unlikely. Several conditions such as low gluten intake, certain drugs (immunosuppressants), age (infants younger than 2 years) may impact on antibody results and should be taken into consideration. If symptoms and suspicion persists a SBB may be necessary independently of antibody results. Thus is seems reasonable that at this stage a pediatric gastroenterologist should be involved in decision taking.

For high **IgA anti-TG2 levels above 10 times ULN** the pediatric gastroenterologist should consider and discuss with the parents the option of

omitting the biopsies but performing additional investigations; this means that on a second (and thus different) blood sample HLA and EMA should be tested. If positive EMA antibodies and HLA-DQ2 or -DQ8, are found then the diagnosis of CD is confirmed and a GFD should be recommended; follow up is mandatory to ascertain improvement of symptoms and decline of antibodies, but no GC is further required. If any of them or both are negative, either a false positive anti-TG2 or a false negative EMA and/or HLA has to be considered; an extended workup including repeated testing and a SBB together with clinical follow up is mandatory to establish a definite diagnosis (Figure 1).

Skipping the biopsy is an option but not obligatory thus a SBB can be preferred for diagnosis confirmation despite very high anti-TG2. It remains mandatory if EMA or HLA-DQ2 and -DQ8 is not available.

If patients have positive **anti-TG2 antibodies but levels are lower than 10 times ULN**, a SBB and histological evaluation of the mucosa is mandatory to confirm CD diagnosis and this because low positive anti-TG2 can be related to non-CD conditions such as other autoimmune diseases, infections, tumors, or tissue damage and do not necessarily predict villous atrophy.

In **totally asymptomatic children or adolescents** who are being investigated because of pertaining to high-risk groups or associated conditions, the second algorithm (Figure 2) should be applied. In this group, HLA testing as the first step is probably cost-effective as HLA-DQ2 and -DQ8 negative individuals can be excluded from further follow-up studies, because of a minimal risk of developing CD. If HLA testing is not feasible the screening procedure may start with CD-specific antibody testing.

In HLA-DQ2 or -DQ8 positive individuals IgA anti-TG2 and serum total IgA determination should be performed or the corresponding IgG test in IgA deficient cases (Figure 2). If anti-TG2 are negative, as disease may still develop later in life, serological testing should be repeated at regular intervals. Truly there is no evidence on how frequently the testing should be performed. If high anti-TG2 are found, as persons belonging to this population more

often have false-positive anti-TG2 results, they should always be diagnosed after performing a SBB so as to have histological proof of CD diagnosis and thus support the need for a lifelong adherence to a strict gluten free diet[73]. If anti-TG2 levels are positive but low, that is <3 times ULN, a false-positive result has to be considered. In the absence of any signs or symptoms, follow up while still on a normal gluten-containing diet with repeated serological testing should be advised; in these cases, anti-EMA testing may be helpful to distinguish between false- and true-positive anti-TG2. If EMA is positive, the likelihood for CD increases and the patient should be referred for SBB. If EMA is negative, follow up and repeated testing is advisable.

A simple scoring system (Table 7) was also proposed by the working group which aimed at simplifying the diagnosis in obvious cases, thus enabling CD recognition at initial assessment, even in cases with no initial SBB and to avoid overdiagnosis in patients presenting only with non specific findings[1]. However prospectively validation in a large series of cases is required before it can be routinely recommended in clinical practice.

Another score system proposed by Catassi et al. (Table 8) is further discussed in section 3.2.9.; as compared to the previous one, histology evaluation is required in all cases[82].

3.2. Adults

Despite evidence of increasing rates of diagnosis, CD continues being an infradiagnosed disease in adults. It has been estimated that at least 75% of the cases remain undiagnosed[83]. Furthermore, there is often a delay in the diagnosis of the disease with a mean of 5 to 11 years from the onset of symptoms to diagnosis[18, 84]. These data may be explained by several facts:

1. The classic presentation of the disease is uncommon in adults. The major mode of presentation is diarrhea and nonspecific gastrointestinal symptoms which bear a large degree of overlap with functional dyspepsia, irritable bowel syndrome (IBS) or functional diarrhea[28].

Table 7. Diagnostic score[1]. The scoring takes into account 4 items: symptoms, antibodies, HLA, and biopsy findings, each contributing once. To make the diagnosis, a sum of 4 points is required.

	Points
Symptoms	
Malabsorption syndrome	2
Other CD- relevant symptom OR having T1DM OR being a 1 st-degree family member	1
Asymptomatic	0
Serum antibodies*	
EMA positivity and/ or high positivity (>10 ULN) for anti-TG2	2
Low positivity for anti-TG2 antibodies or isolated anti-DGP positivity	1
Serology was not performed	0
Serology performed but all* coeliac-specific antibodies negative	-1
HLA	
Full HLA- DQ2 (in cis or trans) or HLA-DQ8 heterodimers present	1
No HLA performed OR half DQ2 (only HLA-DQB1* 0202) present	0
HLA neither DQ2 nor DQ8	-1
Histology	
Marsh 3b or 3c (subtotal villous atrophy, flat lesion)	2
Marsh 2 or 3a (moderately decreased villous height/crypt depth ratio) OR Marsh 0-1 plus intestinal TG2 antibodies	1
Marsh 0-1 OR no biopsy performed	0

*Refers in IgA deficiency to IgG class EMA, anti-TG2 and DGP antibodies.

Comments and Explanations for Use.

*Biopsy items were graded by taking into account Villanacci scoring and the clinical utility of the results. We assumed that Marsh 0 or 1 results without any further information could be nonspecific. In contrast, demonstration of antibodies bound to tissue TG2 in the small bowel adds information to the diagnosis (when available). It is possible to diagnose CD as before even without this possibility. It is not necessary to have an EMA testing facility, but it is a clear advantage.

*Some findings that make CD improbable are resulting in negative scoring points.

*The sum of 4 points may be collected from findings registered at different time points during follow-up if they can be assumed to be gluten dependent. For example, an infant having villous atrophy before the introduction of gluten and normal biopsy at the age of 6 years while normally eating gluten will receive 0 for biopsy.

Table 8. Diagnostic criteria for Celiac Disease according to Catassi et al.[82].

	At least 4 out of 5, or 3 out of 4 if there are no HLA genotypes
1	Typical symptoms of celiac disease[1]
2	Positivity of serum celiac disease IgA class autoantibodies at high titer[2]
3	HLA DQ2 or DQ8 genotypes[3]
4	Celiac enteropathy at the small intestinal biosy[4]
5	Response to the gluten-free diet[5]

Notes: A family history of celiac disease adds evidence to the diagnosis; in symptom-free patients, particularly young children, it is advisable to confirm antibody positivity on 2 or more blood samples taken at least 3 months apart; in selected cases a gluten challenge after at least 2 years of gluten-free diet might be required for diagnosis confirmation.
[1] Examples of typical symptoms are chronic diarrhea, growth delay (children) or weight loss (adults) or iron deficiency anemia.
[2] Both IgA class anti-TG and EMA in IgA-sufficient or IgG class anti-TG and EMA in IgA-deficient subjects. The finding of IgG class anti-deamidated gliadin peptide adds evidence to the diagnosis.
[3] HLA-DQ2 positivity includes subjects with only half the heterodimer (positive HLA-DQB1*02).
[4] Including Marsh-Oberhuber 3 lesions, Marsh Oberhuber 1-2 lesions associated with positive celiac at low/high titer, or Marsh-Oberhuber 1-3 lesions associated with IgA subepithelial deposits.
[5] Histological in patients with sero-negative celiac disease or associated IgA deficiency.

2. Some antecedents, such as growth failure in childhood, iron-deficiency anemia, premature reduced bone mineral density, recurrent oral apthous, dermatological lesions or infertility may be often overlooked. Gastroenterologist's questions have focused exclusively on gastrointestinal symptoms, forgetting that CD is a disorder with a multisystemic expression.
3. Currently, active case-finding (serologic testing for CD in patients with symptoms or conditions closely associated with CD) is the favored strategy to increase detection of CD. However many adults with CD has mild forms of enteropathy (Marsh 1, 2 and 3a) in which a positive result of CD-specific serology may be lower than 30%[85,86].

Consequently, active case-finding may increase detection of CD among patients with symptoms although this strategy might be insufficient to detect all adults with CD[10].

With the objective of improve the recognition and diagnosis of CD several guides to clinical practice have been published[12-15]. In general, these guidelines recommend offering serologic testing for CD in patients with symptoms or conditions closely associated with CD (Table 2). The confirmation of a diagnosis of CD should be based on a combination of findings from the clinical scenario, CD-specific antibodies, upper endoscopy with duodenal biopsies, HLA-DQ2/DQ8 genotyping, and the response to a GFD. A summary of the specific recommendations from these guidelines to improve the diagnosis of CD is showed below.

3.2.1. When to Test for Celiac Disease?

There is no consensus regarding which symptoms, laboratory abnormalities, and/or associated diseases require evaluation for CD. The frequency of CD in common clinical scenarios varies from modestly elevated, such as irritable bowel syndrome, to substantially elevated, such as unexplained iron-deficiency anemia. Clinical guidelines[13,14] recommends to offer serological testing in patients with conditions in which CD occurs more frequently than in the general population and/or for whom a GFD may be beneficial (Table 2).

1. Patients with symptoms, signs, or laboratory evidence suggestive of malabsorption, such as chronic diarrhea with weight loss, steathorrhea, postprandial abdominal pain, and bloating.
2. Patients with unexplained gastrointestinal symptoms including dyspepsia, nausea and vomiting or recurrent abdominal pain.
3. Patients with extraintestinal symptoms such as unexplained iron-deficiency anemia, or other unspecified anemia, premature reduced bone mineral density, elevated serum aminotransferase levels when no other etiology is found or recurrent oral apthous ulcers.

4. Patients with any of the following conditions: Dermatitis herpetiformis, irritable bowel syndrome, autoimmune thyroid disease, type 1 diabetes, peripheral neuropathy, growth failure, discolored teeth or developmentally synchronous enamel loss, Down's and Turner's syndromes. Considerer offering serological test in the rest of associated conditions
5. Patients with a first-degree family member (parents, siblings or children) who has confirmed diagnosis of CD, specially if the show possible signs or symptoms or laboratory evidence of CD

3.2.2. How to Make the Diagnosis of Celiac Disease?

As in children, serologic testing of CD-specific antibodies is the preferred initial approach to find CD in adults, and TG2-based assays (EMA and anti-TG2) the most accurate tests. The sensitivity and specificity of the IgA anti-TG2 for untreated CD is about 95%, but its sensitivity is lower in case of mild histological lesions (no villous atrophy)[85,86]. The higher the titer of the test, the greater the likelihood of a true positive result. There are recognized differences in test performance between the various commercially available test kits, but overall there is consistency in the sensitivity and specificity of the test[87]. Antibodies directed against native gliadin are not recommended for the primary detection of CD[14].

IgA deficiency is more common in CD than in the general population. In patients in whom there is a high pre-test prevalence of CD, the measurement of IgA levels should be considered, especially if IgA-based celiac serology test is negative. One approach is to measure total IgA at the beginning of testing to determine whether IgA levels are sufficient and, if not, to incorporate IgG-based testing into the serology testing cascade (DGP-IgG and/or IgG anti-TG2)[14].

The antibodies directed against deaminated gliadin products as well as the self-antigen TG2 are dependent on the ingestion of gluten. The reduction or cessation of dietary gluten leads to a decrease in the levels of all these celiac-associated antibodies to normal concentrations. Therefore, all diagnostic

serologic testing should be done with patients on a gluten-containing diet. Combining several tests for CD in lieu of IgA anti-TG2 alone may marginally increase the sensitivity for CD but reduces specificity and therefore are not recommend in low-risk populations[14].

If the suspicion of CD is high, intestinal biopsy should be pursued even when serologies are negative (Figure 3).

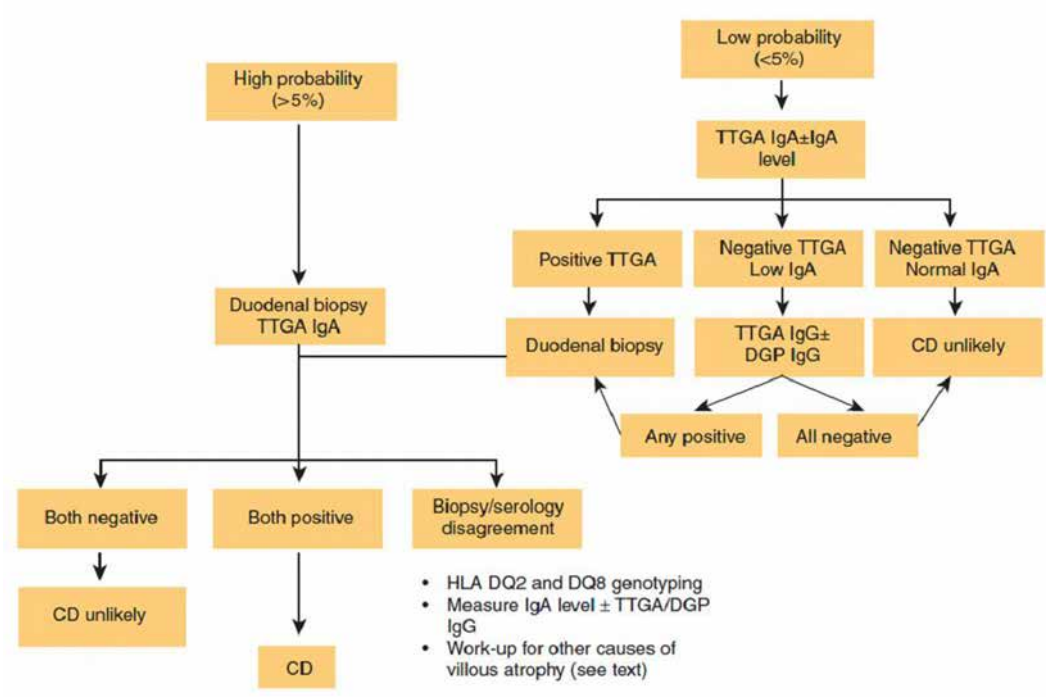


Figure 3. Celiac disease diagnostic testing algorithm according to American Journal Gastroenterology Clinical Guideline[14]. DGP: deamidated gliadin peptide; HLA: human leukocyte antigen; Ig: immunoglobulin; TTGA: tissue transglutaminase antibody. Reprinted by permission from Macmillan Publishers Ltd: American Journal Gastroenterology, Rubio-Tapia A et al, ACG Clinical Guidelines: Diagnosis and Management of Celiac Disease, 2013.

3.2.3. Confirmatory Testing in Celiac Disease

The confirmation of a diagnosis of CD should be based on a combination of findings from the medical history, physical examination, serology, and upper endoscopy with histological analysis of multiple biopsies of the duodenum[14].

Upper endoscopy with small bowel biopsy is a critical component of the diagnostic evaluation for persons with suspected CD and is recommend to confirm the diagnosis. Histological changes associated with the disease can be classified according to Marsh, Marsh modified (Oberhuber), or the more recent, simplified Corazza classification (Table 6)[88]. A positive CD-specific serology in patients with villous atrophy confirms the diagnosis of CD. However, a negative CD-specific serology in patients with enteropathy does not completely exclude the diagnosis of CD though it does make it much less likely. Histological response to GFD and HLA genotyping may help to rule out or confirm the diagnosis of CD in patients with sero-negative CD[14].

Histological abnormalities associated with CD can be patchy, therefore multiple biopsies of the duodenum (one or two biopsies of the bulb and at least four biopsies of the distal duodenum) are recommended to confirm the diagnosis of CD[89, 90]. Lymphocytic infiltration of the intestinal epithelium in the absence of villous atrophy is not specific for CD and other causes should be considered (Table 5)[91, 92].

The diagnosis may be confirmed when there is concordance between the serologic results and the histological findings and the symptoms resolve subsequently on a GFD. However, there are other situations in which it is possible to establish a diagnosis of CD although the result of CD-specific serology is negative[82].

3.2.4. Role of HLA-DQ2/DQ8 Genotyping in Celiac Disease

HLA-DQ2/DQ8 heterodimers are present in almost all patients with CD. Testing negative for both HLA-DQ genotypes makes CD diagnosis very unlikely. Among patients not carrying these heterodimers, the majority encoded half of the HLA-DQ2 heterodimer. Because HLA-DQ2 is present in

approximately 25-30% of the white population, testing for CD with either HLA-DQ type should not be used routinely in the initial diagnosis of CD because the predictive positive value is very low[14].

HLA-DQ2/DQ8 testing has been useful for exclusion of CD in selected clinical situations[14]. Examples of such clinical situations include but are not limited to: 1) Equivocal small-bowel histological finding (Marsh 1 or 2) in seronegative patients[93]; 2) Evaluation of patients on a GFD in whom no testing for CD was done before GFD[94]; 3) Patients with discrepant CD-specific serology and histology[95]; 4) Patients with suspicion of refractory CD where the original diagnosis of celiac remains in question; 5) Screening of CD in at-risk groups such as persons affected by Down' syndrome.[96]. The utility of HLA testing in other at-risk groups (such as type I diabetics or family members) is more limited because a high proportion of these subjects carry the CD susceptibility alleles.

3.2.5. Patients With Enteropathy But Negative CD-Specific Serology

This is a matter of crucial importance, especially in the adult population. In fact, the true prevalence of CD in this population has been underestimated, because both in population screening programs, as in symptomatic or high genetic risk people, intestinal biopsy is indicated only for positive serology. However, there is evidence that serology sensitivity is lower among adults with mild histological lesions (no villous atrophy; Marsh-Oberhuber 1 and 2)[85, 86].

The presence of mild histological lesions represents a difficult to interpret "gray area". Current data suggest that patients with lymphocytic duodenosis (>25 IELs per 100 epithelial cells), may suffer from gastrointestinal and extraintestinal symptoms, such as osteopenia or anemia, as frequently as patients with villous atrophy[97,98]. However, It should be noted that lymphocytic duodenosis, is common in the general population (prevalence of 5.4%)[99] and there are conditions other than CD in which lymphocytic duodenosis is possible. Examples of these include *H.pylori* infection,

medications (e.g., non-steroidal anti-inflammatory drugs), small-bowell bacterial overgrowth, food protein intolerance or autoimmune disorders[91,92]. Furthermore, celiac serology is positive only in 10-30% of patients with lymphocytic duodenosis secondary to CD. Consequently, diagnosis of CD in these patients is not easy and requires the following conditions[93]: First, it is necessary to exclude other possible etiologies such as *H. pylori* infection, medications or small-bowell bacterial overgrowth; Second, to prove the presence of either HLA-DQ2 or –DQ8 heterodimers; Finally, an unequivocal clinical and histological response to a gluten-free diet. The subset characterization of $\gamma\gamma\delta$+ IELs by immunohistochemical analysis or flow cytometry, as well as the presence of IgA anti-TG2 subepithelial deposits in the mucosa seem to be specific for CD[100,101]. However, these techniques require frozen or fresh nonfixed biopsies, and they are not straightforward for use in clinical practice.

3.2.6. Positive CD-Specific Serology But Absence of Enteropathy

False positive anti-TG2 results are rare but do occur and are usually low titer (typically less than twice the upper limit of normal). Repeating the test using an assay that uses human TG2 as the capture antigen may resolve the discrepancy. The duodenal biopsy should be reviewed by a pathologist familiar with CD to look for subtle abnormalities.

If these two steps do not reconcile the results, the patient can be placed on a high gluten diet and, after 6 to 12 weeks, it should be repeated the upper endoscopy with multiple additional biopsies of bulb and distal duodenum. Patients with positive serologic test and only mild histological lesions may respond to a GFD[102]. HLA-DQ2/DQ8 genotyping may also be useful for CD diagnosis in these patients with positive celiac-specific serology and normal duodenal histology[95].

3.2.7. Diagnosis Among Patients on a Gluten Free Diet

While standard diagnostic tests (specific serology and intestinal biopsy) have a high positive predictive value for CD, they should not be relied upon to exclude CD in patients already adhering to a GFD. The specific serologic and histologic features of CD do not normalize immediately upon the initiation of a GFD, but some patients might quickly revert to normal on a GFD. Hence, normal serologic and histologic findings on a GFD cannot be used to exclude CD definitively[14].

HLA-DQ2/DQ8 genotyping are not influenced by diet and can be used to evaluate the likelihood of CD in patients either on a normal or on a GFD. HLA-DQ2/DQ8 testing should be performed to try to exclude CD prior to embarking on a formal GC as a negative result will obviate the need for further workup[14].

Gluten challenge remains the gold standard for CD diagnosis in HLA-DQ2 or –DQ8-positive patients who have normal serologic and histologic findings when tested on a GFD. It must be noted that patients who develop severe symptoms following gluten ingestion are not suitable candidates for GC. Although gluten challenge with a diet containing at least 10 g of gluten per day for 6-8 weeks has long been the norm, there are few data to indicate the diagnostic efficacy of this approach or the optimum dose or duration of challenge[103].

Despite the disadvantages of neither confirming nor excluding a diagnosis of CD, some patients will opt to continue on a strictly GFD without undergoing formal gluten challenge; such patients should be managed in a similar fashion to those with known CD[14].

3.2.8. Differentiation of Celiac Disease from Non-celiac Gluten Sensitivity

Celiac disease should be differentiated from non-celiac gluten sensitivity in order to identify the risk for nutritional deficiency states, complications of CD, risk for CD and associated disorders in family members, and to influence the degree and duration of adherence to the GFD. Symptoms or symptoms

response to a GFD alone should not be used to diagnose CD, as there is often substantial overlap in symptoms between the two conditions. A diagnosis of non-celiac gluten sensitivity should be considered only after CD has been excluded with appropriate testing[14]. Objective tests including CD-specific serology and small-intestinal histology (both obtained while the patient is consuming a gluten-rich diet) and HLA-DQ2/DQ8 testing (to rule out CD if negative) are needed to differentiate between the two disorders[104].

3.2.9. Celiac Disease Diagnostic Approach in Clinical Practice

The diagnostic approach to an adult patient with suspected CD is complex, given the diversity of possible clinical settings. Figure 3 shows CD diagnostic testing algorithm proposed by ACG clinical guideline[14]. CD-specific serology (anti-TG2, EMA or anti-DGP) should be the initial diagnostic test to be performed in patients with signs, symptoms and/or conditions associated to CD.

When IgA anti-TG2 titers are higher than 10 times the upper limit of normal, the intestinal biopsy could be excluded, since the probability of detecting villous atrophy is quite high. Hills et al.[67] showed in adults that a IgA anti-TG2 level > 30U/ml (>10 UNL) using the Celikey test kit is absolutely predictive for CD (positive predictive value of 100%). Before taking this decision it is prudent to investigate and confirm the presence of EMA (performing the extraction at a different time of the first time) and checking for the HLA-DQ2/DQ8 heterodimers, since a positive result reinforces the diagnosis. In contrast, when IgA anti-TG2 level are lower than 10 UNL, multiple biopsies of duodenum should be performed, including one or two biopsies of the bulb (either 9- or 12-oclock position) and at least four biopsies of post-bulbar duodenum (2 bulb biopsies and 4 duodenal 2nd portion biopsies). If the histological results show enteropathy, a GFD should be started.

Further assessment is needed when specific serologic tests are negative but clinical suspicion of CD is high. In this situation, patients should undergo an upper endoscopy with duodenal biopsies to confirm the diagnosis of CD,

because sensitivity of CD-specific serology is lower among adults with non-atrophic lesions. HLA-DQ2/DQ8 genotyping might be useful to evaluate the likelihood of CD in these patients and performed intestinal biopsy only in HLA-DQ2 or –DQ8-positive patients. In patients with enteropathy but negative serologic test negative, HLA-DQ2/DQ8 genotyping might be useful to confirm or exclude a diagnosis of CD because testing negative for both HLA-DQ types makes diagnosis very unlikely[93].

It should be considered that, in any case, serology, genetic testing or duodenal biopsy results are pathognomonic. This means that in, certain cases, it is extremely difficult to confirm or rule out the disease. The wide variability of CD related findings suggests that it is difficult to conceptualize the diagnostic process into rigid algorithms that do not always cover the whole spectrum of clinical situations. Sometimes, it may be preferable the application of simple rules, which, in the hands of an experienced gastroenterologist, may be equally efficient. In this sense, Catassi and Fasano[82] proposed a 5-point scoring system that incorporates: 1) symptoms of CD; 2) positive CD serologies at high titer; 3) the presence of a HLA-DQ2 or -DQ8 haplotype; 4) characteristic histopathologic findings; and 5) a serologic or histologic response to the GFD. The presence of 4 out of the 5 criteria (or 3 out of 4, if HLA-DQ2/DQ8 testing is not performed) would meet diagnostic criteria for CD according to this proposed system, which has not yet been validated prospectively (Table 8).

References

1. Husby S, Koletzko S, Korponay-Szabo IR, Mearin ML, Phillips A, Shamir R et al. *European Society for Pediatric Gastroenterology, Hepatology, and Nutrition guidelines for the diagnosis of coeliac disease.* J Pediatr Gastroenterol Nutr. 2012; 54: 136-60.
   http://dx.doi.org/10.1097/MPG.0b013e31821a23d0
   PMid:22197856
2. Dicke WK. *Treatment of celiac disease.* Ned Tijdschr Geneeskd. 1951; 95: 124-30.
   PMid:14806692
3. Karell K, Louka AS, Moodie SJ, Ascher H, Clot F, Greco L et al. *HLA types in celiac disease patients not carrying the DQA1*05-DQB1*02 (DQ2) heterodimer: results from the European Genetics Cluster on Celiac Disease.* Hum Immunol. 2003; 64: 469-77.
   http://dx.doi.org/10.1016/S0198-8859(03)00027-2
4. Petersen J, Montserrat V, Mujico JR, Loh KL, Beringer DX, van Lummel M et al. *T-cell receptor recognition of HLA-DQ2-gliadin complexes associated with celiac disease.* Nat Struct Mol Biol. 2014; 21: 480-8.
   http://dx.doi.org/10.1038/nsmb.2817
   PMid:24777060
5. de Meij TG, Budding AE, Grasman ME, Kneepkens CM, Savelkoul PH, Mearin ML. *Composition and diversity of the duodenal mucosa-associated microbiome in children with untreated coeliac disease.* Scand J Gastroenterol. 2013; 48: 530-6.
   http://dx.doi.org/10.3109/00365521.2013.775666
   PMid:23534388
6. Lebwohl B, Ludvigsson JF, Green PH. *The unfolding story of celiac disease risk factors.* Clin Gastroenterol Hepatol. 2014; 12: 632-5.
   http://dx.doi.org/10.1016/j.cgh.2013.10.031
   PMid:24211288 PMCid:PMC3960307
7. Steens RF, Csizmadia CG, George EK, Ninaber MK, Hira Sing RA, Mearin ML. *A national prospective study on childhood celiac disease in the Netherlands 1993-2000: an increasing recognition and a changing clinical picture.* J Pediatr. 2005; 147: 239-43.
   http://dx.doi.org/10.1016/j.jpeds.2005.04.013
   PMid:16126057
8. Csizmadia CG, Mearin ML, von Blomberg BM, Brand R, Verloove-Vanhorick SP. *An iceberg of childhood coeliac disease in the Netherlands.* Lancet. 1999; 353: 813-4.
   http://dx.doi.org/10.1016/S0140-6736(99)00243-3
9. Schweizer JJ, von Blomberg BM, Bueno-de Mesquita HB, Mearin ML. *Coeliac disease in The Netherlands.* Scand J Gastroenterol. 2004; 39: 359-64.
   http://dx.doi.org/10.1080/00365520310008503
   PMid:15125468

10. Catassi C, Kryszak D, Louis-Jacques O, Duerksen DR, Hill I, Crowe SE et al. *Detection of Celiac disease in primary care: a multicenter case-finding study in North America.* Am J Gastroenterol. 2007; 102: 1454-60.

http://dx.doi.org/10.1111/j.1572-0241.2007.01173.x
PMid:17355413

11. Ivarsson A, Myleus A, Norstrom F, van der Pals M, Rosen A, Hogberg L et al. *Prevalence of childhood celiac disease and changes in infant feeding.* Pediatrics. 2013; 131: e687-94.

http://dx.doi.org/10.1542/peds.2012-1015
PMid:23420914

12. Rostom A, Murray JA, Kagnoff MF. *American Gastroenterological Association (AGA) Institute technical review on the diagnosis and management of celiac disease.* Gastroenterology. 2006; 131: 1981-2002.

http://dx.doi.org/10.1053/j.gastro.2006.10.004
PMid:17087937

13. National Institute for Health and Clinical Excellence. *Coeliac disease: recognition and assessment of coeliac disease.* London: National Institute for Health and Clinical Excellence. 2009. www.nice.org.uk/CG86

14. Rubio-Tapia A, Hill ID, Kelly CP, Calderwood AH, Murray JA. *ACG Clinical Guidelines: Diagnosis and Management of Celiac Disease.* Am J Gastroenterol. 2013; 108: 656-76.

http://dx.doi.org/10.1038/ajg.2013.79
PMid:23609613 PMCid:PMC3706994

15. Ludvigsson JF, Bai JC, Biagi F, Card TR, Ciacci C, Ciclitira PJ et al. *Diagnosis and management of adult coeliac disease: guidelines from the British Society of Gastroenterology.* Gut. 2014; 63: 1210-28.

http://dx.doi.org/10.1136/gutjnl-2013-306578
PMid:24917550 PMCid:PMC4112432

16. Sapone A, Bai JC, Ciacci C, Dolinsek J, Green PH, Hadjivassiliou M et al. *Spectrum of gluten-related disorders: consensus on new nomenclature and classification.* BMC Med. 2012; 10: 13.

http://dx.doi.org/10.1186/1741-7015-10-13
PMid:22313950 PMCid:PMC3292448

17. Ludvigsson JF, Leffler DA, Bai JC, Biagi F, Fasano A, Green PH et al. *The Oslo definitions for coeliac disease and related terms.* Gut. 2013; 62: 43-52.

http://dx.doi.org/10.1136/gutjnl-2011-301346
PMid:22345659 PMCid:PMC3440559

18. Sanders DS, Hurlstone DP, Stokes RO, Rashid F, Milford-Ward A, Hadjivassiliou M et al. *Changing face of adult coeliac disease: experience of a single university hospital in South Yorkshire.* Postgrad Med J. 2002; 78: 31-3.

http://dx.doi.org/10.1136/pmj.78.915.31
PMid:11796869 PMCid:PMC1742229

19. Vivas S, Ruiz de Morales JM, Fernandez M, Hernando M, Herrero B, Casqueiro J et al. *Age-related clinical, serological, and histopathological features of celiac disease.* Am J Gastroenterol. 2008; 103: 2360-5.
http://dx.doi.org/10.1111/j.1572-0241.2008.01977.x
PMid:18702652

20. McGowan KE, Castiglione DA, Butzner JD. *The Changing Face of Childhood Celiac Disease in North America: Impact of Serological Testing.* Pediatrics. 2009; 124: 1572-1578.
http://dx.doi.org/10.1542/peds.2008-2373
PMid:19948628

21. Reilly NR, Fasano A, Green PH. *Presentation of celiac disease.* Gastrointest Endosc Clin N Am. 2012; 22: 613-21.
http://dx.doi.org/10.1016/j.giec.2012.07.008
PMid:23083982

22. Pelleboer RA, Janssen RL, Deckers-Kocken JM, Wouters E, Nissen AC, Bolz WE et al. *Celiac disease is overrepresented in patients with constipation.* J Pediatr (Rio J). 2012; 88: 173-6.
http://dx.doi.org/10.2223/JPED.2155

23. Reilly NR, Aguilar K, Hassid BG, Cheng J, Defelice AR, Kazlow P et al. *Celiac disease in normal-weight and overweight children: clinical features and growth outcomes following a gluten-free diet.* J Pediatr Gastroenterol Nutr. 2013; 53: 528-31.

24. Schaart MW, Mearin ML. *Early nutrition: prevention of celiac disease?* J Pediatr Gastroenterol Nutr. 2014; 59 Suppl 1: S18-20.
http://dx.doi.org/10.1097/01.mpg.0000450398.53650.f4
PMid:24979195

25. Ivarsson A, Persson LA, Nystrom L, Ascher H, Cavell B, Danielsson L et al. *Epidemic of coeliac disease in Swedish children.* Acta Paediatr. 2000; 89: 165-71.
http://dx.doi.org/10.1111/j.1651-2227.2000.tb01210.x
PMid:10709885

26. Vriezinga SL, Auricchio R, Bravi E, Castillejo G, Chmielewska A, Crespo Escobar P et al. *Randomized feeding intervention in infants at high risk for celiac disease.* N Engl J Med. 2014; 371: 1304-15.
http://dx.doi.org/10.1056/NEJMoa1404172
PMid:25271603

27. Lionetti E, Castellaneta S, Francavilla R, Pulvirenti A, Tonutti E, Amarri S et al. *Introduction of gluten, HLA status, and the risk of celiac disease in children.* N Engl J Med. 2014; 371: 1295-303.
http://dx.doi.org/10.1056/NEJMoa1400697
PMid:25271602

28. Green PH. *The many faces of celiac disease: clinical presentation of celiac disease in the adult population.* Gastroenterology. 2005; 128: S74-8.

http://dx.doi.org/10.1053/j.gastro.2005.02.016
PMid:15825130

29. M. Domínguez-Cajal S, Santolaria-Piedrafita I, Lera-Omiste B, Cuartero-Cuenca G, Pérez-Clavijo T. Guevara-López et al. *Patrones de presentación en una serie retrospectiva de pacientes con enfermedad celíaca según la nomenclatura aceptada en el consenso de Oslo, 2012. El comienzo del proyecto aretaea.* Gastroenterologia y Hepatologia. 2013; 36: 216.

30. Ciacci C, Cirillo M, Sollazzo R, Savino G, Sabbatini F, Mazzacca G. *Gender and clinical presentation in adult celiac disease.* Scand J Gastroenterol. 1995; 30: 1077-81.

http://dx.doi.org/10.3109/00365529509101610
PMid:8578167

31. Zipser RD, Patel S, Yahya KZ, Baisch DW, Monarch E. *Presentations of adult celiac disease in a nationwide patient support group.* Dig Dis Sci. 2003; 48: 761-4.

http://dx.doi.org/10.1023/A:1022897028030
PMid:12741468

32. Ford AC, Ching E, Moayyedi P. *Meta-analysis: yield of diagnostic tests for coeliac disease in dyspepsia.* Aliment Pharmacol Ther. 2009; 30: 28-36.

http://dx.doi.org/10.1111/j.1365-2036.2009.04008.x
PMid:19416130

33. Santolaria S, Alcedo J, Cuartero B, Diez I, Abascal M, Garcia-Prats MD et al. *Spectrum of gluten-sensitive enteropathy in patients with dysmotility-like dyspepsia.* Gastroenterol Hepatol. 2013; 36: 11-20.

http://dx.doi.org/10.1016/j.gastrohep.2012.07.011
PMid:23103052

34. O'Leary C, Wieneke P, Buckley S, O'Regan P, Cronin CC, Quigley EM et al. *Celiac disease and irritable bowel-type symptoms.* Am J Gastroenterol. 2002; 97: 1463-7.

http://dx.doi.org/10.1111/j.1572-0241.2002.05690.x
PMid:12094866

35. Ford AC, Chey WD, Talley NJ, Malhotra A, Spiegel BMR, Moayyedi P. *Yield of Diagnostic Tests for Celiac Disease in Individuals With Symptoms Suggestive of Irritable Bowel Syndrome Systematic Review and Meta-analysis.* Archives of Internal Medicine. 2009; 169: 651-658.

http://dx.doi.org/10.1001/archinternmed.2009.22
PMid:19364994

36. Fernandez-Bañares F, Esteve M, Salas A, Alsina M, Farre C, Gonzalez C et al. *Systematic evaluation of the causes of chronic watery diarrhea with functional characteristics.* Am J Gastroenterol. 2007; 102: 2520-8.

http://dx.doi.org/10.1111/j.1572-0241.2007.01438.x
PMid:17680846

37. Nachman F, Vazquez H, Gonzalez A, Andrenacci P, Compagni L, Reyes H et al. *Gastroesophageal reflux symptoms in patients with celiac disease and the effects of a gluten-free diet.* Clin Gastroenterol Hepatol. 2011; 9: 214-9.
http://dx.doi.org/10.1016/j.cgh.2010.06.017
PMid:20601132

38. Usai P, Manca R, Cuomo R, Lai MA, Russo L, Boi MF. *Effect of gluten-free diet on preventing recurrence of gastroesophageal reflux disease-related symptoms in adult celiac patients with nonerosive reflux disease.* J Gastroenterol Hepatol. 2008; 23: 1368-1372.
http://dx.doi.org/10.1111/j.1440-1746.2008.05507.x
PMid:18853995

39. Crowe SE. *In the clinic. Celiac disease.* Ann Intern Med. 2011; 154: ITC51-ITC5-15; quiz ITC5-16.

40. Meeuwisse GW. *Diagnostic criteria in coeliac disease.* Acta Paediatr Scand 1970; 59: 461-463.

41. Guandalini S, Ventura A, Ansaldi N, Giunta AM, Greco L, Lazzari R et al. *Diagnosis of coeliac disease: time for a change?* Arch Dis Child. 1989; 64: 1320-4; discussion 1324-5.
http://dx.doi.org/10.1136/adc.64.9.1320
PMid:2817956 PMCid:PMC1792735

42. Working Group of European Society of Paediatric Gastroenterology and Nutrition. *Revised criteria for diagnosis of coeliac disease.* Report. Arch Dis Child. 1990; 65: 909-11.
http://dx.doi.org/10.1136/adc.65.8.909
PMid:2205160 PMCid:PMC1792502

43. Koninckx CR, Giliams JP, Polanco I, Pena AS. *IgA antigliadin antibodies in celiac and inflammatory bowel disease.* J Pediatr Gastroenterol Nutr. 1984; 3: 676-82.
http://dx.doi.org/10.1097/00005176-198411000-00006
PMid:6502368

44. Troncone R, Ferguson A. *Anti-gliadin antibodies.* J Pediatr Gastroenterol Nutr. 1991; 12: 150-8.
http://dx.doi.org/10.1097/00005176-199102000-00002
PMid:2051264

45. Grodzinsky E, Jansson G, Skogh T, Stenhammar L, Falth-Magnusson K. *Anti-endomysium and anti-gliadin antibodies as serological markers for coeliac disease in childhood: a clinical study to develop a practical routine.* Acta Paediatr. 1995; 84: 294-8.
http://dx.doi.org/10.1111/j.1651-2227.1995.tb13631.x
PMid:7780251

46. Rossi TM, Albini CH, Kumar V. *Incidence of celiac disease identified by the presence of serum endomysial antibodies in children with chronic diarrhea, short stature, or insulin-dependent diabetes mellitus.* J Pediatr. 1993; 123: 262-4.
http://dx.doi.org/10.1016/S0022-3476(05)81699-3

47. de Lecea A, Ribes-Koninckx C, Polanco I, Calvete JF. *Serological screening (antigliadin and antiendomysium antibodies) for non-overt coeliac disease in children of short stature.* Acta Paediatr. Suppl 1996; 412: 54-5.

http://dx.doi.org/10.1111/j.1651-2227.1996.tb14252.x
PMid:8783760

48. Korponay-Szabo IR, Kovacs JB, Czinner A, Goracz G, Vamos A, Szabo T. *High prevalence of silent celiac disease in preschool children screened with IgA/IgG antiendomysium antibodies.* J Pediatr Gastroenterol Nutr. 1999; 28: 26-30.

http://dx.doi.org/10.1097/00005176-199901000-00008
PMid:9890464

49. Dieterich W, Ehnis T, Bauer M, Donner P, Volta U, Riecken EO et al. *Identification of tissue transglutaminase as the autoantigen of celiac disease.* Nat Med. 1997; 3: 797-801.

http://dx.doi.org/10.1038/nm0797-797
PMid:9212111

50. Troncone R, Maurano F, Rossi M, Micillo M, Greco L, Auricchio R et al. *IgA antibodies to tissue transglutaminase: An effective diagnostic test for celiac disease.* J Pediatr. 1999; 134: 166-71.

http://dx.doi.org/10.1016/S0022-3476(99)70410-5

51. Rostom A, Dube C, Cranney A, Saloojee N, Sy R, Garritty C et al. *The diagnostic accuracy of serologic tests for celiac disease: a systematic review.* Gastroenterology. 2005; 128: S38-46.

http://dx.doi.org/10.1053/j.gastro.2005.02.028
PMid:15825125

52. Prause C, Ritter M, Probst C, Daehnrich C, Schlumberger W, Komorowski L et al. *Antibodies against deamidated gliadin as new and accurate biomarkers of childhood coeliac disease.* J Pediatr Gastroenterol Nutr. 2009; 49: 52-8.

http://dx.doi.org/10.1097/MPG.0b013e318195dae3
PMid:19465869

53. Barker CC, Mitton C, Jevon G, Mock T. *Can tissue transglutaminase antibody titers replace small-bowel biopsy to diagnose celiac disease in select pediatric populations?* Pediatrics. 2005; 115: 1341-6.

http://dx.doi.org/10.1542/peds.2004-1392
PMid:15867045

54. Donaldson MR, Book LS, Leiferman KM, Zone JJ, Neuhausen SL. *Strongly positive tissue transglutaminase antibodies are associated with Marsh 3 histopathology in adult and pediatric celiac disease.* J Clin Gastroenterol. 2008; 42: 256-60.

http://dx.doi.org/10.1097/MCG.0b013e31802e70b1

55. Dahlbom I, Korponay-Szabo IR, Kovacs JB, Szalai Z, Maki M, Hansson T. *Prediction of clinical and mucosal severity of coeliac disease and dermatitis herpetiformis by quantification of IgA/IgG serum antibodies to tissue transglutaminase.* J Pediatr Gastroenterol Nutr. 2010; 50: 140-6.
http://dx.doi.org/10.1097/MPG.0b013e3181a81384
PMid:19841593

56. Mearin ML, Koninckx CR, Biemond I, Polanco I, Pena AS. *Influence of genetic factors on the serum levels of antigliadin antibodies in celiac disease.* J Pediatr Gastroenterol Nutr. 1984; 3: 373-7.
http://dx.doi.org/10.1097/00005176-198406000-00012
PMid:6588183

57. Sollid LM, Markussen G, Ek J, Gjerde H, Vartdal F, Thorsby E. *Evidence for a primary association of celiac disease to a particular HLA-DQ alpha/beta heterodimer.* J Exp Med. 1989; 169: 345-50.
http://dx.doi.org/10.1084/jem.169.1.345
PMid:2909659

58. Margaritte-Jeannin P, Babron MC, Bourgey M, Louka AS, Clot F, Percopo S et al. *HLA-DQ relative risks for coeliac disease in European populations: a study of the European Genetics Cluster on Coeliac Disease.* Tissue Antigens. 2004; 63: 562-7.
http://dx.doi.org/10.1111/j.0001-2815.2004.00237.x
PMid:15140032

59. Wolters VM, van de Nadort C, Gerritsen SA, Kneepkens CM, Ten Kate FJ, Gijsbers CF et al. *Is gluten challenge really necessary for the diagnosis of coeliac disease in children younger than age 2 years?* J Pediatr Gastroenterol Nutr. 2009; 48: 566-70.
http://dx.doi.org/10.1097/MPG.0b013e31818c526b
PMid:19367182

60. Ribes-Koninckx C, Mearin ML, Korponay-Szabo IR, Shamir R, Husby S, Ventura A et al. *Coeliac disease diagnosis: ESPGHAN 1990 criteria or need for a change? Results of a questionnaire.* J Pediatr Gastroenterol Nutr. 2011; 54: 15-9.
http://dx.doi.org/10.1097/MPG.0b013e31822a00bb
PMid:21716133

61. Giersiepen K, Lelgemann M, Stuhldreher N, Ronfani L, Husby S, Koletzko S et al. *Accuracy of diagnostic antibody tests for coeliac disease in children: summary of an evidence report.* J Pediatr Gastroenterol Nutr. 2012; 54: 229-41.
http://dx.doi.org/10.1097/MPG.0b013e318216f2e5
PMid:22266486

62. Klapp G, Masip E, Bolonio M, Donat E, Polo B, Ramos D et al. *Celiac disease: the new proposed ESPGHAN diagnostic criteria do work well in a selected population.* J Pediatr Gastroenterol Nutr. 2013; 56: 251-6.
http://dx.doi.org/10.1097/MPG.0b013e318279887b
PMid:23111763

63. Lewis NR, Scott BB. *Systematic review: the use of serology to exclude or diagnose coeliac disease (a comparison of the endomysial and tissue transglutaminase antibody tests).* Aliment Pharmacol Ther. 2006; 24: 47-54.

http://dx.doi.org/10.1111/j.1365-2036.2006.02967.x
PMid:16803602

64. Kaukinen K, Maki M, Partanen J, Sievanen H, Collin P. *Celiac disease without villous atrophy: revision of criteria called for.* Dig Dis Sci. 2001; 46: 879-87.

http://dx.doi.org/10.1023/A:1010729207320
PMid:11330428

65. Kurppa K, Ashorn M, Iltanen S, Koskinen LLE, Saavalainen P, Koskinen O et al. *Celiac Disease without Villous Atrophy in Children: A Prospective Study.* Journal of Pediatrics. 2010; 157: 373-U48.

66. Vivas S, Ruiz de Morales JG, Riestra S, Arias L, Fuentes D, Alvarez N et al. *Duodenal biopsy may be avoided when high transglutaminase antibody titers are present.* World J Gastroenterol. 2009; 15: 4775-80.

http://dx.doi.org/10.3748/wjg.15.4775
PMid:19824110 PMCid:PMC2761554

67. Hill PG, Holmes GK. *Coeliac disease: a biopsy is not always necessary for diagnosis.* Aliment Pharmacol Ther. 2008; 27: 572-7.

http://dx.doi.org/10.1111/j.1365-2036.2008.03609.x
PMid:18194500

68. Baviera LC, Aliaga ED, Ortigosa L, Litwin N, Pena-Quintana L, Mendez V et al. *Celiac disease screening by immunochromatographic visual assays: results of a multicenter study.* J Pediatr Gastroenterol Nutr. 2007; 45: 546-50.

http://dx.doi.org/10.1097/MPG.0b013e31814794b9
PMid:18030231

69. Korponay-Szabo IR, Szabados K, Pusztai J, Uhrin K, Ludmany E, Nemes E et al. *Population screening for coeliac disease in primary care by district nurses using a rapid antibody test: diagnostic accuracy and feasibility study.* Bmj. 2007; 335: 1244-7.

http://dx.doi.org/10.1136/bmj.39405.472975.80
PMid:18063612 PMCid:PMC2137074

70. Ravelli A, Villanacci V, Monfredini C, Martinazzi S, Grassi V, Manenti S. *How patchy is patchy villous atrophy?: distribution pattern of histological lesions in the duodenum of children with celiac disease.* Am J Gastroenterol. 2010; 105: 2103-10.

http://dx.doi.org/10.1038/ajg.2010.153
PMid:20372112

71. Bonamico M, Thanasi E, Mariani P, Nenna R, Luparia RPL, Barbera C et al. *Duodenal Bulb Biopsies in Celiac Disease: A Multicenter Study.* J Pediatr Gastroenterol Nutr. 2008; 47: 618-622.

http://dx.doi.org/10.1097/MPG.0b013e3181677d6e
PMid:18979585

72. Corazza GR, Villanacci V, Zambelli C, Milione M, Luinetti O, Vindigni C et al. *Comparison of the interobserver reproducibility with different histologic criteria used in celiac disease.* Clin Gastroenterol Hepatol. 2007; 5: 838-43.
http://dx.doi.org/10.1016/j.cgh.2007.03.019
PMid:17544877

73. Kaukinen K, Collin P, Maki M. *Latent coeliac disease or coeliac disease beyond villous atrophy?* Gut. 2007; 56: 1339-1340.

74. Weir DC, Glickman JN, Roiff T, Valim C, Leichtner AM. *Variability of histopathological changes in childhood celiac disease.* Am J Gastroenterol. 2010; 105: 207-12.
http://dx.doi.org/10.1038/ajg.2009.557
PMid:19809405

75. Marsh MN. *Grains of truth: evolutionary changes in small intestinal mucosa in response to environmental antigen challenge.* Gut. 1990; 31: 111-4.
http://dx.doi.org/10.1136/gut.31.1.111
PMid:2180789 PMCid:PMC1378351

76. Oberhuber G, Granditsch G, Vogelsang H. *The histopathology of coeliac disease: time for a standardized report scheme for pathologists.* Eur J Gastroenterol Hepatol. 1999; 11: 1185-94.
http://dx.doi.org/10.1097/00042737-199910000-00019
PMid:10524652

77. Lio D, Bonanno CT, D'Anna C, De Luca S, Gervasi F, Cavataio F et al. *Gluten stimulation induces an in vitro expansion of peripheral blood T gamma delta cells from HLA-DQ2-positive subjects of families of patients with celiac disease.* Exp Clin Immunogenet. 1998; 15: 46-55.
http://dx.doi.org/10.1159/000019052
PMid:9619399

78. Koskinen O, Collin P, Korponay-Szabo I, Salmi T, Iltanen S, Haimila K et al. *Gluten-dependent small bowel mucosal transglutaminase 2-specific IgA deposits in overt and mild enteropathy coeliac disease.* J Pediatr Gastroenterol Nutr. 2008; 47: 436-42.
http://dx.doi.org/10.1097/MPG.0b013e31817b6dec
PMid:18852635

79. Koskinen O, Collin P, Lindfors K, Laurila K, Maki M, Kaukinen K. *Usefulness of small-bowel mucosal transglutaminase-2 specific autoantibody deposits in the diagnosis and follow-up of celiac disease.* J Clin Gastroenterol. 2010; 44: 483-8.

80. Salmi TT, Collin P, Korponay-Szabo IR, Laurila K, Partanen J, Huhtala H et al. *Endomysial antibody-negative coeliac disease: clinical characteristics and intestinal autoantibody deposits.* Gut. 2006; 55: 1746-53.
http://dx.doi.org/10.1136/gut.2005.071514
PMid:16571636 PMCid:PMC1856451

81. Jarvinen TT, Collin P, Rasmussen M, Kyronpalo S, Maki M, Partanen J et al. *Villous tip intraepithelial lymphocytes as markers of early-stage coeliac disease.* Scand J Gastroenterol. 2004; 39: 428-33.

http://dx.doi.org/10.1080/00365520310008773
PMid:15180179

82. Catassi C, Fasano A. *Celiac disease diagnosis: simple rules are better than complicated algorithms.* Am J Med. 2010; 123: 691-3.

http://dx.doi.org/10.1016/j.amjmed.2010.02.019
PMid:20670718

83. Katz KD, Rashtak S, Lahr BD, Melton LJ, 3rd, Krause PK, Maggi K et al. *Screening for celiac disease in a North American population: sequential serology and gastrointestinal symptoms.* Am J Gastroenterol. 2011; 106: 1333-9.

http://dx.doi.org/10.1038/ajg.2011.21
PMid:21364545 PMCid:PMC3130886

84. Green PHR, Stavropoulos SN, Panagi SG, Goldstein SL, McMahon DJ, Absan H et al. *Characteristics of adult celiac disease in the USA: results of a national survey.* Am J Gastroenterol. 2001; 96: 126-31.

http://dx.doi.org/10.1111/j.1572-0241.2001.03462.x
PMid:11197241

85. Rostami K, Kerckhaert J, Tiemessen R, von Blomberg BM, Meijer JW, Mulder CJ. *Sensitivity of antiendomysium and antigliadin antibodies in untreated celiac disease: disappointing in clinical practice.* Am J Gastroenterol. 1999; 94: 888-94.

http://dx.doi.org/10.1111/j.1572-0241.1999.983_f.x
PMid:10201452

86. Tursi A, Brandimarte G, Giorgetti GM. *Prevalence of antitissue transglutaminase antibodies in different degrees of intestinal damage in celiac disease.* J Clin Gastroenterol. 2003; 36: 219-21.

http://dx.doi.org/10.1097/00004836-200303000-00007
PMid:12590232

87. Naiyer AJ, Hernandez L, Ciaccio EJ, Papdakis K, Manavalan JS, Bhagat G et al. *Comparison of Commercially Available Serologic Kits for the Detection of Celiac Disease.* J Clin Gastroenterol. 2009; 43: 225-232.

http://dx.doi.org/10.1097/MCG.0b013e31816200e5
PMid:18724250

88. Walker MM, Murray JA. *An update in the diagnosis of coeliac disease.* Histopathology. 2010; 59: 166-79.

89. Hopper AD, Cross SS, Sanders DS. *Patchy villous atrophy in adult patients with suspected gluten-sensitive enteropathy: is a multiple duodenal biopsy strategy appropriate?* Endoscopy. 2008; 40: 219-24.

90. Evans KE, Aziz I, Cross SS, Sahota GR, Hopper AD, Hadjivassiliou M et al. *A prospective study of duodenal bulb biopsy in newly diagnosed and established adult celiac disease.* Am J Gastroenterol. 2011; 106: 1837-742.
http://dx.doi.org/10.1038/ajg.2011.171
PMid:21606978

91. Santolaria S, Dominguez M, Alcedo J, Abascal M, Garcia-Prats MD, Marigil M et al. *Lymphocytic duodenosis: etiological study and clinical presentations.* Gastroenterol Hepatol. 2013.

92. Rosinach M, Esteve M, Gonzalez C, Temino R, Marine M, Monzon H et al. *Lymphocytic duodenosis: Aetiology and long-term response to specific treatment.* Dig Liver Dis. 2012; 44: 643-8.
http://dx.doi.org/10.1016/j.dld.2012.03.006
PMid:22497904

93. Collin P, Wahab PJ, Murray JA. *Intraepithelial lymphocytes and coeliac disease.* Best Pract Res Clin Gastroenterol. 2005; 19: 341-50.
http://dx.doi.org/10.1016/j.bpg.2005.01.005
PMid:15925840

94. Green PH, Cellier C. *Celiac disease.* N Engl J Med. 2007; 357: 1731-43.
http://dx.doi.org/10.1056/NEJMra071600
PMid:17960014

95. Kaukinen K, Partanen J, Maki M, Collin P. *HLA-DQ typing in the diagnosis of celiac disease.* Am J Gastroenterol. 2002; 97: 695-99.
http://dx.doi.org/10.1111/j.1572-0241.2002.05471.x
PMid:11922565

96. Wouters J, Weijerman ME, van Furth AM, Schreurs MW, Crusius JB, von Blomberg BM et al. *Prospective human leukocyte antigen, endomysium immunoglobulin A antibodies, and transglutaminase antibodies testing for celiac disease in children with Down syndrome.* J Pediatr. 2009; 154: 239-42.
http://dx.doi.org/10.1016/j.jpeds.2008.08.007
PMid:18822429

97. Esteve M, Rosinach M, Fernandez-Bañares F, Farre C, Salas A, Alsina M et al. *Spectrum of gluten-sensitive enteropathy in first-degree relatives of patients with coeliac disease: clinical relevance of lymphocytic enteritis.* Gut. 2006; 55: 1739-45.
http://dx.doi.org/10.1136/gut.2006.095299
PMid:16709658 PMCid:PMC1856469

98. Zanini B, Caselani F, Magni A, Turini D, Ferraresi A, Lanzarotto F et al. *Celiac disease with mild enteropathy is not mild disease.* Clin Gastroenterol Hepatol. 2013; 11: 253-8.
http://dx.doi.org/10.1016/j.cgh.2012.09.027
PMid:23022697

99. Walker MM, Murray JA, Ronkainen J, Aro P, Storskrubb T, D'Amato M et al. *Detection of celiac disease and lymphocytic enteropathy by parallel serology and histopathology in a population-based study.* Gastroenterology. 2010; 139: 112-9.

http://dx.doi.org/10.1053/j.gastro.2010.04.007
PMid:20398668 PMCid:PMC2902605

100. Olivencia-Palomar P, Cano-Ruiz A, Martin-Scapa MA, Leon-Prieto F, Roy-Arino G, Redondo-Verge C. *Adult celiac disease and intraepithelial lymphocytes. New options for diagnosis?* Gastroenterol Hepatol. 2008; 31: 555-9.

101. Salmi TT, Collin P, Reunala T, Maki M, Kaukinen K. *Diagnostic methods beyond conventional histology in coeliac disease diagnosis.* Dig Liver Dis. 2010; 42: 28-32.

http://dx.doi.org/10.1016/j.dld.2009.04.004
PMid:19473894

102. Kurppa K, Collin P, Viljamaa M, Haimila K, Saavalainen P, Partanen J et al. *Diagnosing Mild Enteropathy Celiac Disease: A Randomized, Controlled Clinical Study.* Gastroenterology. 2009; 136: 816-823.

http://dx.doi.org/10.1053/j.gastro.2008.11.040
PMid:19111551

103. Leffler D, Schuppan D, Pallav K, Najarian R, Goldsmith JD, Hansen J et al. *Kinetics of the histological, serological and symptomatic responses to gluten challenge in adults with coeliac disease.* Gut. 2013; 62: 996-1004.

http://dx.doi.org/10.1136/gutjnl-2012-302196
PMid:22619366 PMCid:PMC3525791

104. Biesiekierski JR, Muir JG, Gibson PR. *Is Gluten a Cause of Gastrointestinal Symptoms in People Without Celiac Disease?* Curr Allergy Asthma Rep. 2013.

Chapter 10

Extraintestinal Manifestations of Celiac Disease and Associated Disorders

Alfredo J. Lucendo[1], Luis Rodrigo[2], A. Salvador Peña[3]

[1] Gastroenterology Department, Tomelloso General Hospital, Tomelloso, Ciudad Real, Spain.

[2] Emeritus Professor of Medicine, Department of Gastroenterology and Medicine, University of Oviedo, Oviedo, Spain

[3] Emeritus Professor, Laboratory of Immunogenetics, Department of Medical Microbiology and Infection Control, "Vrije Universiteit" Medical Center (VUmc), Amsterdam, The Netherlands.

alucendo@vodafone.es, lrodrigosaez@gmail.com, pena.as@gmail.com

Doi: http://dx.doi.org/10.3926/oms.258

How to cite this chapter

Lucendo AJ, Rodrigo L, Peña AS. *Extraintestinal Manifestations of Celiac Disease and Associated Disorders.* In Arranz E, Fernández-Bañares F, Rosell CM, Rodrigo L, Peña AS, editors. *Advances in the Understanding of Gluten Related Pathology and the Evolution of Gluten-Free Foods.* Barcelona, Spain: OmniaScience; 2015. p. 341-407.

Abstract

Celiac disease is not limited to the gastrointestinal tract and belongs to the group of autoimmune systemic diseases. It is frequently accompanied by a variety of extra digestive manifestations. More than half of the patients with adult celiac disease present with extra intestinal manifestations. The majority improve on a gluten-free diet. It is therefore advisable to have a low threshold of suspicion for the diagnosis.

The most frequent extraintestinal manifestations are iron deficiency anemia, osteoporosis, and dermatitis herpetiformis. The causes for the onset and manifestation of associated diseases are diverse; some share a similar genetic base, like type-1 diabetes mellitus; others share pathogenic mechanisms, and yet, others are of unknown nature. The implementation of a gluten-free diet improves the overall clinical course and influences the evolution of the associated diseases. In some cases, such as iron deficiency anemia, the gluten-free diet cures the manifestations and in other disorders, like in type-1 diabetes allows a better control of the disease. In several associated diseases, an adequate adherence to a gluten-free diet may slow their evolution, especially if implemented at an early stage.

We have reviewed in this chapter, first, the intra and extra intestinal manifestations of celiac disease, such as oral manifestations, hematological disorders, and osteoporosis. Secondly, the gluten-related associated diseases with genetic links, such as dermatitis herpetiformis and gluten ataxia. Finally, from the associated diseases we have reviewed type-1 diabetes mellitus, thyroid diseases, and malignancy.

Keywords

Celiac disease, extraintestinal manifestations, associated disorders, gluten-related diseases, anemia, osteoporosis, malignancy.

1. Introduction

Celiac disease is a process of autoimmune nature, induced by the ingestion of gluten genetically predisposed individuals[1]. It usually affects the digestive tract, which is classically associated with the presence of diarrhea, malabsorption, and weight-loss. In the last few decades the protean clinical presentation accompanied by a series of extraintestinal manifestations has substituted the classical presentation. The most frequent extraintestinal manifestations are iron deficiency anemia, osteoporosis, and dermatitis herpetiformis[2].

Recent studies have confirmed that autoimmune diseases are between 3 to 10 times more frequent in patients with celiac disease than in the general population[3,4].

The most prevalent are type-1 diabetes mellitus[5-7], autoimmune thyroid disease[8,9], Sjögren's syndrome[10], Addison's disease[11], autoimmune hepatitis[12-14], autoimmune cholestatic liver disease[15] and primary biliary cirrhosis[16-18]. Some reports of patients rheumatoid arthritis with celiac disease and other reports with dermatitis herpetiformis have been published. However, there is no evidence of a systematic association. Interestingly, from the genetic point of view, sharing of several genes within these diseases[19,20] are significantly increased. A study on the causes of mortality has identified an important number of patients who died of celiac disease, also had rheumatoid arthritis[21]. These observations suggest that the study of the association between rheumatoid arthritis and gluten related disorders should be systematically approached.

Several hypotheses have been put forward to explain the increased prevalence of autoimmune disease in patients with celiac disease. One of the hypotheses posits that a longer duration in the exposure to gluten before diagnosis, could be a risk factor for the development and emergence of related diseases[22,23]. However, other authors found that the prevalence of autoimmune diseases in patients with a late celiac disease diagnosis does not correlate with the duration of gluten intake[4].

Also the presence of the HLA-DQ2 and HLA-DQ8 in common with type-1 diabetes mellitus, autoimmune thyroid and Addison's disease shows a genetic link. It still remains to define which are the immunological mechanisms involved in the emergence and development of other autoimmune diseases in patients with celiac disease. The association of celiac disease with the HLA antigens may help to understand the mechanisms that link food-proteins intolerance to autoimmunity. It even has been suggested that celiac disease is a model for understanding autoimmune disease[24-27]. Like all autoimmune disorders celiac disease has a multifactorial etiology as well as the genetics of a complex disease[28].

From the immunological point of view, in celiac disease there is an overexpression of interleukin (IL)-15 in the mucosa of the small intestine. There is some evidence that due to the presence of this cytokine, effector T cells in the intestinal epithelium are not suppressed by regulatory T cells causing loss of tolerance to gluten and antibodies to self-antigens[29].

Another factor that has been implicated in the pathogenesis of autoimmunity in celiac disease is deficiency of vitamin D as this deficiency is commonly found in patients suffering from celiac disease and in other autoimmune disorders. Vitamin D was used to treat osteoporosis. Presently it has become an important biological inhibitor of inflammatory hyperactivity even in the presence of several malignant tumors. Its role is not yet fully understood[30].

In this chapter the following medical disorders will be reviewed:

First, the intra and extraintestinal manifestations of celiac disease, such as oral manifestations, hematological disorders, and osteoporosis.

Second, gluten-related associated diseases with genetic links, such as dermatitis herpetiformis and gluten ataxia.

Third, associated diseases such as Type-1 diabetes mellitus, Thyroid diseases, and malignancy.

In the available medical literature casual associations to other diseases have been found although no proper systematic studies have been published and will not be described.

Non-celiac gluten sensitivity and gluten allergy are comprehensibly described in other chapters of this book.

2. Oral and Dental Manifestations of Celiac Disease

The mouth and teeth are now widely recognized as tissues characteristically affected by celiac disease. In fact, several oral disorders have been related with celiac disease, including delayed eruption of teeth, enamel defects, recurrent aphthous oral ulcers, oral lichen planus, cheilosis, atrophic glossitis, glosodinia, and Sjögren syndrome. A celiac disease-characteristic pattern of T-cell inflammation has been also described in the oral mucosa of celiac patients[31,32].

2.1. The Oral Mucosa of Celiac Patients

Several studies have assessed the presence of histopathological changes in the oral mucosa of celiac patients[33,34], where a dense infiltration by T-lymphocytes, similar to that documented in the small bowel mucosa, has been repeatedly demonstrated; furthermore, a gluten-free diet was able to modify the T-cell populations[35]. Beyond the challenging potential of the oral mucosa as an easily accessible tissue to simplify the diagnosis of celiac disease[36], the involvement of the oral cavity and the capacity to produce anti-endomisial and anti-transglutaminase antibodies[37], allows the screening of celiac disease through salivary samples[38].

2.2. Aphthous Stomatitis

The association between recurrent aphthous ulcerations and celiac disease was described 4 decades ago, after documenting an unexpectedly high proportion of atrophic jejunal mucosa in patients with troublesome recurrent aphthous ulceration[37]. All patients remitted completely on a gluten-free diet, and the aphthous ulceration did not recur. Gluten withdrawal also showed a favorable response in many patients without villous atrophy[39]. Further

research has strengthened the link between oral aphthae and symptomatic as well as subclinical celiac disease[40,41]. The presence of recurrent aphthae is now recognized as one of the most frequent atypical associated conditions[42] which affects up to 20% - 40% of celiac patients at some stage in life. A significant association between oral aphthous ulcers and enamel defects in celiac patients has been described in some but not in all studies[43].

Aphthous oral ulcers have been recognized as risk factor for celiac disease. This justifies the screening of celiac disease in cases of recurrent or troublesome aphthous ulcers. A serological screening test is recommended as the initial method. Small intestinal biopsies should also be considered even if serology is negative. Favorable responses to a gluten-free diet have been documented in patients who showed an increase in intra-epithelial lymphocytes within the small bowel epithelium (stages Marsh 1/Coraza A)[44]. The improvement of oral aphthous stomatitis after a gluten-free diet has been demonstrated[45].

2.3. Delayed Dental Age in Celiac Children

Celiac disease in childhood may deprive of several nutritional factors, which are essential not only to promote body development, but also dental eruption. In fact, teeth development appears delayed or is slowed down in celiac children compared to healthy subjects[46]. Celiac disease has been also reported to influence the mineralization of permanent teeth[47].

2.4. Enamel Defects

Dental enamel defects are the imperfections in the enamel, which is the hard mineralized surface of teeth that makes up the normally visible part of the tooth, covering the crown. The tooth enamel is the hardest substance in the human body and contains the highest percentage of minerals, 96%, with water and organic material composing the rest. Dental enamel defects, mainly characterized by pitting, grooving and sometimes by complete loss of enamel, were firstly reported in children with celiac disease by Aine in 1986[48]. Since

this publication, repeated reports have led to *The North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition* to include the presence of specific dental enamel defects as a risk factor for celiac disease[49].

Enamel defects include both discoloration and structural changes, as shown in Table 1.

Table 1. Classification of Systemic and Chronologic Enamel Defects. Modified from Aine in 1986[48].

Classification	Enamel Defect
Grade 0	No defect
Grade I	Defect in color of enamel consisting of single or multiple cream, yellow or brown opacities (marks) and loss of normal enamel glaze.
Grade II	Slight structural defects consisting of a rough surface with horizontal grooves or shallow pits; light opacities and color changes may also be found. Part of or the entire surface of enamel is without glaze.
Grade III	Obvious structural defects with partly or entire surface of the enamel is rough and filled with deep horizontal grooves. This may vary in width or have large vertical pits; large opacities of different colors or linear discoloration may be present in combination.
Grade IV	Severe structural defects. Shape of the tooth is changed. The tips are sharp-pointed and/or the incisal edges are unevenly thinned and rough. The thinning of the enamel material is easily detectable and the lesion may be strongly discolored.

The exact mechanism leading to these defects remains unclear; immune-mediated damage has been involved as the primary origin[50]. Also nutritional disturbances, especially hypocalcemia, seem to play an important role[51]. A gluten-induced stimulation of naïve lymphocytes in the oral cavity has also been hypothesized[31]. Ultrastructural analyses have demonstrated that the enamel hypoplasia of deciduous and permanent teeth in celiac disease patients is highly hypomineralized with shorter prisms of hydroxyapatite,

more irregularly distributed and less interprismatic substance than observed in the non-celiac enamel hypoplasia[52].

A significant increased prevalence of enamel defects has been repeatedly reported in children, adolescents and adult subjects with celiac disease[43,53], and also in patients with dermatitis herpetiformis. There is no correlation between the degree of enamel defects and that of the mucosal damage in small bowel biopsy specimens[53].

Finally, dental enamel defects are not specific for celiac disease, having been also associated with an excessive fluoride intake, tetracycline exposition or bulimia[43]. In these conditions, dental enamel defects are not as severe as those identified in celiac patients[54,55].

2.5. Caries Risk and Celiac Disease

Dental caries remains today as one the most common diseases throughout the world, affecting 1/3 of the population of their permanent teeth[56]. It consists in a bacterial infection with production of acids, which in case of excess of remineralization factors, leads to demineralization and destruction of the hard tissues of the teeth, including the enamel, dentine and cementum.

In spite of having been recognized as the most common childhood disease, a significantly increased prevalence of caries has been reported in subjects with celiac disease compared to matched controls in several observational studies[32]. The proportion of caries-free subjects in the control group was found to be 2-fold higher than in the celiac disease group before starting on a gluten-free diet[57].

2.6. Oral Lichen planus

Lichen planus is a disease of the skin or mucous membranes that resembles vegetal lichen. It presents with a variety of lesions, the most common is a well-defined area of purple-colored, itchy, flat-topped papules with interspersed lacy white lines[58]. This is possibly the result of an autoimmune process with an unknown initial trigger. Lichen planus in the mouth may

persist for many years, and is difficult to treat, with relapses being common[59]. Atrophic/erosive lichen planus is associated with a small risk of malignant transformation. There is no cure for lichen planus, so treatment is for symptomatic relief or due to cosmetic concerns.

From the first description of the association of celiac disease and oral lichen planus in 1993[60], an increased prevalence of celiac disease in patients with oral lichen planus has been repeatedly reported, generally using serological screening[61]. These authors conclude that celiac disease screening should be considered in oral lichen planus patients, since untreated celiac disease can present many complications and reduce a patient's quality of life.

2.7. Atrophic Glossitis and Other Tongue-related Symptoms

Atrophic glossitis is an inflammatory condition of the tongue mucosa that is characterized by a smooth, glossy appearance with a red or pink background. This is due to the atrophy of filiform papillae that causes the development of circinate erythematous ulcer-like lesions of the dorsum and the lateral border of the tongue[62]. Several diseases have been primarily related with atrophic glossitis, including chemical irritations, local and systemic infections such as candidiasis, amyloidosis, drug reactions, nutritional deficiencies, pernicious anemia, malnutrition, sarcoidosis, Sjögren's syndrome, psoriasis[63], and celiac disease[64]. The tongue was the most frequently affected site in a series of 128 patients with celiac disease who were examined for oral mucosal lesions and symptoms, with 29.6% of the patients describing soreness or a burning sensation and 8.6% having erythema or atrophy[33]. This recognition should lead dentists to play a more significant role in screening for celiac disease, to widen the possibility of a correct diagnosis and subsequent treatment.

2.8. Sjögren's Syndrome

This chronic autoimmune disease is characterized by a destruction of exocrine glands, specifically salivary and lacrimal glands, caused by

lymphocytic infiltration[65]. The association of celiac disease with primary Sjögren's syndrome, as with other immune-mediated disorders, has been described in the literature. In Hungary it was found in 111 patients with Sjögren syndrome that celiac disease was significantly higher than in the non-Sjögren syndrome European population (4.5: 100 *vs.* 4.5-5.5: 1,000)[66]. Even when following a gluten-free diet does not usually result in the resolution as both disorder evolved independently, the evaluation of celiac disease in patients with must be considered[67].

3. Hematological Manifestations of Celiac Disease

Within the hematological manifestations of celiac disease, anemia remains the most common due to iron, folate, and occasionally vitamin B12 deficiency. Anemia may be the sole presenting symptom. Other manifestations include thrombocytosis, leukopenia, thromboembolism, increased bleeding tendency, immunoglobulin (Ig)A deficiency, spleen dysfunction, and lymphoma[68]. In a recent nationwide prospective population-based cohort study in Sweden has been found that individuals with IgA deficiency more often had celiac disease (6.7 % *vs.* 0.19 % in controls) and type-1 diabetes (5.9 % *vs.* 0.57 %) corresponding to a 35-fold higher prevalence ratios for celiac disease and 10-fold higher for type-1 diabetes. These individuals with IgA deficiency have a higher prevalence of several other autoimmune disorders[69]. These findings should be taken into account in screening programs to detect celiac disease.

3.1. Anemia and Celiac Disease

Anemia without other clinical clues of intestinal malabsorption is one of the most common extraintestinal manifestations of celiac disease[70,71]. Although folate and vitamin B12 deficiency are known complications of celiac disease, the most common nutritional type of anemia associated with celiac disease is iron deficiency.

Celiac disease is frequently diagnosed in patients referred for evaluation of iron deficiency anemia, being reported in 1.8%-14.6% of patients[72].

In one large study in Italy of 42 centers with patients presenting subclinical celiac disease, iron-deficiency anemia appeared to be the most frequent extraintestinal symptom in children and in adults[71]. A characteristic feature of the iron deficiency anemia associated with celiac disease is its refractoriness to oral iron treatment[73].

Since anemia is a common presenting feature of celiac disease, what is the chance of finding celiac disease in patients presenting with iron deficiency anemia? This question is of particular importance for hematologists and general practitioners who are often consulted for unexplained iron deficiency anemia. Table 2 shows that celiac disease in this group of patients occurs between 4.8 and 6%. Most of the studies included a majority of premenopausal females. The most consistent clinical feature in the series of Table 2 was the complete refractoriness to oral iron treatment and the complete absence of a rise in serum iron two hours after an oral iron doses of 100 mg ferrous sulphate tablets[74-79].

Table 2. Prevalence of celiac disease in patients with chronic iron deficiency anemia.

Year and Ref	n	Serology	Biopsy	Celiac Disease (%)
1995[74]	200	+	+	5.0
1998[75]	85	–	+	5.8
2001[76]	71	–	+	5.6
2002[77]	258	+	+	4.8
2005[78]	150	+	+	5.3
2008[79]	116	+	+	6.0

n = number of included patients; (–) means not performed. Modified from Hershko and Patz[80].

A prospective study of patients with iron deficiency anemia published in 2005[78] found a prevalence of celiac disease of 5%. Subsequent studies have confirmed that about 4% to 6% of patients with obscure refractory iron deficiency anemia have celiac disease. Autoimmune gastritis is encountered in 20% to 27% of patients, 50% of these have active *H. pylori* infection and are permanently cured by eradication[81].

The most obvious cause of anemia is an impaired absorption of iron and other nutrients including folate and cobalamin. Villous atrophy of the intestinal mucosa is an important cause of abnormal iron absorption which is reflected in the laboratory evidence of iron deficiency anemia in most anemic patients with celiac disease[79].

Abnormal iron absorption is also supported by the failure to increase serum iron following an oral iron supplement and refractoriness to oral iron treatment. Other factors may contribute to cause anemia, which in many cases is multifactorial in etiology[82].

Occult gastrointestinal blood loss as a cause of anemia in celiac disease is doubtful, since the evidence supporting an increased fecal blood loss in celiac disease is controversial. Although abnormal intestinal bleeding may occur in some celiac patients, it does not appear to play a significant role in the cause of anemia[83].

Bergamaschi et al., focused on the role of anemia of chronic disease in the differential diagnosis in series of 150 anemic patients with celiac disease at presentation. The authors found 45 patients who had uncomplicated iron deficiency anemia and 2 had vitamin B12 or folate deficiency. The iron status parameters which identified anemia of chronic disease alone or anemia in combination with iron deficiency (6 patients) showed a prevalence of 17% concluding that the anemia of chronic disease plays a significant role in celiac disease. A gluten-free diet resolved the different mechanisms leading to anemia in these patients[84].

From a practical point of view, in absence of markers of chronic disease, such as increased C-reactive protein, elevated sedimentation rate or high fibrinogen levels, presence of underlying inflammatory gastrointestinal disease,

celiac disease, chronic autoimmune and/or *H. pylori* gastritis cannot be excluded. The sensitive and accurate indicators employed by Bergamaschi et al.[84] such as the measure of the ferritin/transferrin ratio, serum levels of interferon gamma (IFN-γ) and other markers of inflammation may facilitate the differential diagnosis and the identification of an underlying inflammatory condition that may explain the cause of the anemia and guide to an effective treatment.

4. Bone Metabolism and Bone Mineral Density in Celiac Disease

The association of celiac disease with metabolic bone disorders has been known even before the origin and treatment of celiac disease; Osteomalacia, a disease characterized by low bone mineral density (BMD), marked deformities and rickets, has been repeatedly described among children with celiac disease in the early literature[85]. Rarely it is part of the initial presentation of celiac disease in children[86]. The development and availability of the bone density scan as a non-invasive diagnostic technique has confirmed the link between low BMD and celiac disease. For adult patients the BMD-scan is used since 2005[87]. Today, metabolic bone disease remains a significant and common complication of celiac disease found at the time of diagnosis in both children and adults. Low BMD leads to an impaired deterioration in quality of life[88], aggravated by its clinical manifestation such as fractures.

At present, a low BMD constitutes the first diagnostic criterion for osteoporosis, a skeletal metabolic disease further defined by impaired bone microarchitecture, increased bone fragility and susceptibility to bone fractures. The WHO establishes a diagnosis of osteoporosis when bone mass values are below -2.5 standard deviation (SD) of peak bone mass (i.e. the maximum BMD value reached by an adult), and osteopenia when those values are located between -1 SD and -2.5 SD (Table 3).

Table 3. The World Health Organization (WHO) diagnostic criteria for post-menopausal Caucasian women.

Diagnosis	BMD criteria (T-score)
Normal	BMD T > –1 SD
Osteopenia or low bone density	BMD T < –1 SD and > –2.5 SD
Osteoporosis	BMD T < –2.5 SD
Severe osteoporosis	BMD T< –2.5 SD + fracture

T-score: comparison with BMD value in average reference population. SD: Standard deviation. BMD: Bone mineral density.

Severe or established osteoporosis associates with a current or past fragility fracture. A low BMD defining osteoporosis in children and adolescents consists in an area of BMD of less than 2 standard deviations (SD) below the age-adjusted mean value (Z-score < -2 SD)[89]. Osteoporosis is similar to celiac disease in terms of missed diagnosis and therefore a lower prevalence than expected is found. It has been hypothesized that celiac disease could explain part of the considerable idiopathic osteoporosis "mixed bag"[90]. Nonetheless, despite many studies on this subject, a description of how celiac disease –a primarily digestive disorder– can affect bone metabolism has yet to be fully elucidated.

4.1. Prevalence of Osteoporosis Among Patients With Celiac Disease

It is estimated that at the moment of diagnosis, one-third of pediatric patients have osteoporosis, one-third osteopenia and only the remaining one-third of patients with celiac disease has a normal BMD[91]. Despite the fact that more than half of the children with celiac disease present with low BMD at the moment of diagnosis[92], once the gluten-free diet is instituted, most celiac children catch up to their height-weight growth curve and accelerate their rate of bone mineralization, so that most achieve normal peak bone mass by the time bone growth is completed[93]. The main problem arises when celiac

disease is diagnosed during adulthood, once bone growth is complete and peak bone mass has been reached[94]. The prevalence of osteoporosis in adult patients with celiac disease is twice that of the non-affected population in the same age group[95]. The average prevalence of low BMD among adult celiac patients compared to the general population is around 40%. In some series of patients with celiac disease this prevalence reached up to 75%[96]. This low BMD also affects patients with dermatitis herpetiformis[97].

A low BMD has been demonstrated in celiac patients with classic symptoms[98], in patients with sub-clinical manifestions[99], and even in asymptomatic patients with celiac disease[100,101]. Therefore, the type of celiac disease-related symptoms cannot predict the presence of low BMD, and justifies attempts to reach the low BMD diagnosis by further searching for other determinants.

Since osteoporosis is a common complication of celiac disease, it is appropriate to consider whether or not to screen for celiac disease in patients with idiopathic osteoporosis. Although there is no definitive consensus, the greater weight of opinion is in favor of the screening strategy since the frequency of celiac disease is 10 times higher than expected in patients with osteoporosis[102]. A similar frequency of celiac disease among type-1 diabetic mellitus already justifies universal screening among these patients (see later). In fact, celiac disease screening through specific antibodies in patients with osteoporosis has led to an increase in the diagnosis of celiac disease between 4[103] and 17[102] times higher prevalence.

4.2. Etiology and Pathogenesis of Low BMD in Celiac Disease

The origin of osteoporosis in celiac disease has been classically associated with malabsorption caused by intestinal villous atrophy and poor absorption of calcium and vitamin D[104], as well as secondary hyperparathyroidism, even in patients with normal vitamin D serum levels[105]. Low consumption of dairy products[106], failure to ever reach peak theoretical bone mass[107], higher degree of duodenal injury in biopsy specimens[108], and greater delay in the diagnosis

of celiac disease[109] have also been directly related to the pathogenesis of low BMD in celiac patients.

Vitamin D deficiency is common among patients with celiac disease, although there are no changes in the expression of vitamin D receptors[110] nor a greater number of receptor gene mutations interfering with the metabolism of this vitamin in the celiac population[111]. Restricted milk intake may exacerbate vitamin D deficiency; in fact, co-occurrence of lactose intolerance is common among celiac patients and is estimated at 10%, but may increase to 50% in the presence of obvious symptoms of malabsorption[112]. However, one must bear in mind that diet only provides 5-10% of the required vitamin D, the rest being obtained from exposure to sunlight. Even so, studies of celiac patients have failed to establish a clear association between vitamin D levels and bone impairment, as demonstrated for inflammatory bowel disease[113].

Deficits in other fat-soluble vitamins (A, K and E) and water-soluble vitamins (C, B12, folic acid and B6) or minerals (such as iron, calcium, phosphorus, copper, zinc, boron, fluorine), which are required for normal bone metabolism[112,114], may be the result from the intestinal malabsorption and contribute to impaired BMD.

Celiac patients on a gluten-free diet frequently exhibit high serum parathyroid hormone (PTH) levels[114]. Secondary hyperparathyroidism may explain the higher prevalence of bone loss in the appendicular skeleton compared with the axial skeleton in celiac disease[115].

Reduced serum levels of insulin-like growth factor-1 (IGF-1) also called somatomedin C[116], constitute an additional hormonal factor which has been involved in patients with a lower bone mass. This reduced level was associated with decreased serum levels of zinc[117], which normalized after introduction of a gluten-free diet.

Chronic inflammation determines changes in bone metabolism via several pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α), IL-1beta, IL-6 or gamma interferon. TNF-related cytokines include the receptor activator of nuclear factor kappa-B (RANK), its ligand (RANKL), and osteoprotegerin (OPG). RANKL is secreted by activated T lymphocytes

and is a key molecule in the regulation of bone metabolism. RANKL has proved to be a survival factor whose primary function is activation of osteoclasts, the cells involved in bone resorption[118].

Serum levels of RANKL and OPG are high in patients with celiac disease[119], The OPG/RANKL ratio is directly associated with IL-6 serum levels and lumbar bone mass[120]. Thus, adult women with celiac disease have OPG/RANKL ratios significantly lower than controls, despite adherence to a gluten-free diet; this correlates with a lower lumbar BMD[121].

Finally, the etiology of osteoporosis in celiac disease coincides with the factors shared with the rest of the population such as family history, age, menopause, physical activity, smoking, as well as other specific factors such as genetic influence, the above-mentioned vitamin deficiencies, hormonal changes and the inflammatory process itself.

The years of exposure to gluten in the diet before the diagnosis of celiac disease do not appear to influence BMD significantly nor does early menopause. There is little data on the influence of patient gender on BMD, but most studies show no difference in this respect. Another factor associated with poor bone condition is a low body mass index (BMI). Patients with persistent villous atrophy despite proper adherence to the gluten-free diet (refractory celiac disease) are particularly susceptible to osteoporosis, with a prevalence of 58% compared to the 22% reported among gluten-free diet responsive patients[122].

4.3. Diagnosis of Low Bone Mineral Density in Celiac Disease

All patients in whom there is clinical suspicion of osteoporosis should undergo a thorough history-taking and physical examination so as to identify other risk factors and/or consequences. In the case of celiac disease, it has been suggested that all patients diagnosed in adulthood should undergo bone density scans[123], since conventional radiography has not proven to be a specific or sensitive method in assessing changes in bone mass. However, some studies, seeing the low risk of bone fracture among celiac patients, have questioned the utility of routine bone density scans[124]. Recent studies advocate densitometric

assessment in all celiac patients diagnosed during adulthood who have villous atrophy on duodenal biopsies and/or laboratory values suggestive of malnutrition or malabsorption, regardless of their symptoms[108]. The greatest benefit from bone density scans is determining whether there is osteoporosis and the degree of impairment, so that a treatment regimen can be planned. However, the optimal timing to perform bone density scans in celiac patients, whether at the time of celiac disease diagnosis or after a period of adherence to the gluten-free diet, has motivated some controversy. Celiac children show a great bone recovery capacity after starting a gluten-free diet, so no further studies seem to be necessary until their growth period is completed.

4.4. Bone Fracture Risk in Celiac Disease

Due to the increased prevalence of osteoporosis, celiac patients have a high risk of bone fractures compared with the unaffected population of the same age and gender. Up to one in four adult patients may have an established history of fractures[125,126], which produces a significant deterioration in quality of life.

As in other aspects of the relationship between celiac disease and osteoporosis, quantification of fracture risks in different studies shows mixed results. These discrepancies are largely due to differences in data collection, mainly from fracture reports, questionnaires, or hospital admissions. It is therefore possible that the prevalence of fractures (vertebral, hip, and overall) is underestimated in the celiac population. One of the common issues of fracture risk studies is that they lack proper morphometric assessment of the spine, which underestimates fractures at that level[127], or failure to use validated questionnaires or methods, such as the FRAX® (Fracture Risk Assessment Tool) index proposed by the WHO[128-130]. Several studies have estimated the incidence and prevalence of bone fractures among patients with celiac disease[131] (Table 4). The results have been summarized in two systematic reviews: The first one included 20,955 celiac disease patients, 1,819 (8.7%) had fractures and 96,777 controls with 5,955 (6.1%) fractures, which resulted in a pooled odds ratio of 1.43 (95% confidence interval (CI), 1.15 to 1.78), with a significant heterogeneity among the studies[132]. The baseline was associated with a 30%

increase (95%CI, 14% to 50%) in the risk of any fracture, and a 69% increase in the risk of hip fracture 136 (95% CI, 10% to 259%).

Table 4. Studies of fracture risk available in adult patients with celiac disease. Adapted from Scott, 2000[142].

Year Country	Subjects CD-Controls (C)	Design of study	Diagnostic methods	Fractures	Risk of fracture OR(95%CI)
2000 Argentina[127]	165 CD – 165 C Matched controls with GI symptoms	Cross-sectional Retrospective Analysis	Dual energy x-ray Densitometry Spine radiography	Peripheral Lumbar spine	3.5 (1.8 - 7.2) 2.8 (0.7-11.5)
2001 UK[133]	75 CD – 75 C Control matched by Age and sex	Cross-sectional Retrospective Analysis	Dual energy x-ray Absorptiometry of Lumbar spine and Femoral neck	Any location	21% in CD versus 3% in C
2002 Denmark[134]	1,021 CD – 3,063 C Control matched by Age and sex	Computerized Registered of national hospitals admissions & discharges	Diagnoses of fractures in cases and controls in the same national registry	Any Lumbar Distal radius (Colles) Neck of femur	RRI 0.7 (0.45-1.09) RRI 2.14 (0.70-6.57) RRI 2.00 (0.58-6.91) RRI 0.71 (0.27-1.89)
2003 UK [135]	244 CD – 161 C Controls paired for age and sex	Analysis of celiac population records	Lifestyle and general health questionnaire, with specific questions about history of fractures	Any location Forearm	1.05 (0.68-1.62) 1.21 (0.66-2.25)
2003 UK[126]	4,732 CD – 23,620 C 1,589 CD "incidents" controls matched by age and sex	Population cohort study from a database	Codified registry of fractures in patients with CD and controls	Any location Hip Ulna, radius	HR 1.30 (1.16-1.46) HR 1.90 (1.20-3.02) HR 1.77 (1.35-2.34)
2004 Argentina[136]	148 CD – 292 C Matched controls with GI symptoms	Cross-sectional study of cases and controls	History of fracture based on interview with a predefined questionnaire	any	5.2 (2.8 - 9.8) in "classic" CD 1.7 (0.7 - 4.4) in "asymptomatic" CD
2005 UK[137]	383* – 445 C *celiac women over 50 years of age	Cross-sectional study	Detailed questionnaire about history of fractures	Any location	1.51 (1.13-1.5)

Year Country	Subjects CD-Controls (C)	Design of study	Diagnostic methods	Fractures	Risk of fracture OR(95%CI)
2007 Sweden[138]	13,000 CD – 6,500 C 4,819 adults CD controls matched by age and sex	Cross-sectional population cohort study based on hospital discharge records	Records of 1st documented fracture at any location	Any location Hip	HR 1.4 (1.3-1.5) HR 2.1 (1.8-2.4)
2008 USA[139]	83 CD – 166 C	Retrospective cohort, retrospective case-control	Clinical history and the radiologist's report of each fracture	All fractures. Fractures of the hip, spine, or distal forearm that result from minimal or moderate trauma in patients <35 years were considered osteoporotic fractures	2.0 (1.0–3.9)
2011 Argentina[140]	265 CD – 530 C	Retrospective cohort	Standard questionnaire on CD and fracture history through in-person interviews	All fractures	HR=1.78 (1.23–2.56) (before diagnosis of CD)
2011 Finland[141]	35 CD screen-detected CD patients	Case series study	Dual energy x-ray absorptiometry of lumbar spine and femoral neck	All fractures	Low-energy fractures in 8/35 of CD patients; 22.8 %
2012 Spain[108]	40 CD patients with a diagnosis of CD in adulthood	Prospective cross-sectional	Dual energy x-ray densitometry, FRAX® tool	Risk of hip fracture Risk of major osteoporotic fracture (lumbar, femoral neck, forearm and shoulder)	3.5 times greater in Marsh 3 on 1-2 1.34 times greater in Marsh 3 on 1-2

CD, coeliac disease; OR, odds ratio; RRI, relative risk increase; HR: hazard ratio; (95%CI), 95% confidence interval.

The risk of fracture at 10 years estimated at the time of celiac disease diagnosis was determined by using the FRAX® tool in a recent Spanish

research study[108]. A moderate risk of fracture was demonstrated among patients with duodenal villous atrophy (Marsh stage 3), which was 3.5 times higher than in patients without villous atrophy (Marsh stage 1 or 2). More recently, a Swedish cohort study, has found that persistent villous atrophy on follow-up biopsy is predictive for hip fracture risk but not overall fractures, irrespective of patients' age[143]. The authors stated that persistent villous atrophy could result in a decreased body mass index and a reduction on its protective role against fall and trauma.

4.5. Treatment of Low Bone Mineral Density in Patients with Celiac Disease

The first-line treatment for osteoporosis in celiac disease is gluten-free diet. Many studies have demonstrated its effect on bone density and calcium absorption in both children and adults[144-146]. The greatest bone mass gain described in these studies is during the first year of instituting a gluten-free diet. It leads to a 5% increase in bone mass after 1 year[144], although this is not enough for bone mass to normalize. In clinical practice, the degree of adherence to the gluten-free diet also determines the recovery of bone mass, which is generally estimated to be around 30%[147-149]. Furthermore, the recovery rate is higher in young celiac patients[150] than among adults[144]. This is largely explained by the fact that 97% of bone mass is gained in the first two decades of life and full recovery is difficult after this time.

BMD loss associated with pediatric celiac disease responds to gluten-free diet continuously and gradually, with almost complete restoration of bone mass after about two years' treatment[151]. The earlier the age at which the gluten-free diet is started, the better and faster is the response[152]. In fact, it is estimated that an increase in BMD will only take place if the gluten-free diet is started before the age of 25[104]. The strict adherence to a gluten-free diet is so important for bone metabolism that lack of improvement in BMD after its introduction has been associated with persistent duodenal lesions[153].

In addition to the gluten-free diet, an adequate daily intake of calcium and vitamin D should be ensured, as it is a critical factor for bone mass

acquisition and maintenance. Untreated adult celiac patients have shown a 45% reduction in calcium absorption followed by an improvement of 52% after 6 months of gluten-free diet adherence[104]. Regarding vitamin D, at the time of diagnosis, less than 5% of Spanish adult celiac disease patients had normal serum levels[108]. A daily intake of 1,200-1,500 mg calcium and 400U vitamin D3 is recommended and as in all other forms of osteoporosis. Adherence to drug therapy, as to the gluten-free diet, is a crucial aspect of treatment, so patients must be kept motivated. In fact, these patients will most commonly abandon treatment with calcium and vitamin D, as it must be taken daily, while hormonal therapy and bisphosphonates (which are administered weekly) are usually adhered to correctly. Drug treatment would be indicated for patients who do not achieve bone mass recovery goals, and would not differ from that established for other causes of osteoporosis.

In these cases bisphosphonates are the recommended first-line therapy. However, as far as we know there is no data on the effect of bisphosphonates in celiac disease-associated osteoporosis.

5. Gluten-Related Disorders

5.1. Dermatitis Herpetiformis

Dermatitis herpetiformis was firstly described in 1884 by the French dermatologist Louis Duhring[154]. In some countries, this disease is still called Duhring's disease. In 1966, Marks et al. identified the presence of histological abnormalities in the small bowel, identical to those observed in patients with celiac disease[155]. The patients have gluten-induced IgA autoantibodies against tissue transglutaminase (tTG)-2 and tTG-3[156]. Dermatitis herpetiformis is regarded as the skin manifestation of gluten sensitivity[157,158]. The autoimmune basis is confirmed by the characteristic findings of the presence of IgA deposits and tTG at the dermo-epidermal junctional level. Its etiology is multifactorial and has a polygenetic basis. Dermatitis herpetiformis is like celiac disease associated with a number of autoimmune diseases such as IgA

deficiency, type-1 diabetes mellitus, autoimmune hypothyroidism and Addison's disease[159-161].

Primary cutaneous lesions appear as erythematous papules associated with vesicles filled with liquid, in patches distributed symmetrically on extensor surfaces[162]. As the vesicles are very itchy, patients scratch themselves rupturing the blisters, releasing its liquid content and give rise to erosions and abrasions. Subsequently, papules become scabs and fall off leaving a slightly pigmented area. It usually predominates in young adults but children and the elderly may be affected, especially in atopic children. The vast majority of patients report the onset of symptoms in hot months, from early spring to late summer. [163,164].

Usually the eruptions are symmetrical, affecting mainly the surface extension of the upper and lower limbs, predominantly in elbows and knees but also ankles, waist, neck and buttocks. The face, scalp and groins can be affected. The localization of lesions on the palms of the hands but not on the back is also relatively frequent. They also may appear on the fingers, these lesions appear in the form of petechial pads. The aspect of the lesions adopts a very similar appearance in the great majority of affected patients, which facilitates its early diagnosis[165]. Mucosal involvement is rare. The diagnosis of dermatitis herpetiformis is established clinically, histologically and immunopathologically.

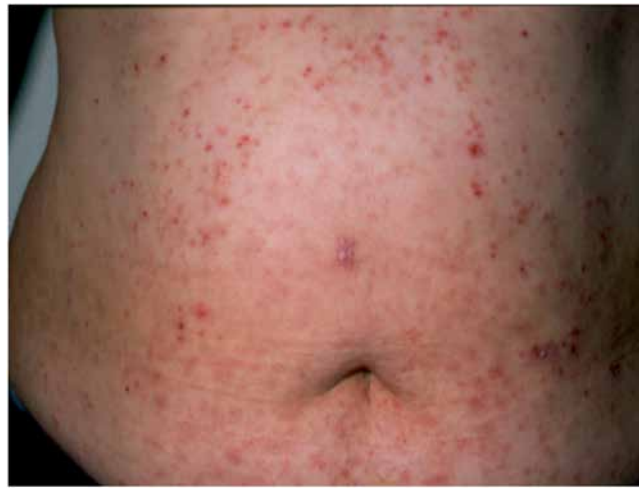


Figure 1. Many characteristic lesions in different stages of evolution in the abdomen in a patient with dermatitis herpetiformis, an unusual localization.

The majority of patients with dermatitis herpetiformis does not have, or present few intestinal manifestations. Sometimes the patients only have iron deficiency anemia. Males are affected more than females (1.5-2 to 1) as opposed to celiac disease, which shows a clear predominance in females (2-4 to 1)[166].

The most characteristic histological finding is the confirmation of the presence of granular IgA deposits localized at the level of the papillae of the dermis and along the basement membrane, demonstrable by direct immunofluorescence in skin biopsies. These accumulations promote an inflammatory response with infiltration of neutrophils and vesicles in the affected areas[167]. The immunological basis for its development is closely linked to the pathogenesis of gluten intolerance in celiac disease. tTG-3 antibody is the main auto-antigen and it is located on the skin of these patients, triggering an inflammatory response[168].

The association with genetic markers from HLA class-II, mainly HLA-DQ2 and/or HLA-DQ8, is the same as seen in celiac disease. A genome-wide association study (GWAS) in celiac disease in North America has provided suggestive statistical evidence for the association of dermatitis herpetiformis and microscopic colitis with SNPs at chromosomes 3p21.31, 6q15, 6q25, 1q24.3 and 10p11.23[169].

The main treatment of dermatitis herpetiformis is a gluten-free diet, which should be strictly maintained during lifetime. The skin lesions disappear within various weeks after initiating a gluten-free diet. Some cases may require a short complementary treatment with dapsone. This drugs target the skin eruption inhibiting neutrophil migration and is used temporarily, until the complete disappearance of the skin lesions[170]. A survey in Finland from 1971 to 2010 on the mortality rate and causes of death in 476 consecutive patients with dermatitis herpetiformis documented significantly reduced all-cause and cerebrovascular disease mortality. The standardized mortality rate for all causes of death was significantly reduced, being 0.70 (95% CI, 0.55 to 0.87), similar in both sexes and was equal in patients with dermatitis herpetiformis with (0.73) and without (0.77) small bowel villous atrophy[171].

The authors have suggested that strict adherence to a gluten-free diet (the questionnaire survey documented that 97.7% of the patients with dermatitis herpetiformis adhered to a gluten-free diet), less smoking and less hypercholesterolemia played a role in the observed substantial health benefit.

5.2. Gluten Ataxia and Neurological Phenotypes in Gluten-related Disorders

In the diagnosis of gluten ataxia cases formerly known as "idiopathic sporadic ataxia" accompanying circulating antibodies against gluten, are included. It is a type of cerebellar ataxia caused by exposure to gluten in sensitive patients and may complicate celiac disease but also other gluten-related disorders[172]. In the USA and Europe gluten ataxia may occur in 24% of patients with cerebellar ataxia[173] but it was considered to be rare in Asia. Japanese neurologists have recently speculated that more than 10% of cerebellar ataxia patients in Japan, have gluten ataxia[174].

The most common clinical form of presentation is the typical pure cerebellar ataxia with abnormal gait and balance, and associated dysarthria. Less frequently as a clinical form of diffuse or focal myoclonus manifestations. It may be accompanied by nystagmus and other ocular signs, over 70% of cases. It usually has a slow start and generally affects individuals older than 50 years without difference between both sexes. A rapid progressive disease may occur but a slow evolution, with a stationary clinical course, punctuated by some transient worsening episodes is seen. In most cases, there is a long previous history of several digestive symptoms of recurrent characteristics, but some patients have not been previously diagnosed as suffering from celiac disease or non-celiac gluten sensitivity. In the majority of patients with gluten ataxia, magnetic resonance imaging of the brain shows the presence of a moderate cerebellar atrophy, mainly in the cerebellar vermis. The Sheffield´s group directed by Dr. Hadjivassiliou, was the first to describe this type of association and has made great contributions to this field[175].

The diagnosis of gluten ataxia is confirmed by the presence of anti-gliadin antibodies (AGA)[176], and anti-tTG-2 and anti-tTG-6 when available. Patients

with gluten ataxia have anti-tTG-2 IgA in less than 40%. When combined with anti-tTG-6 it can reach a positivity of up to 85%[175]. Autoantibodies to tTG-6 have been identified in immune-mediated ataxia in patients with gluten sensitivity, thus suggesting a critical role for transglutaminase 6 in cortical and cerebellar neurons[177,178]. Sometimes gluten ataxia has a familial character with several first-degree members affected[179].

Gluten ataxia is therefore considered an autoimmune disease characterized by the presence of a cerebellar injury, affecting mainly Purkinje cells[180] that produces ataxia. It has been found that there is cross-reactivity between antigens located at the level of Purkinje cells and circulating antibodies related to gluten. The deposits are confined not only to the cerebellum, but also in the pons and spinal cord.

These patients must be treated with a strict gluten-free diet maintained during a life-time. After 1 year of starting the gluten-free diet, stabilization or improvement of clinical signs of ataxia, are good indicators or the confirmation that the patient indeed suffers from gluten ataxia. The degree of response clearly depends on the time elapsed since the start of the occurrence of ataxia and the establishment of the gluten-free diet. If the gluten-free diet is started after the first six months of the diagnosis the improvement is more favorable.

It is important to remember that nutritional deficiency and coexisting autoimmunity may cause neurologic dysfunction in celiac disease. A variety of neurologic phenotypes with different etiologies were found 68 patients with either celiac disease or AGA positive non-celiac disease in a 10 year period (2002-2012): cerebellar ataxia, neuropathy, dementia, myeloneuropathy, autoimmune disease, deficiencies of vitamin E, folate, or copper, genetic disorders, toxic or metabolic syndrome. The authors concluded that gluten exposure may produce neurologic dysfunction even in those patients without established celiac disease[181].

6. Associated Diseases

6.1. Celiac Disease and Type-1 Diabetes Mellitus

The association between type-1 diabetes mellitus and celiac disease has been known since the 1960s. The first reports in adults came from the United States by Ellenberg and Bookman in 1960[182], Vinnik et al.[183] and Green et al. in 1962[184], all cited by Wruble and Kalser[185], who also observed that diabetic steatorrhea is uncommon but a more intense manifestation of diabetic diarrhea. The amount of fecal fat is significantly higher that the observed in cases of celiac disease. According to Wruble and Kalser, Thompson observed 2 cases of diabetes in 119 patients with celiac disease and reported an increased incidence of diabetes in relatives of patients with celiac disease[186]. However Carter et al. could not confirm the familial association between diabetes mellitus and celiac disease[187]. But these were the early days in the diagnosis of atypical celiac disease and the genetics of both conditions was still unknown.

In children, the first cases with celiac disease and type-1 diabetes mellitus were reported in 1969[188-190].

A controlled longitudinal follow-up study of 10 years of progression in 335 celiac adult patients diagnosed in 1980-90 compared with age- and sex-matched control patients with various gastrointestinal symptoms, found a high statistical significant prevalence of endocrine disorders in patients with celiac disease (11.9% in celiac patients and 4.3% in the control group, $p<0.003$)[191, 192]. More recently, other authors found a prevalence of 5.4%-7.4% of type-1 diabetes mellitus in patients with celiac disease [71,193].

The high prevalence of the existence of both diseases can be explained in part by the sharing of common markers for the genetic susceptibility within and outside the HLA system. HLA-DQB1*0201 allele (part of the HLA-DQ2 heterodimer) was present in 17 of 18 patients (94%) with both diseases in Finland[194]. It should be taken into account that during a screening study, most children do not complain of digestive symptoms. Nevertheless, many have retarded growth and some other signs or symptoms of celiac disease, such as delayed puberty, hypertransaminasemia, and/or chronic iron

deficiency anemia, arthralgias, and dental enamel defects[195-198]. Recent studies of genome wide association have found additional genes that are shared by celiac disease and type-1 diabetes mellitus. Both diseases are polygenic in nature and several loci in different chromosomes determine their susceptibility. Since both are T cell mediated diseases, those genes regulating the immune response are likely to be shared and explain their familial association[199-202].

The diagnosis of type-1 diabetes mellitus was established in 90% of children before celiac disease was recognized[191]. The patients with diabetes mellitus and symptoms associated with celiac disease who follow a gluten-free diet notice an overall clear clinical improvement, in children often an increase in the rate of growth, increased hemoglobin levels. There is improvement in the control of diabetes mellitus, as supported by reduced hypoglycemic episodes and daily needs of required insulin[192,203].

More than 5% of patients with type-1 diabetes mellitus have also celiac disease confirmed by the histopathological features of duodenal biopsy specimens and response to gluten-free diet. This strong association between the two diseases would support the systematic screening of celiac disease among patients with insulin-dependent diabetes. Strategies for follow up include periodical serological determinations of specific antibodies, initially at diagnosis, followed by every six months during the first year and repeated at least annually, for five or more years. Patients with specific positive serological tests and with the presence of the genetic markers of susceptibility (HLA-DQ2 and/or HLA-DQ8) require a duodenal biopsy to confirm the diagnosis. Although there are many clinical guidelines that recommend screening implementation, in particular in children, in adolescents and young adults, its application in clinical practice has failed to achieve the desired levels of performance and expectation[204-206]. The American Gastroenterological Association does not recommend to screen all type-1 diabetes mellitus because those without symptoms are not motivated to follow a gluten-free diet and the natural course of asymptomatic celiac disease is unknown[207].

Also no ideal serological technique is available for screening. The best at present are the antibodies against tTG and anti-endomysium antibodies (EmA). The main problem is that the tests for specific antibodies are very sensitive (80%-90%) only in the presence of villous atrophy, but have a low diagnostic sensitivity (10%-30%) in Marsh stages 1 and 2 celiac patients. AGAs have virtually been abandoned for the diagnosis and screening of celiac disease since these antibodies have a low sensitivity and specificity[208,209].

In spite of these shortcomings, the cost of establishing a screening program for celiac disease in patients with type-1 diabetes mellitus is moderate. Assuming that the average prevalence of diabetes mellitus is 0.4% in the general population, for a hospital that serves a population of 200,000 people, about 800 patients would have to be screened. A determination of tTG antibodies costs about 8 euros per determination. Positive cases would have to undergo an endoscopy with duodenal biopsies. The average cost of this procedure is 300 euros per patient. At present, therefore in well-equipped hospitals it can be concluded that the costs for screening are acceptable and should be recommended in symptomatic cases suggesting the presence of celiac disease. In these situations an early diagnosis of celiac disease will prevent a series of unnecessary expenses with less discomfort for the patient on the short term and the prevention of osteoporosis and possible malignancy at a later stage. A recent meta-analysis analyzed the prevalence of celiac disease in 26,605 patients with type-1 diabetes mellitus in different countries. The mean prevalence of biopsy-confirmed celiac disease was 6% (95% CI, 5.0% to 6.9%). However, the heterogeneity observed was large. The prevalence in adults with type-1 diabetes mellitus was 2.7%. In mixed populations with both children and adult diabetic patients the prevalence was 4.7% and the prevalence of children with diabetes mellitus was 6.2% ($p<0.001$). More than one in twenty patients with type-1 diabetes has biopsy-verified celiac disease. The authors concluded that this prevalence is high enough to motivate screening for celiac disease among patients with type-1 diabetes mellitus[205].

A Swedish study has identified that the major histocompatibility complex class II transactivator (CIITA) gene (16p13) is associated to celiac disease and type-1 diabetes mellitus in families and is age dependent[200].

This suggests that advances in human genome and the identification of genes regulating the immune response may help to identify the heterogeneity of the clinical observations in both diseases.

6.2. Thyroid Diseases and Celiac Disease

Celiac disease has been found to be present at an increased rate in patients who have an autoimmune thyroid disease (Grave's disease and Hashimoto's thyroiditis), with a prevalence ranging from 2% to 7%[210-213]. Similar observations have been made in patients with celiac disease, in whom serological signs of autoimmune thyroid disease were present in up to 26%. Occurrence of thyroid dysfunction was detected in up to 10% and the risk of thyroid disease was estimated to be 3-fold higher as compared to controls[214-217].

It has been reported that celiac individuals who are following a gluten-free diet may still develop autoimmune thyroid impairment, suggesting that gluten withdrawal does not protect them in this respect[218,219]. By contrast, the decrease of the thyroid antibodies after 2 or 3 years[220] or the normalization of thyroid function after 1 year of gluten-free diet has been reported in other studies[221]. These different results may depend on longer duration of gluten-free diet in treated patients with celiac disease[222]. The authors prospectively evaluated the presence of thyroid autoimmunity in children and adolescents with celiac disease on a gluten-free diet. At the end of the 2 years follow-up, an increase of 7% in the prevalence of patients with celiac disease with thyroid autoimmunity requiring L-thyroxine was found. Apparently, thyroid autoimmunity is no more common in pediatric and adolescent patients with celiac disease on a gluten-free diet than in the control group. Since its clinical development does not seem to impact on growth, the authors concluded that a long-term regular screening program for thyroid disease may not be necessary for all patients with celiac disease on a gluten-free diet, but only for those who are suspected of having thyroid diseases[222].

Increased prevalence of celiac disease, autoimmune thyroid disorders, and type-1 diabetes mellitus, has been widely reported[223]. However, the authors have also concluded that certain patient groups such as those with autoimmune diseases may be offered screening but active case finding seems to be the most prudent option to follow in most clinical situations. In these cases such associations may lead to adverse effects on the growth, metabolism and fertility, so early detection is necessary to prevent secondary complications of these disorders.

The coexistence of celiac disease and autoimmune thyroid disease has been explained by several mechanisms such as common genetic predisposition and the association of both diseases with the gene encoding cytotoxic T-lymphocyte-associated antigen-4, a gene conferring susceptibility to thyroid autoimmunity. In addition, it has also been demonstrated that tTG-2 IgA antibodies react with thyroid tissue, and this binding could contribute to the development of thyroid disease in celiac disease[224].

7. Malignancy Associated with Celiac Disease

Gluten-free diet is demonstrated as an efficient treatment for the vast majority of celiac patients, leading to normalization of clinical and biochemical disturbances, reversion of inflammatory changes in the small bowel mucosa, and restoring the normal villous architecture. However, many celiac patients remain undiagnosed for several years before an adequate treatment. Additionally, the treatment of celiac disease with a lifelong strict gluten-free diet is difficult to follow, and an inadequate adherence rate of around 30% has been repeatedly reported[225-227].

The presence of gluten cross-contamination should also be considered in patients with persistent symptoms and/or villous atrophy[228,229]. These factors contribute to persistent inflammation with consequent malabsorption of micronutrients, and increased risk of infection, which may explain an excess of mortality and a higher malignancy risk among the celiac population[230,231]. The association of celiac disease with an increased risk for several malignancies has

been repeatedly reported in the medical literature of the last decades. This association is particularly clear in the case of a specific subtype of non-Hodgkin lymphoma, the enteropathy-associated T cell lymphoma which is considered as an established complication of celiac disease[232]. However, controversy exists regarding the increased risk of other neoplasias among patients with celiac disease, including solid tumors.

7.1. Overall Risk of Malignancies in Patients with Celiac Disease

The risk of malignancy in patients with celiac disease has been evaluated in several large epidemiological studies carried out in European and North American populations[232-235], as well as a systematic review with meta-analysis of 3 prospective studies that included 35,582 individuals[236]: According to the authors, the overall risk of presenting any neoplasia among patients with celiac disease was not increased compared to control populations, with a pooled OR of 1.07 (95% CI, 0.89 to 1.29). Relevantly, no significant heterogeneity or publication biases were observed in this meta-analysis. Although celiac patients are at a slightly increased risk of mortality, this cannot be attributed to malignancy in general[236].

7.2. Lymphoproliferative Malignancies of the Small Intestine

Several population based studies have repeatedly found a 2 to 6-fold increased risk of small bowel lymphoproliferative malignancies in celiac disease[237], particularly due to non-Hodgkin lymphoma. A recent meta-analysis summarizing the results of 8 individual cohort and case-control studies has estimated a pooled OR of 2.75 (95% CI, 2.0 to 3.78) for this neoplasia in celiac patients[236].

The highest non-Hodgkin lymphoma relative risk in celiac disease has been described for T-cell non-Hodgkin lymphoma, a particular subtype repeatedly related with celiac disease. The risk estimates for T-cell non-Hodgkin

lymphoma have varied markedly in the literature, and has been summarized with a pool OR of 15.84 (95% CI, 7.85 to 31.94) in a meta-analysis[236].

The increased risk of lymphoproliferative malignancies in celiac disease has been directly related with persistence of chronic inflammation. A multicenter retrospective cohort study demonstrated that the risk of small intestine lymphomas in celiac disease was dependent on small intestinal histopathology, and patients with villous atrophy (Marsh 3 stages in duodenal/jejunal biopsies) had a statistically significantly higher risk of lymphoma than those celiac patients with either Marsh 1 to 2 or Marsh 0 but positive celiac disease serology[234]. The degree of inflammation is then crucial for the development of lymphoproliferative malignancies in celiac disease, as recently shown also for rheumatoid arthritis, in which disease activity and not suppressive treatment was demonstrated as the underlying cause of neoplasia development[238].

The protective role of gluten-free diet in reducing the overall malignancy risk appeared after 5 years of following the diet[239]. A large study found an overall risk of lymphoproliferative malignancies of 2.82 (95% CI, 2.36-3.37) that decreased to 2.25 during 1 to 5 years of follow-up after celiac disease diagnosis and further to 1.98 after more than 5 years of follow-up[234].

On the other hand, individuals with celiac disease and lymphoproliferative malignancies were at an increased risk of death compared with individuals with lymphoproliferative malignancy only. But the increased mortality has been observed in the first year after the diagnosis of lymphoproliferative malignancies in celiac disease patients, which has been related to the predominance of T-non-Hodgkin lymphoma in that population. Thus there is no evidence that co-existing celiac disease influences survival in individuals with lymphoproliferative malignancy[240].

7.3. Small Bowel Carcinoma Risk in Patients with Celiac Disease

Malignant tumors of the small bowel are rare neoplasms comprising only 3% of all gastrointestinal tumors; approximately 25% of which are small bowel adenocarcinomas. Identified risk factors for small bowel adenocarcinoma

include Crohn's disease, celiac disease, and genetic polyposis syndromes[241]. Since the first report of association between small bowel adenocarcinoma and celiac disease in 1958[242], more cases have been reported[243]. Most of the population based epidemiological studies have shown that, although rare, the incidence of small bowel adenocarcinoma in celiac disease is increased between 4- and 11-fold compared to matched control populations[233,235,244].There were no differences among genders[235]. In a 30-year population based study in Finland no increase in the prevalence of small bowel carcinoma was found, possibly due to the overall rarity of this neoplasia. In this study, non-Hodgkin lymphoma emerged in patients with undiagnosed or poorly treated celiac disease[245].

7.4. Colorectal Cancer and Celiac Disease

The risk of colorectal cancer among patients with celiac disease has been evaluated in various reports during the last decade. The first report on this topic specifically assessed the prevalence of colorectal neoplasia (including tubulo-villous adenomas and carcinomas) among older patients with celiac disease who presented with iron deficiency anemia or an altered bowel habit[246]. In this report, a high prevalence of colorectal cancer was demonstrated in older patients presenting with iron deficiency anemia or an altered bowel habit. The prevalence was not superior to that of non-celiac patients with the same presentations. In a parallel study, the incidence of colorectal cancer among Swedish patients hospitalized with celiac disease and dermatitis herpetiformis was assessed in a retrospective population-based study[233]. The authors concluded that the risk of colorectal cancer was slightly increased, mainly in the ascending and transverse colon standardized incidence ratio (SIR) was 1.9 (95% CI, 1.2 to 2.8) among the group of patients with celiac disease, but not in those with dermatitis herpetiformis. Remarkably, an increased risk of rectal cancer was not found for both gluten-related diseases. A recent population based study has also corroborated this increase in the risk of colorectal cancer among celiac patients[235], but to a

lesser extent than the previously reported (SIR was 1.35; 95% CI, 1.13 to 1.58)[247].

In contrast, other studies such as carried out in Scotland[244], Finland[245], United States[248], Canada[249], and Argentina[250,251] have failed to demonstrate an increased incidence of colorectal cancer among patients with celiac disease and dermatitis herpetiformis. Most interestingly, recent research has also identified that the risk of colorectal cancer among Italian patients with celiac disease was even lower than for the general population, with a SIR of 0.29 (95% CI, 0.07 to 0.45)[252].

The discrepancy observed among different epidemiological studies on a possible increased risk of colorectal cancer in subjects with celiac disease when compared with the respective matched control populations may be attributed to a different diet composition and genetic background of the population studied. However, the possible increase in the risk of presenting colorectal cancer in the celiac population can be considered marginal, and do not support specific preventive measures for these patients, different to those established for an average risk general population. In fact, a prospective research aimed to evaluate the yield of colonoscopy for diagnosing additional pathologies in celiac patients on a gluten-free diet and with a newly diagnosed iron deficiency anemia or persisting diarrhea did not demonstrate an increased prevalence of colonic neoplasia regarding control subjects[253]. These authors have concluded until new data becomes available that colonoscopy should be considered in patients with celiac disease (over the age of 45 years) who present with iron deficiency anemia. Whilst, for celiac disease patients with persisting diarrhea (on a gluten-free diet) in the absence of sinister symptoms, a flexible sigmoidoscopy may be the initial investigation in order to exclude microscopic colitis.

In any case, the importance of a strict adherence to a gluten-free diet in preventing colorectal neoplasia has been recently highlighted. A low adherence to a gluten-free diet was an independent factor significantly associated with the presence of colonic adenomas (OR 6.78; CI, 1.39 to 33.20)[251], and those

patients who had a strict adherence to a gluten-free diet showed additional reductions in the risk of presenting colorectal cancer[252].

7.5. Breast Cancer in Women with Celiac Disease and Other Hormone-Dependent Neoplasm

Several population-based cohort studies have repeatedly shown a reduction in the risk of breast cancer development among women with celiac disease compared to matched controls[232,233,235,254-256], with SIR ratios varying from 0.3 to 0.85. This reduction in breast cancer risk has been explained by malnutrition and weight loss, associated with clinical or subclinical nutrient deficiency[145] and the presence of various reproductive disturbances in women with celiac disease, including delayed menarche, early menopause and ovulatory dysfunction[257-259]. These disturbances contribute to limit the lifetime exposition to sex hormones that are implicated in the etiological role for breast cancer.

Estrogens also play an important role in promoting endometrial and ovarian cancer, but in contrast with breast cancer, parallel reductions for these last cancers have not been universally demonstrated among women with celiac disease. Available studies show opposite results[233,235,260].

7.6. Thyroid Cancer and Celiac Disease

The association of celiac disease with thyroid disease, especially autoimmune thyroiditis, is widely recognized[261] as has been described early in this chapter. Few studies have also tried to relate celiac disease with papillary thyroid cancer, providing an increased risk between 2.5[262] and 22.52 fold[263]. In contrast, some other cohort studies from Sweden have not demonstrated such association[233,264].

7.7. Esophageal Cancer

An increased risk for esophageal cancer, especially squamous carcinoma, in celiac disease was described in the early literature[265,266], but has not been reproduced in later published well-designed research studies[235,245].

7.8. Preventing Cancer in Celiac Disease

Delayed diagnosis of celiac disease has shown to increase cancer risk because of the prolonged period of dietary exposure to gluten[267]. This risk is more relevant for the intestine -specific cancers such as small bowel carcinoma and non-Hodgkin lymphoma. Except for lymphoproliferative malignancies, no definitive data support an increased risk of cancer in patients with celiac disease, thus, surveillance and preventive measures for this population are currently not justified. However, the benefits of a gluten-free diet in reducing the overall risk of cancer must be emphasized. For many years it is known that following a gluten-free diet during an extended period reduces the risk of cancer to the level of a control population[239]. The long-term risks of malignancy beyond 10-15 years in people with celiac disease diagnosed in the Lothian region of Scotland, United Kingdom showed that the risk of any malignancy in celiac disease patients compared with the general population was increased 40% (SIR = 1.41; 95% CI, 1.09 to 1.78]. The increased risk for cancer overall persisted for up to 15 years, beyond which no overall increase in malignancy risk was observed, although the risk of non-Hodgkin's lymphoma remained raised beyond 15 years (SIR = 5.15; 95% CI, 1.40-13.2)[244]. Long-term risk studies beyond 25 years of follow-up are needed. For the time being, the above observations provide further support to strongly advise all patients with celiac disease and dermatitis herpetiformis to adhere to a strict gluten-free diet for life.

References

1. Rodrigo-Saez LR. *Celiac disease: an unique autoimmune model.* An R Acad Nac Med (Madr). 2008; 125: 91-100; discussion 100-4.
2. Hernandez L, Green PH. *Extraintestinal manifestations of celiac disease.* Current gastroenterology reports. 2006; 8: 383-9.
   http://dx.doi.org/10.1007/s11894-006-0023-7
   PMid:16968605
3. Bai D, Brar P, Holleran S, Ramakrishnan R, Green PH. *Effect of gender on the manifestations of celiac disease: evidence for greater malabsorption in men.* Scand J Gastroenterol. 2005; 40: 183-7.
   http://dx.doi.org/10.1080/00365520510011498
   PMid:15764149
4. Sategna-Guidetti C, Solerio E, Scaglione N, Aimo G, Mengozzi G. *Duration of gluten exposure in adult coeliac disease does not correlate with the risk for autoimmune disorders.* Gut. 2001; 49: 502-5.
   http://dx.doi.org/10.1136/gut.49.4.502
   PMid:11559646 PMCid:PMC1728461
5. Marchese A, Lovati E, Biagi F, Corazza GR *Coeliac disease and type 1 diabetes mellitus: epidemiology, clinical implications and effects of gluten-free diet.* Endocrine. 2013; 43: 1-2.
   http://dx.doi.org/10.1007/s12020-012-9758-0
   PMid:22820894
6. Greco D, Pisciotta M, Gambina F, Maggio F. *Celiac disease in subjects with type 1 diabetes mellitus: a prevalence study in western Sicily (Italy).* Endocrine. 2013; 43: 108-11.
   http://dx.doi.org/10.1007/s12020-012-9718-8
   PMid:22707396
7. Soyucen E, Yilmaz S, Celtik C, Vatansever U, Oner N, Karasalihoglu S. *Seroprevalence of autoimmune thyroiditis and celiac disease in children with insulin-dependent diabetes mellitus in the Thrace region of Turkey.* Turk J Gastroenterol. 2010; 21: 231-5.
   PMid:20931425
8. Sattar N, Lazare F, Kacer M, Aguayo-Figueroa L, Desikan V, Garcia M. *Celiac disease in children, adolescents, and young adults with autoimmune thyroid disease.* J Pediatr. 2011; 158: 272-5.
   http://dx.doi.org/10.1016/j.jpeds.2010.08.050
   PMid:20961564

9. Ergur AT, Ocal G, Berberoglu M et al. *Celiac disease and autoimmune thyroid disease in children with type 1 diabetes mellitus: clinical and HLA-genotyping results.* J Clin Res Pediatr Endocrinol. 2010; 2: 151-4.

http://dx.doi.org/10.4274/jcrpe.v2i4.151
PMid:21274314 PMCid:PMC3005689

10. D'Onofrio F, Miele L, Diaco M et al. *Sjogren's syndrome in a celiac patient: searching for environmental triggers.* Int.J Immunopathol Pharmacol. 2006; 19: 445-448.

PMid:16831312

11. Reunala T, Salmi J, Karvonen J. *Dermatitis herpetiformis and celiac disease associated with Addison's disease.* Arch Dermatol. 1987; 123: 930-2.

http://dx.doi.org/10.1001/archderm.1987.01660310098023
PMid:3606172

12. Vajro P, Paolella G, Maggiore G, Giordano G. *Meta-analysis: pediatric celiac disease, cryptogenic hypertransaminasemia, and autoimmune hepatitis.* J Pediatr Gastroenterol Nutr. 2013.

http://dx.doi.org/10.1097/MPG.0b013e31828dc5c5
PMCid:PMC3637398

13. Nastasio S, Sciveres M, Riva S, Filippeschi IP, Vajro P, Maggiore G. *Celiac disease-associated autoimmune hepatitis in childhood: long term response to treatment.* J Pediatr Gastroenterol Nutr. 2013.

http://dx.doi.org/10.1097/MPG.0b013e31828b1dfa
PMid:23403438

14. Panetta F, Nobili V, Sartorelli MR, Papa RE, Ferretti F, Alterio A, Diamanti A. *Celiac disease in pediatric patients with autoimmune hepatitis: etiology, diagnosis, and management.* Paediatr Drugs. 2012; 14: 35-41.

http://dx.doi.org/10.2165/11593150-000000000-00000
PMid:22149550

15. Volta U, Rodrigo L, Granito A, Petrolini N, Muratori P, Muratori L et al. *Celiac disease in autoimmune cholestatic liver disorders.* Am J Gastroenterol. 2002; 97: 2609-13.

http://dx.doi.org/10.1111/j.1572-0241.2002.06031.x
PMid:12385447

16. Volta U, Caio G, Tovoli F, De Giorgio R. *Gut-liver axis: an immune link between celiac disease and primary biliary cirrhosis.* Expert Rev Gastroenterol Hepatol. 2013; 7: 253-61.

http://dx.doi.org/10.1586/egh.13.5
PMid:23445234

17. Efe C, Wahlin S, Ozaslan E, Berlot AH, Purnak T, Muratori L, Quarneti C. *Autoimmune hepatitis/primary biliary cirrhosis overlap syndrome and associated extrahepatic autoimmune diseases.* Eur J Gastroenterol Hepatol. 2012; 24: 531-4.

http://dx.doi.org/10.1097/MEG.0b013e328350f95b
PMid:22465972

18. Abenavoli L, Arena V, Giancotti F, Vecchio FM, Abenavoli S. *Celiac disease, primary biliary cirrhosis and helicobacter pylori infection: one link for three diseases.* Int J Immunopathol Pharmacol. 2010; 23: 1261-5.

PMid:21244776

19. Eyre S, Hinks A, Bowes J, Flynn E, Martin P, Wilson A. *Overlapping genetic susceptibility variants between three autoimmune disorders: rheumatoid arthritis, type 1 diabetes and coeliac disease.* Arthritis Res Ther. 2010; 12: R175.

http://dx.doi.org/10.1186/ar3139
PMid:20854658 PMCid:PMC2991006

20. Zhernakova A, Stahl EA, Trynka G, Raychaudhuri S, Festen EA, Franke L. *Meta-analysis of genome-wide association studies in celiac disease and rheumatoid arthritis identifies fourteen non-HLA shared loci.* PLoS Genet. 2011; 7: e1002004.

http://dx.doi.org/10.1371/journal.pgen.1002004
PMid:21383967 PMCid:PMC3044685

21. Peters U, Askling J, Gridley G, Ekbom A, Linet M. *Causes of death in patients with celiac disease in a population-based Swedish cohort.* Arch Intern Med. 2003; 163: 1566-72.

http://dx.doi.org/10.1001/archinte.163.13.1566
PMid:12860579

22. Ventura A, Magazu G, Gerarduzzi T, Greco L. *Coeliac disease and the risk of autoimmune disorders.* Gut. 2002; 51: 897; author reply 897-8.

http://dx.doi.org/10.1136/gut.51.6.897
PMid:12427802 PMCid:PMC1773487

23. Cosnes J, Cellier C, Viola S, Colombel JF, Michaud L, Sarles J. *Incidence of autoimmune diseases in celiac disease: protective effect of the gluten-free diet.* Clin Gastroenterol Hepatol. 2008; 6: 753-8.

http://dx.doi.org/10.1016/j.cgh.2007.12.022
PMid:18255352

24. Abadie V, Sollid LM, Barreiro LB, Jabri B. *Integration of genetic and immunological insights into a model of celiac disease pathogenesis.* Annu Rev Immunol. 2011; 29: 493-525.

http://dx.doi.org/10.1146/annurev-immunol-040210-092915
PMid:21219178

25. Jabri B, Kasarda DD, Green PH. *Innate and adaptive immunity: the yin and yang of celiac disease.* Immunol Rev. 2005; 206: 219-31.

http://dx.doi.org/10.1111/j.0105-2896.2005.00294.x
PMid:16048552

26. Koning F, Gilissen L, Wijmenga C. *Gluten: a two-edged sword. Immunopathogenesis of celiac disease.* Springer Semin Immunopathol. 2005; 27: 217-32.

http://dx.doi.org/10.1007/s00281-005-0203-9
PMid:16091925

27. Meresse B, Malamut G, Cerf-Bensussan N. *Celiac disease: an immunological jigsaw.* Immunity. 2012; 36: 907-19.

http://dx.doi.org/10.1016/j.immuni.2012.06.006
PMid:22749351

28. Sollid LM, Jabri B. *Is celiac disease an autoimmune disorder?* Curr Opin Immunol. 2005; 17: 595-600.

http://dx.doi.org/10.1016/j.coi.2005.09.015
PMid:16214317

29. Hmida NB, Ben Ahmed M, Moussa A, Rejeb MB, Said Y, Kourda N. *Impaired control of effector T cells by regulatory T cells: a clue to loss of oral tolerance and autoimmunity in celiac disease?* Am J Gastroenterol. 2012; 107: 604-11.

http://dx.doi.org/10.1038/ajg.2011.397
PMid:22108452

30. Arnson Y, Itzhaky D, Mosseri M, Barak V, Tzur B, Agmon-Levin N et al. *Vitamin D inflammatory cytokines and coronary events: a comprehensive review.* Clin Rev Allergy Immunol. 2013; 45: 236-47.

http://dx.doi.org/10.1007/s12016-013-8356-0
PMid:23314982

31. Rashid M, Zarkadas M, Anca A, Limeback H. *Oral manifestations of celiac disease: a clinical guide for dentists.* J Mich Dent Assoc. 2011; 93: 42-6.

PMid:22073722

32. Costacurta M, Maturo P, Bartolino M, Docimo R. *Oral manifestations of coeliac disease: A clinical-statistic study.* Oral Implantol (Rome). 2010; 3: 12-9.

33. Lahteenoja H, Toivanen A, Viander M, Mäki M, Irjala K, Räihä I et al. *Oral mucosal changes in coeliac patients on a gluten-free diet.* Eur.J Oral Sci. 1998; 106: 899-906.

http://dx.doi.org/10.1046/j.0909-8836.1998.eos106501.x

34. Bardellini E, Amadori F, Ravelli A, Salemme M, Lonardi S, Villanacci V et al. *Histopathological findings in the oral mucosa of celiac patients.* Rev Esp Enferm Dig. 2014; 106: 86-91.

http://dx.doi.org/10.4321/S1130-01082014000200003
PMid:24852733

35. Lahteenoja H, Toivanen A, Viander M, Raiha I, Rantala I, Syrjanen S et al. *Increase in T-cell subsets of oral mucosa: a late immune response in patients with treated coeliac disease?* Scand.J Immunol. 2000; 52: 602-8.
http://dx.doi.org/10.1046/j.1365-3083.2000.00794.x
PMid:11119267

36. Lahteenoja H, Maki M, Viander M, Raiha I, Vilja P, Rantala I et al. *Local challenge on oral mucosa with an alpha-gliadin related synthetic peptide in patients with celiac disease.* Am J Gastroenterol. 2000; 95: 2880-7.
http://dx.doi.org/10.1016/S0002-9270(00)01049-2

37. Carroccio A, Campisi G, Iacono G, Iacono OL, Maresi E, DI Prima L et al. *Oral mucosa of coeliac disease patients produces antiendomysial and antitransglutaminase antibodies: the diagnostic usefulness of an in vitro culture system.* Aliment Pharmacol Ther. 2007; 25: 1471-7.
http://dx.doi.org/10.1111/j.1365-2036.2007.03335.x
PMid:17539987

38. Bonamico M, Nenna R, Luparia RP, Perricone C, Montuori M, Lucantoni F et al. *Radioimmunological detection of anti-transglutaminase autoantibodies in human saliva: a useful test to monitor coeliac disease follow-up.* Aliment Pharmacol Ther. 2008; 28(3): 364-70.
http://dx.doi.org/10.1111/j.1365-2036.2008.03720.x
PMid:19086333

39. Wray D. *Gluten-sensitive recurrent aphthous stomatitis.* Dig Dis Sci. 1981; 26: 737-40.
http://dx.doi.org/10.1007/BF01316864
PMid:7261838

40. Sedghizadeh PP, Shuler CF, Allen CM, Beck FM, Kalmar JR. *Celiac disease and recurrent aphthous stomatitis: a report and review of the literature.* Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2002; 94: 474-8.
http://dx.doi.org/10.1067/moe.2002.127581
PMid:12374923

41. Patial RK. *Recurrent aphthous ulcers in subclinical coeliac disease.* J Assoc Physicians India. 2003; 51: 80.
PMid:12693466

42. Admou B, Essaadouni L, Krati K, Zaher K, Sbihi M, Chabba L et al. *Atypical celiac disease: from recognizing to managing.* Gastroenterol Res Pract. 2012; 637187.
http://dx.doi.org/10.1155/2012/637187

43. Cheng J, Malahias T, Brar P, Minaya MT, Green PH. *The association between celiac disease, dental enamel defects, and aphthous ulcers in a United States cohort.* J Clin Gastroenterol. 2010; 44: 191-4.
http://dx.doi.org/10.1097/MCG.0b013e3181ac9942
PMid:19687752

44. Veloso FT, Saleiro JV. *Small-bowel changes in recurrent ulceration of the mouth.* Hepatogastroenterology. 1987; 34: 36-7.

PMid:3570141

45. Campisi G, Di LC, Carroccio A, Compilato D, Iacono G, Procaccini M et al. *Coeliac disease: oral ulcer prevalence, assessment of risk and association with gluten-free diet in children.* Dig Liver Dis. 2008; 40: 104-7.

http://dx.doi.org/10.1016/j.dld.2007.10.009
PMid:18063428

46. Condo R, Costacurta M, Maturo P, Docimo R. *The dental age in the child with coeliac disease.* Eur J Paediatr Dent. 2011; 12: 184-8.

PMid:22077688

47. Rasmussen P, Espelid I. *Coeliac disease and dental malformation.* ASDC J Dent.Child. 1980; 47: 190-2.

PMid:6929800

48. Aine L. *Dental enamel defects and dental maturity in children and adolescents with coeliac disease.* Proc.Finn.Dent.Soc. 1986; 82(3): 1-71.

PMid:3725749

49. Hill ID, Dirks MH, Liptak GS, Colletti RB, Fasano A, Guandalini S et al. *Guideline for the diagnosis and treatment of celiac disease in children: recommendations of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition.* J Pediatr Gastroenterol Nutr. 2005; 40: 1-19.

http://dx.doi.org/10.1097/00005176-200501000-00001
PMid:15625418

50. Munoz F, Del Rio N, Sonora C, Tiscornia I, Marco A, Hernández A. *Enamel defects associated with coeliac disease: putative role of antibodies against gliadin in pathogenesis.* Eur J Oral Sci. 2012; 120: 104-12.

http://dx.doi.org/10.1111/j.1600-0722.2012.00949.x
Pmid:22409216

51. Fraser D, Nikiforuk G. *The etiology of enamel hypoplasia in children - a unifying concept.* J Int Assoc Dent Child. 2014; 13: 1-11.

52. Bossu M, Bartoli A, Orsini G, Luppino E, Polimeni A. *Enamel hypoplasia in coeliac children: a potential clinical marker of early diagnosis.* Eur J Paediatr Dent. 2007; 8: 31-7.

Pmid:17359212

53. Ventura A, Martelossi S. *Dental enamel defects and coeliac disease.* Arch.Dis Child. 1997; 77: 91.

http://dx.doi.org/10.1136/adc.77.1.91

54. Aine L. *Permanent tooth dental enamel defects leading to the diagnosis of coeliac disease.* Br.Dent.J. 1994; 177: 253-4.

http://dx.doi.org/10.1038/sj.bdj.4808578
PMid:7917634

55. Aine L, Maki M, Collin P, Keyriläinen O. *Dental enamel defects in celiac disease.* J Oral Pathol.Med. 1990; 19: 241-5.
http://dx.doi.org/10.1111/j.1600-0714.1990.tb00834.x
PMid:2401959

56. Vos T, Flaxman AD, Naghavi M et al. *Years lived with disability (YLDs) for 1160 sequelae of 289 diseases and injuries 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010.* 2012; 380: 2163-96.

57. Avsar A, Kalayci AG. *The presence and distribution of dental enamel defects and caries in children with celiac disease.* Turk J Pediatr. 2008; 50: 45-50.
PMid:18365591

58. Usatine RP, Tinitigan M. *Diagnosis and treatment of lichen planus.* Am Fam Physician. 2011; 84: 53-60.
PMid:21766756

59. Alam F, Hamburger J. *Oral mucosal lichen planus in children.* Int J Paediatr Dent. 2001; 11: 209-14.
http://dx.doi.org/10.1046/j.1365-263X.2001.00266.x
PMid:11484471

60. Fortune F, Buchanan JA. *Oral lichen planus and coeliac disease.* Lancet. 1993; 341: 1154-5.
http://dx.doi.org/10.1016/0140-6736(93)93175-Z

61. Cigic L, Gavic L, Simunic M, Ardalic Z, Biocina-Lukenda D. *Increased prevalence of celiac disease in patients with oral lichen planus.* Clin Oral Investig. 2014.
http://dx.doi.org/10.1007/s00784-014-1288-0

62. Reamy BV, Derby R, Bunt CW. *Common tongue conditions in primary care. Am Fam Physician.* 2010; 81: 627-34.
PMid:20187599

63. Erriu M, Canargiu F, Orru G, Garau V, Montaldo C. *Idiopathic atrophic glossitis as the only clinical sign for celiac disease diagnosis: a case report.* J Med Case Rep. 2012; 6: 185.
http://dx.doi.org/10.1186/1752-1947-6-185
PMid:22762365 PMCid:PMC3407713

64. Pastore L, Lo ML, Serpico R. *Atrophic glossitis leading to the diagnosis of celiac disease.* N Engl.J Med. 2007; 356: 2547.
http://dx.doi.org/10.1056/NEJMc070200
PMid:17568041

65. Borchers AT, Naguwa SM, Keen CL, Gershwin ME. *Immunopathogenesis of Sjögren's syndrome.* Clin Rev Allergy Immunol. 2003; 25: 89-104.
http://dx.doi.org/10.1385/CRIAI:25:1:89

66. Szodoray P, Barta Z, Lakos G, Szakáll S, Zeher M. *Coeliac disease in Sjogren's syndrome-a study of 111 Hungarian patients.* Rheumatol Int. 2004; 24: 278-82.
http://dx.doi.org/10.1007/s00296-003-0360-x
PMid:13680146

67. Roblin X, Helluwaert F, Bonaz B. *Celiac disease must be evaluated in patients with Sjögren syndrome.* Arch Intern Med. 2004; 164: 387.

http://dx.doi.org/10.1001/archinte.164.21.2387-b

68. Baydoun A, Maakaron JE, Halawi H, Abou Rajal J, Taher AT. *Hematological manifestations of celiac disease.* Scand J Gastroenterol. 2012; 47: 1401-11.

http://dx.doi.org/10.3109/00365521.2012.706828
PMid:22861356

69. Ludvigsson JF, Neovius M, Hammarstrom L. *Association between IgA deficiency & other autoimmune conditions: a population-based matched cohort study.* J Clin Immunol. 2014; 34: 444-51.

http://dx.doi.org/10.1007/s10875-014-0009-4
PMid:24584841

70. Halfdanarson TR, Litzow MR, Murray JA. *Hematologic manifestations of celiac disease.* Blood. 2007; 109: 412-21.

http://dx.doi.org/10.1182/blood-2006-07-031104
PMid:16973955 PMCid:PMC1785098

71. Bottaro G, Cataldo F, Rotolo N, Spina M, Corazza GR. *The clinical pattern of subclinical/silent celiac disease: an analysis on 1026 consecutive cases.* The American journal of gastroenterology. 1999; 94: 691-6.

http://dx.doi.org/10.1016/S0002-9270(98)00819-3

72. Fernandez-Banares F, Monzon H, Forne M. *A short review of malabsorption and anemia.* World J Gastroenterol. 2009; 15: 4644-52.

http://dx.doi.org/10.3748/wjg.15.4644
PMid:19787827 PMCid:PMC2754512

73. Mody RJ, Brown PI, Wechsler DS. *Refractory iron deficiency anemia as the primary clinical manifestation of celiac disease.* J Pediatr Hematol Oncol. 2003; 25: 169-72.

http://dx.doi.org/10.1097/00043426-200302000-00018
PMid:12571473

74. Corazza GR, Valentini RA, Andreani ML et al. *Subclinical coeliac disease is a frequent cause of iron-deficiency anaemia.* Scand.J Gastroenterol. 1995; 30: 153-6.

http://dx.doi.org/10.3109/00365529509093254
PMid:7732338

75. Carroccio A, Iannitto E, Cavataio F, Montalto G, Tumminello M, Campagna P et al. *Sideropenic anemia and celiac disease: one study, two points of view.* Dig Dis Sci. 1998; 43: 673-8.

http://dx.doi.org/10.1023/A:1018896015530
PMid:9539667

76. Annibale B, Capurso G, Chistolini A, D'Ambra G, DiGiulio E, Monarca B et al. *Gastrointestinal causes of refractory iron deficiency anemia in patients without gastrointestinal symptoms.* Am J Med. 2001; 111: 439-45.

http://dx.doi.org/10.1016/S0002-9343(01)00883-X

77. Howard MR, Turnbull AJ, Morley P, Hollier P, Webb R, Clarke A. *A prospective study of the prevalence of undiagnosed coeliac disease in laboratory defined iron and folate deficiency.* J Clin Pathol. 2002; 55: 754-7.
http://dx.doi.org/10.1136/jcp.55.10.754
PMid:12354801 PMCid:PMC1769776

78. Hershko C, Hoffbrand AV, Keret D, Souroujon M, Maschler I, Monselise Y et al. *Role of autoimmune gastritis, Helicobacter pylori and celiac disease in refractory or unexplained iron deficiency anemia.* Haematologica. 2005; 90: 585-95.
PMid:15921373

79. Carter D, Maor Y, Bar-Meir S, Avidan B. *Prevalence and predictive signs for gastrointestinal lesions in premenopausal women with iron deficiency anemia.* Dig Dis Sci. 2008; 53: 3138-44.
http://dx.doi.org/10.1007/s10620-008-0298-7
PMid:18465236

80. Hershko C, Patz J. *Ironing out the mechanism of anemia in celiac disease.* Haematologica. 2008; 93: 1761-5.
http://dx.doi.org/10.3324/haematol.2008.000828
PMid:19050064

81. Hershko C, Camaschella C. *How I treat unexplained refractory iron deficiency anemia.* Blood. 2014; 123: 326-33.
http://dx.doi.org/10.1182/blood-2013-10-512624
PMid:24215034

82. Harper JW, Holleran SF, Ramakrishnan R, Bhagat G, Green PH. *Anemia in celiac disease is multifactorial in etiology.* Am J Hematol. 2007; 82: 996-1000.
http://dx.doi.org/10.1002/ajh.20996
PMid:17636474

83. Fine KD. *The prevalence of occult gastrointestinal bleeding in celiac sprue.* N Engl J Med. 1996; 334: 1163-7.
http://dx.doi.org/10.1056/NEJM199605023341804
PMid:8602182

84. Bergamaschi G, Markopoulos K, Albertini R, Di Sabatino A, Biagi F, Ciccocioppo R et al. *Anemia of chronic disease and defective erythropoietin production in patients with celiac disease.* Haematologica. 2008; 93: 1785-91.
http://dx.doi.org/10.3324/haematol.13255
Pmid:18815191

85. Salvesen HA, Boe J. *Osteomalacia in sprue.* Acta Med Scand. 1953; 146: 290-9.
http://dx.doi.org/10.1111/j.0954-6820.1953.tb10243.x
PMid:13091741

86. Rabelink NM, Westgeest HM, Bravenboer N, Jacobs MA, Lips P. *Bone pain and extremely low bone mineral density due to severe vitamin D deficiency in celiac disease.* Arch Osteoporos. 2011; 6: 209-213.

http://dx.doi.org/10.1007/s11657-011-0059-7
PMid:22207878 PMCid:PMC3235277

87. Corazza GR, Di SM, Maurino E, Bai JC. *Bones in coeliac disease: diagnosis and treatment.* Best.Pract Res.Clin Gastroenterol. 2005; 19: 453-465.

http://dx.doi.org/10.1016/j.bpg.2005.01.002
PMid:15925849

88. Dorn SD, Hernandez L, Minaya MT, Morris CB, Leserman J, Lewis S et al. *The development and validation of a new coeliac disease quality of life survey (CD-QOL).* Aliment Pharmacol Ther. 2010; 31: 666-75.

http://dx.doi.org/10.1111/j.1365-2036.2009.04220.x
PMid:20015103

89. Gordon CM, Baim S, Bianchi ML, Bishop NJ, Hans DB, Kalkwarf H et al. *Special report on the 2007 Pediatric position development conference of the International Society for Clinical Densitometry.* South Med J. 2008; 101: 740-3.

http://dx.doi.org/10.1097/SMJ.0b013e31817a8b55
PMid:18580718

90. Lucendo AJ, Garcia-Manzanares A. *Bone mineral density in adult coeliac disease: an updated review.* Revista espanola de enfermedades digestivas: organo oficial de la Sociedad Espanola de Patologia Digestiva. 2013; 105: 154-62.

http://dx.doi.org/10.4321/S1130-01082013000300006
PMid:23735022

91. Jatla M, Zemel BS, Bierly P, Verma R. *Bone mineral content deficits of the spine and whole body in children at time of diagnosis with celiac disease.* J Pediatr Gastroenterol Nutr. 2009; 48: 175-80.

http://dx.doi.org/10.1097/MPG.0b013e318177e621
PMid:19179879

92. Trovato CM, Albanese CV, Leoni S, Celletti I, Valitutti F, Cavallini C et al. *Lack of Clinical Predictors for Low Mineral Density in Children With Celiac Disease.* J Pediatr Gastroenterol Nutr. 2014.

http://dx.doi.org/10.1097/MPG.0000000000000541
PMid:25162363

93. Mora S, Weber G, Barera G, Bellini A, Pasolini D, Prinster C et al. *Effect of gluten-free diet on bone mineral content in growing patients with celiac disease.* Am J Clin Nutr. 1993; 57: 224-8.

PMid:8424392

94. McFarlane XA, Bhalla AK, Robertson DA. *Effect of a gluten free diet on osteopenia in adults with newly diagnosed coeliac disease.* Gut. 1996; 39: 180-4.

http://dx.doi.org/10.1136/gut.39.2.180
PMid:8991855 PMCid:PMC1383295

95. Sundar N, Crimmins R, Swift G. *Clinical presentation and incidence of complications in patients with coeliac disease diagnosed by relative screening.* Postgrad.Med J. 2007; 83: 273-6.
http://dx.doi.org/10.1136/pgmj.2006.052977
PMid:17403956 PMCid:PMC2600030

96. Corazza GR, Di Stefano M, Maurino E, Bai JC. *Bones in coeliac disease: diagnosis and treatment.* Best Pract Res Clin Gastroenterol. 2005; 19: 453-65.
http://dx.doi.org/10.1016/j.bpg.2005.01.002
PMid:15925849

97. Lorinczy K, Juhasz M, Csontos A, Fekete B. Terjék O, Lakatos PL et al. *Does dermatitis herpetiformis result in bone loss as coeliac disease does? A cross sectional study.* Rev Esp Enferm Dig. 2013; 105: 187-93.
http://dx.doi.org/10.4321/S1130-01082013000400002
PMid:23859446

98. Mustalahti K, Collin P, Sievanen H et al. *Osteopenia in patients with clinically silent coeliac disease warrants screening.* Lancet. 1999; 354: 744-5.
http://dx.doi.org/10.1016/S0140-6736(99)01990-X

99. Corazza GR, Di SA, Cecchetti L, Jorizzo RA, Di Stefano M, Minguzzi L et al. *Influence of pattern of clinical presentation and of gluten-free diet on bone mass and metabolism in adult coeliac disease.* Bone. 1996; 18: 525-30.
http://dx.doi.org/10.1016/8756-3282(96)00071-3

100. Turner J, Pellerin G, Mager D. *Prevalence of metabolic bone disease in children with celiac disease is independent of symptoms at diagnosis.* J Pediatr Gastroenterol Nutr. 2009; 49: 589-93.
http://dx.doi.org/10.1097/MPG.0b013e31819ca18e
PMid:19644400

101. Kurppa K, Paavola A, Collin P, Sievänen H, Laurika K, Huhtala H et al. *Benefits of a gluten-free diet for asymptomatic patients with serologic markers of celiac disease.* Gastroenterology. 2014; 147: 610-7.
http://dx.doi.org/10.1053/j.gastro.2014.05.003
PMid:24837306

102. Stenson WF, Newberry R, Lorenz R, Baldus C, Civitelli R. *Increased prevalence of celiac disease and need for routine screening among patients with osteoporosis.* Arch Intern Med. 2005; 165: 393-9.
http://dx.doi.org/10.1001/archinte.165.4.393
PMid:15738367

103. Lindh E, Ljunghall S, Larsson K, Lavö B. *Screening for antibodies against gliadin in patients with osteoporosis.* J Intern Med. 1992; 231: 403-6.
http://dx.doi.org/10.1111/j.1365-2796.1992.tb00951.x
PMid:1588266

104. Ciacci C, Maurelli L, Klain M, Savino G, Salvatore M, Mazzacca G et al. *Effects of dietary treatment on bone mineral density in adults with celiac disease: factors predicting response.* Am J Gastroenterol. 1997; 92: 992-6.

PMid:9177517

105. Selby PL, Davies M, Adams JE, Mawer EB. *Bone loss in celiac disease is related to secondary hyperparathyroidism.* J Bone Miner Res. 1999; 14: 652-7.

http://dx.doi.org/10.1359/jbmr.1999.14.4.652
PMid:10234588

106. Kinsey L, Burden ST, Bannerman E. *A dietary survey to determine if patients with coeliac disease are meeting current healthy eating guidelines and how their diet compares to that of the British general population.* Eur J Clin Nutr. 2008; 62: 1333-42.

http://dx.doi.org/10.1038/sj.ejcn.1602856
PMid:17700651

107. Fisher AA, Davis MW, Budge MM. *Should we screen adults with osteoporotic fractures for coeliac disease?* Gut. 2004; 53: 154-5.

http://dx.doi.org/10.1136/gut.53.1.154-a
PMid:14684593 PMCid:PMC1773923

108. Garcia-Manzanares A, Tenias JM, Lucendo AJ. *Bone mineral density directly correlates with duodenal Marsh stage in newly diagnosed adult celiac patients.* Scandinavian journal of gastroenterology. 2012; 47: 927-36.

http://dx.doi.org/10.3109/00365521.2012.688217
PMid:22587226

109. Younes M, Ben Youssef H, Safer L, Fadoua H, Zrour S, Beija I et al. *Prevalence of bone loss in adult celiac disease and associated factors: a control case study.* La Tunisie medicale. 2012; 90: 129-35.

PMid:22407624

110. Colston KW, Mackay AG, Finlayson C, Wu JC, Maxwell JD. *Localisation of vitamin D receptor in normal human duodenum and in patients with coeliac disease.* Gut. 1994; 35: 1219-25.

http://dx.doi.org/10.1136/gut.35.9.1219
PMid:7959227 PMCid:PMC1375697

111. Vogelsang H, Suk EK, Janisiw M, Stain C. Mayr WR, Panzer S. *Calcaneal ultrasound attenuation and vitamin-D-receptor genotypes in celiac disease.* Scand J Gastroenterol. 2000; 35: 172-6.

http://dx.doi.org/10.1080/003655200750024344
PMid:10720115

112. Garcia-Manzanares A, Lucendo AJ. *Nutritional and dietary aspects of celiac disease.* Nutrition in clinical practice: official publication of the American Society for Parenteral and Enteral Nutrition. 2011; 26: 163-73.

http://dx.doi.org/10.1177/0884533611399773
PMid:21447770

113. Nilsen EM, Jahnsen FL, Lundin KE, Johansem FE, Fausa O, Sollid LM et al. *Gluten induces an intestinal cytokine response strongly dominated by interferon gamma in patients with celiac disease.* Gastroenterology. 1998; 115: 551-63.

http://dx.doi.org/10.1016/S0016-5085(98)70134-9

114. Stazi AV, Trecca A, Trinti B. *Osteoporosis in celiac disease and in endocrine and reproductive disorders.* World J Gastroenterol. 2008; 14: 498-505.

http://dx.doi.org/10.3748/wjg.14.498
PMid:18203279 PMCid:PMC2681138

115. Wishart J, Horowitz M, Need A et al. *Relationship between forearm and vertebral mineral density in postmenopausal women with primary hyperparathyroidism.* 1990; 150: 1329-31.

116. Valdimarsson T, Arnqvist HJ, Toss G, Järnerot G, Nyström F, Ström M. *Low circulating insulin-like growth factor I in coeliac disease and its relation to bone mineral density.* Scand J Gastroenterol. 1999; 34: 904-8.

http://dx.doi.org/10.1080/003655299750025381
PMid:10522610

117. Jameson S. *Coeliac disease, insulin-like growth factor, bone mineral density, and zinc.* Scand J Gastroenterol. 2000; 35: 894-6.

http://dx.doi.org/10.1080/003655200750023291
PMid:10994632

118. Buckley KA, Fraser WD. *Receptor activator for nuclear factor kappaB ligand and osteoprotegerin: regulators of bone physiology and immune responses/potential therapeutic agents and biochemical markers.* Ann Clin Biochem. 2002; 39: 551-6.

http://dx.doi.org/10.1177/000456320203900602

119. Taranta A, Fortunati D, Longo M, Rucci N, Iacomino E, Aliberti F et al. *Imbalance of osteoclastogenesis-regulating factors in patients with celiac disease.* Journal of bone and mineral research: the official journal of the American Society for Bone and Mineral Research. 2004; 19: 1112-21.

http://dx.doi.org/10.1359/JBMR.040319
PMid:15176994

120. McCormick RK. *Osteoporosis: integrating biomarkers and other diagnostic correlates into the management of bone fragility.* Alternative medicine review: a journal of clinical therapeutic. 2007; 12: 113-45.

121. Fiore CE, Pennisi P, Ferro G, Ximenes B, Privitelli L, Mangiafico RA et al. *Altered osteoprotegerin/RANKL ratio and low bone mineral density in celiac patients on long-term treatment with gluten-free diet.* Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme. 2006; 38: 417-22.

http://dx.doi.org/10.1055/s-2006-944548
PMid:16823725

122. Kaukinen K, Peraaho M, Lindfors K, Partanen J, Wooleey N, Pikkarainen P et al. *Persistent small bowel mucosal villous atrophy without symptoms in coeliac disease.* Aliment Pharmacol Ther. 2007; 25: 1237-45.

http://dx.doi.org/10.1111/j.1365-2036.2007.03311.x
PMid:17451570

123. American Gastroenterological Association. *American Gastroenterological Association medical position statement: guidelines on osteoporosis in gastrointestinal diseases.* Gastroenterology. 2003; 124: 791-4.

PMid:12612916

124. Murray JA. *Celiac disease in patients with an affected member, type 1 diabetes, iron-deficiency, or osteoporosis?* Gastroenterology. 2005; 128: S52-6.

http://dx.doi.org/10.1053/j.gastro.2005.02.029
PMid:15825127

125. Logan R, West J. *Risk of fracture in coeliac disease.* Gut. 2003; 52: 1532.

http://dx.doi.org/10.1136/gut.52.10.1532-a
PMid:12970154 PMCid:PMC1773841

126. West J, Logan RF, Card TR, Smith C, Hubbard R. *Fracture risk in people with celiac disease: a population-based cohort study.* Gastroenterology. 2003; 125: 429-36.

http://dx.doi.org/10.1016/S0016-5085(03)00891-6

127. Vasquez H, Mazure R, Gonzalez D, Flores D, Pedreira S, Niveloni S et al. *Risk of fractures in celiac disease patients: a cross-sectional, case-control study.* The American journal of gastroenterology. 2000; 95: 183-9.

http://dx.doi.org/10.1111/j.1572-0241.2000.01682.x
PMid:10638580

128. Brennan SL, Leslie WD, Lix LM, Johansson H, Oden A, McCloskey E et al. *FRAX provides robust fracture prediction regardless of socioeconomic status.* Osteoporos Int. 2014; 25: 61-9.

http://dx.doi.org/10.1007/s00198-013-2525-0
PMid:24190425

129. Kanis JA, Johansson H, Oden A, Cooper C, McCloskey EV, Epidemiology and Quality of Life Working Group of IOF. *Worldwide uptake of FRAX.* Arch Osteoporos. 2014; 9: 166.

http://dx.doi.org/10.1007/s11657-013-0166-8
PMid:24420978

130. Sund R, Honkanen R, Johansson H, Odén A, McCloskey E, Kanis J et al. *Evaluation of the FRAX model for hip fracture predictions in the population-based Kuopio Osteoporosis Risk Factor and Prevention Study (OSTPRE).* Calcif Tissue Int. 2014; 95: 39-45.

http://dx.doi.org/10.1007/s00223-014-9860-9
PMid:24792689

131. Hjelle AM, Apalset E, Mielnik P, Bollerslev J, Lundin KE, Tell GS. *Celiac disease and risk of fracture in adults-a review.* Osteoporosis international: a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA. 2014; 25: 1667-76.

http://dx.doi.org/10.1007/s00198-014-2683-8
PMid:24691647

132. Olmos M, Antelo M, Vazquez H, Smecuol E, Mauriño E, Bai JC. *Systematic review and meta-analysis of observational studies on the prevalence of fractures in coeliac disease.* Digestive and liver disease: official journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver. 2008; 40: 46-53.

http://dx.doi.org/10.1016/j.dld.2007.09.006
PMid:18006396

133. Fickling WE, McFarlane XA, Bhalla AK, Robertson, DA. *The clinical impact of metabolic bone disease in coeliac disease.* Postgraduate medical journal. 2001; 77: 33-6.

http://dx.doi.org/10.1136/pmj.77.903.33
PMid:11123392 PMCid:PMC1741871

134. Vestergaard P, Mosekilde L. *Fracture risk in patients with celiac disease, Crohn's disease, and ulcerative colitis: a nationwide follow-up study of 16,416 patients in Denmark.* Am J Epidemiol. 2002; 156: 1-10.

http://dx.doi.org/10.1093/aje/kwf007
PMid:12076883

135. Thomason K, West J, Logan RF, Coupland C, Holmes GK. *Fracture experience of patients with coeliac disease: a population based survey.* Gut. 2003; 52: 518-22.

http://dx.doi.org/10.1136/gut.52.4.518
PMid:12631662 PMCid:PMC1773600

136. Moreno ML, Vazquez H, Mazure R, Smecuol E, Niveloni S, Pedreira S et al. *Stratification of bone fracture risk in patients with celiac disease.* Clinical gastroenterology and hepatology: the official clinical practice journal of the American Gastroenterological Association. 2004; 2: 127-34.

http://dx.doi.org/10.1016/S1542-3565(03)00320-3

137. Davie MW, Gaywood I, George E, Jones PW, Masud T, Price T et al. *Excess non-spine fractures in women over 50 years with celiac disease: a cross-sectional, questionnaire-based study.* Osteoporosis international: a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA. 2005; 16: 1150-5.

http://dx.doi.org/10.1007/s00198-004-1822-z
PMid:15692728

138. Ludvigsson JF, Michaelsson K, Ekbom A, Montgomery SM. *Coeliac disease and the risk of fractures - a general population-based cohort study.* Alimentary pharmacology & therapeutics. 2007; 25: 273-85.

http://dx.doi.org/10.1111/j.1365-2036.2006.03203.x
PMid:17269989

139. Jafri MR, Nordstrom CW, Murray JA, Van Dyke CT, Dierkhising RA, Zinsmeister AR et al. *Long-term fracture risk in patients with celiac disease: a population-based study in Olmsted County, Minnesota.* Digestive diseases and sciences. 2008; 53: 964-71.

http://dx.doi.org/10.1007/s10620-007-9976-0
PMid:17934823 PMCid:PMC2556244

140. Sanchez MI, Mohaidle A, Baistrocchi A, Matoso D, Vázquez H, González A et al. *Risk of fracture in celiac disease: gender, dietary compliance, or both?* World journal of gastroenterology: WJG. 2011; 17: 3035-42.

http://dx.doi.org/10.3748/wjg.v17.i25.3035
PMid:21799650 PMCid:PMC3132255

141. Vilppula A, Kaukinen K, Luostarinen L, Krekelä I, Patrikainen H, Valve R et al. *Clinical benefit of gluten-free diet in screen-detected older celiac disease patients.* BMC gastroenterology. 2011; 11: 136.

http://dx.doi.org/10.1186/1471-230X-11-136
PMid:22176557 PMCid:PMC3377922

142. Scott EM, Gaywood I, Scott BB. *Guidelines for osteoporosis in coeliac disease and inflammatory bowel disease. British Society of Gastroenterology.* Gut. 2000; 46(1): i1-8.

http://dx.doi.org/10.1136/gut.46.suppl_1.I1
PMid:10647595 PMCid:PMC1766735

143. Lebwohl B, Michaelsson K, Green PH, Ludvigsson JF. *Persistent mucosal damage and risk of fracture in celiac disease.* The Journal of clinical endocrinology and metabolism. 2014; 99: 609-16.

http://dx.doi.org/10.1210/jc.2013-3164
PMid:24432993

144. Pantaleoni S, Luchino M, Adriani A, Pellicano R, Stradella D, Ribaldone DG et al. *Bone mineral density at diagnosis of celiac disease and after 1 year of gluten-free diet.* Scientific World Journal. 2014; 2014: 173082.

http://dx.doi.org/10.1155/2014/173082
PMid:25379519 PMCid:PMC4213989

145. Bardella MT, Fredella C, Prampolini L, Molteni N, Giunta AM, Bianchi PA. *Body composition and dietary intakes in adult celiac disease patients consuming a strict gluten-free diet.* Am J Clin Nutr. 2000; 72: 937-9.

PMid:11010934

146. Molteni N, Bardella MT, Vezzoli G, Pozzoli E, Bianchi P. *Intestinal calcium absorption as shown by stable strontium test in celiac disease before and after gluten-free diet.* Am J Gastroenterol. 1995; 90: 2025-8.
PMid:7485015

147. Alaedini A, Green PH. *Narrative review: celiac disease: understanding a complex autoimmune disorder.* Ann Intern Med. 2005; 142: 289-98.
http://dx.doi.org/10.7326/0003-4819-142-4-200502150-00011
PMid:15710962

148. Green PH, Jabri B. *Coeliac disease.* Lancet. 2003; 362: 383-91.
http://dx.doi.org/10.1016/S0140-6736(03)14027-5

149. Green PH, Jabri B. *Celiac disease.* Annu Rev Med. 2006; 57: 207-21.
http://dx.doi.org/10.1146/annurev.med.57.051804.122404
PMid:16409146

150. Mora S, Barera G, Beccio S, Menni L, Proverbio MC, Bianchi C et al. *A prospective, longitudinal study of the long-term effect of treatment on bone density in children with celiac disease.* J Pediatr. 2001; 139: 516-21.
http://dx.doi.org/10.1067/mpd.2001.116298
PMid:11598597

151. Mora S, Barera G, Ricotti A, Weber G, Bianchi C, Chiumello G. *Reversal of low bone density with a gluten-free diet in children and adolescents with celiac disease.* Am J Clin Nutr. 1998; 67: 477-81.
PMid:9497193

152. Mazure R, Vazquez H, Gonzalez D, Mautalen C, Pedreira S, Boerr L et al. *Bone mineral affection in asymptomatic adult patients with celiac disease.* Am J Gastroenterol. 1994; 89: 2130-4.
PMid:7977227

153. Walters JR, Banks LM, Butcher GP, Fowler CR. *Detection of low bone mineral density by dual energy x ray absorptiometry in unsuspected suboptimally treated coeliac disease.* Gut. 1995; 37: 220-4.
http://dx.doi.org/10.1136/gut.37.2.220
PMid:7557572 PMCid:PMC1382722

154. Duhring LA. *Landmark article, Aug 30, 1884: Dermatitis herpetiformis.* JAMA. 1983; 250: 212-16.
http://dx.doi.org/10.1001/jama.1983.03340020028029
PMid:6345814

155. Marks J, Shuster S, Watson AJ. *Small-bowel changes in dermatitis herpetiformis.* Lancet. 1966; 2: 1280-2.
http://dx.doi.org/10.1016/S0140-6736(66)91692-8

156. Karpati S. *Dermatitis herpetiformis.* Clin Dermatol. 2012; 30: 56-9.
http://dx.doi.org/10.1016/j.clindermatol.2011.03.010
PMid:22137227

157. Reunala T. *Dermatitis herpetiformis: coeliac disease of the skin.* Annals of medicine. 1998; 30: 416-8.

http://dx.doi.org/10.3109/07853899809002482
PMid:9814827

158. Vassileva S, Drenovska K, Manuelyan K. *Autoimmune blistering dermatoses as systemic diseases.* Clinics in dermatology. 2014; 32: 364-75.

http://dx.doi.org/10.1016/j.clindermatol.2013.11.003
PMid:24767184

159. Gaspari AA, Huang CM, Davey RJ, Bondy C, Lawley TJ, Katz S. *Prevalence of thyroid abnormalities in patients with dermatitis herpetiformis and in control subjects with HLA-B8/-DR3.* The American journal of medicine. 1990; 88: 145-50.

http://dx.doi.org/10.1016/0002-9343(90)90464-O

160. Zettinig G, Weissel M, Flores J, Dudczak R, Vogelsang H. *Dermatitis herpetiformis is associated with atrophic but not with goitrous variant of Hashimoto's thyroiditis.* Eur J Clin Invest. 2000; 30: 53-7.

http://dx.doi.org/10.1046/j.1365-2362.2000.00590.x
PMid:10620002

161. Hervonen K, Viljamaa M, Collin P, Knip M, Reunala T. *The occurrence of type 1 diabetes in patients with dermatitis herpetiformis and their first-degree relatives.* The British journal of dermatology. 2004; 150: 136-8.

http://dx.doi.org/10.1111/j.1365-2133.2004.05642.x
PMid:14746628

162. Zone JJ. *Skin manifestations of celiac disease.* Gastroenterology. 2005; 128: S87-91.

http://dx.doi.org/10.1053/j.gastro.2005.02.026
PMid:15825132

163. Reunala TL. *Dermatitis herpetiformis.* Clin Dermatol. 2001; 19: 728-36.

http://dx.doi.org/10.1016/S0738-081X(00)00184-X

164. Smith JB, Tulloch JE, Meyer LJ, Zone JJ. *The incidence and prevalence of dermatitis herpetiformis in Utah.* Archives of dermatology. 1992; 128: 1608-10.

http://dx.doi.org/10.1001/archderm.1992.04530010046006
PMid:1456754

165. Hofmann SC, Nashan D, Bruckner-Tuderman L. *Petechiae on the fingertips as presenting symptom of dermatitis herpetiformis Duhring.* J Eur Acad Dermatol Venereol. 2009; 23: 732-3.

http://dx.doi.org/10.1111/j.1468-3083.2009.03200.x
PMid:19470074

166. Llorente-Alonso MJ, Fernandez-Acenero MJ, Sebastian M. *Gluten intolerance: sex and age-related features.* Can J Gastroenterol. 2006; 20: 719-22.

PMid:17111054 PMCid:PMC2660827

167. Zone JJ, Egan CA, Taylor TB, Meyer LJ. *IgA autoimmune disorders: development of a passive transfer mouse model.* J Investig Dermatol Symp Proc. 2004; 9: 47-51.

http://dx.doi.org/10.1111/j.1087-0024.2004.00840.x
PMid:14870985

168. Hitomi K. *Transglutaminases in skin epidermis.* Eur J Dermatol. 2005; 15: 313-9.

PMid:16172037

169. Garner C, Ahn R, Ding YC, Steele L, Stoven S, Green PH. *Genome-wide association study of celiac disease in North America confirms FRMD4B as new celiac locus.* PloS one. 2014; 9: e101428.

http://dx.doi.org/10.1371/journal.pone.0101428
PMid:24999842 PMCid:PMC4084811

170. Plotnikova N, Miller JL. *Dermatitis herpetiformis.* Skin therapy letter. 2013; 18: 1-3.

PMid:23674144

171. Hervonen K, Alakoski A, Salmi TT, Helakorpi S, Kautiainen H, Kaukinen K et al. *Reduced mortality in dermatitis herpetiformis: a population-based study of 476 patients.* The British journal of dermatology. 2012; 167: 1331-7.

http://dx.doi.org/10.1111/j.1365-2133.2012.11105.x
PMid:22708883

172. Aziz I, Hadjivassiliou M. *Coeliac disease: noncoeliac gluten sensitivity-food for thought.* Nat Rev Gastroenterol Hepatol. 2014; 11: 398-9.

http://dx.doi.org/10.1038/nrgastro.2014.91
PMid:24935422

173. Hadjivassiliou M, Grunewald R, Sharrack B, Sanders D, Lobo A, Williamson C et al. *Gluten ataxia in perspective: epidemiology, genetic susceptibility and clinical characteristics.* Brain. 2003; 126: 685-91.

http://dx.doi.org/10.1093/brain/awg050
PMid:12566288

174. Nanri K, Mitoma H, Ihara M, Tanaka N, Taguchi T, Takeguchi M et al. *Gluten ataxia in Japan.* Cerebellum. 2014; 13: 623-7.

http://dx.doi.org/10.1007/s12311-014-0582-3
PMid:24997752

175. Hadjivassiliou M, Aeschlimann P, Strigun A, Sanders DS, Woodroofe N, Aeschlimann D. *Autoantibodies in gluten ataxia recognize a novel neuronal transglutaminase.* Ann Neurol. 2008; 64: 332-43.

http://dx.doi.org/10.1002/ana.21450
PMid:18825674

176. Burk K, Bosch S, Muller CA, Melms A, Zühlke C, Stern M et al. *Sporadic cerebellar ataxia associated with gluten sensitivity.* Brain. 2001; 124: 1013-9.

http://dx.doi.org/10.1093/brain/124.5.1013
PMid:11335703

177. Hadjivassiliou M, Grunewald RA, Kandler RH, Chattopadhyay AK, Jarratt JA, Sanders DS et al. *Neuropathy associated with gluten sensitivity.* J Neurol Neurosurg Psychiatry. 2006; 77: 1262-6.

http://dx.doi.org/10.1136/jnnp.2006.093534
PMid:16835287 PMCid:PMC2077388

178. Thomas H, Beck K, Adamczyk M, Langley M, Oita RC, Thiebach L et al. *Transglutaminase 6: a protein associated with central nervous system development and motor function.* Amino Acids. 2013; 44: 161-77.

http://dx.doi.org/10.1007/s00726-011-1091-z
PMid:21984379 PMCid:PMC3535377

179. Hernandez-Lahoz C, Rodrigo-Saez L, Vega-Villar J, Mauri-Capdevila G, Mier-Juanes J. *Familial gluten ataxia.* Mov Disord. 2014; 29: 308-10.

http://dx.doi.org/10.1002/mds.25783
PMid:24375771

180. Lock RJ, Tengah DP, Williams AJ, Ward JJ, Bingley PJ, Wills AJ et al. *Cerebellar ataxia, peripheral neuropathy, "gluten sensitivity" and anti-neuronal autoantibodies.* Clin Lab. 2006; 52: 589-92.

PMid:17175889

181. McKeon A, Lennon VA, Pittock SJ, Kryzer TJ, Murray J. *The neurologic significance of celiac disease biomarkers.* Neurology. 2014; 83: 1789-96.

http://dx.doi.org/10.1212/WNL.0000000000000970
PMid:25261501

182. Ellenberg M, Bookman JJ. *Diabetic diarrhea with malabsorption syndrome.* Diabetes. 1960; 9: 14-9.

http://dx.doi.org/10.2337/diab.9.1.14
PMid:13819963

183. Vinnik IE, Kern F, Jr., Struthers JE, Jr. *Malabsorption and the diarrhea of diabetes mellitus.* Gastroenterology. 1962; 43: 507-20.

PMid:13997379

184. Green PA, Wollager EE, Sprague RG, Brown AL Jr. *Diabetes mellitus associated with nontropical sprue. Report of four cases.* Diabetes. 1962; 11: 388-92.

PMid:13950109

185. Wruble LD, Kalser MH. *Diabetic Steatorrhea: A Distinct Entity.* Am J Med. 1964; 37: 118-29.

http://dx.doi.org/10.1016/0002-9343(64)90216-5

186. Thompson MW. *Heredity, maternal age, and birth order in the etiology of celiac disease.* Am J Hum Genet. 1951; 3: 159-66.

PMid:14902759 PMCid:PMC1716325

187. Carter C, Sheldon W, Walker C. *The inheritance of coeliac disease.* Ann Hum Genet. 1959; 23: 266-78.

http://dx.doi.org/10.1111/j.1469-1809.1959.tb01470.x
PMid:13808015

188. Hooft C, Roels H, Devos E. *Diabetes and coeliac disease.* Lancet. 1969; 2: 1192.
PMid:4187221
189. Visakorpi JK. *Diabetes and coeliac disease.* Lancet. 1969; 2: 1192.
http://dx.doi.org/10.1016/S0140-6736(69)92512-4
190. Walker-Smith JA, Grigor W. *Coeliac disease in a diabetic child.* Lancet. 1969; 1: 1021.
http://dx.doi.org/10.1016/S0140-6736(69)91817-0
191. Koletzko S, Burgin-Wolff A, Koletzko B, Knapp M, Burger W, Grüneklee D et al. *Prevalence of coeliac disease in diabetic children and adolescents. A multicentre study.* Eur J Pediatr. 1988; 148: 113-7.
http://dx.doi.org/10.1007/BF00445915
PMid:3069470
192. Lorini R, Scaramuzza A, Vitali L, d'Annunzio G, Avanzini MA, De Giacomo C et al. *Clinical aspects of coeliac disease in children with insulin-dependent diabetes mellitus.* J Pediatr Endocrinol Metab. 1996; 9(1): 101-11.
http://dx.doi.org/10.1515/JPEM.1996.9.S1.101
193. de Freitas IN, Sipahi AM, Damiao AO, de Brito T, Cançado EL, Leser PG et al. *Celiac disease in Brazilian adults.* Journal of clinical gastroenterology. 2002; 34: 430-4.
http://dx.doi.org/10.1097/00004836-200204000-00009
PMid:11907355
194. Saukkonen T, Savilahti E, Reijonen H, Ilonen J, Tuomilehto-Wolf E, Akerblom HK et al. *Coeliac disease: frequent occurrence after clinical onset of insulin-dependent diabetes mellitus. Childhood Diabetes in Finland Study Group.* Diabet Med. 1996; 13: 464-70.
http://dx.doi.org/10.1002/(SICI)1096-9136(199605)13:5<464::AID-DIA101>3.0.CO;2-R
195. Acerini CL, Ahmed ML, Ross KM, Sullivan PB, Bird G, Dunger DB. *Coeliac disease in children and adolescents with IDDM: clinical characteristics and response to gluten-free diet.* Diabet Med. 1998; 15: 38-44.
http://dx.doi.org/10.1002/(SICI)1096-9136(199801)15:1<38::AID-DIA520>3.0.CO;2-L
196. Barera G, Bonfanti R, Viscardi M, Bazzigaluppi E, Calori G, Meschi F et al. *Occurrence of celiac disease after onset of type 1 diabetes: a 6-year prospective longitudinal study.* Pediatrics. 2002; 109: 833-8.
http://dx.doi.org/10.1542/peds.109.5.833
PMid:11986443
197. Hansen D, Bennedbaek FN, Hansen LK, HoierMadsen M, Hegedü LS et al. *High prevalence of coeliac disease in Danish children with type I diabetes mellitus.* Acta Paediatr. 2001; 90: 1238-43.
http://dx.doi.org/10.1111/j.1651-2227.2001.tb01568.x
PMid:11808892

198. Pocecco M, Ventura A. *Coeliac disease and insulin-dependent diabetes mellitus: a causal association?* Acta Paediatr. 1995; 84: 1432-3.

http://dx.doi.org/10.1111/j.1651-2227.1995.tb13583.x
PMid:8645965

199. Mori T, Iwasaki Y, Seki Y, Iseki M, Katayama H, Yamamoto K et al. *Lnk/Sh2b3 controls the production and function of dendritic cells and regulates the induction of IFN-gamma-producing T cells.* J Immunol. 2014; 193: 1728-36.

http://dx.doi.org/10.4049/jimmunol.1303243
PMid:25024389

200. Gyllenberg A, Asad S, Piehl F, Swanberg M, Padyukov L, Van Yserloo B, Rutledge EA et al. *Age-dependent variation of genotypes in MHC II transactivator gene (CIITA) in controls and association to type 1 diabetes.* Genes Immun. 2012; 13: 632-40.

http://dx.doi.org/10.1038/gene.2012.44
PMid:23052709

201. Hemminki K, Li X, Sundquist J, Sundquist K. *Familial association between type 1 diabetes and other autoimmune and related diseases.* Diabetologia. 2009; 52: 1820-8.

http://dx.doi.org/10.1007/s00125-009-1427-3
PMid:19543881

202. Heap GA, van Heel DA. *The genetics of chronic inflammatory diseases.* Hum Mol Genet. 2009; 18: R101-6.

http://dx.doi.org/10.1093/hmg/ddp001
PMid:19297396

203. Westman E, Ambler GR, Royle M, Peat J, Chan A. *Children with coeliac disease and insulin dependent diabetes mellitus-growth, diabetes control and dietary intake.* J Pediatr Endocrinol Metab. 1999; 12: 433-42.

http://dx.doi.org/10.1515/JPEM.1999.12.3.433
PMid:10821223

204. Atherton R, Ross A, Jessop F, Williams R, Heuschkel R, Zilbauer M. *Coeliac disease in children with type 1 diabetes: are current guidelines proving difficult to implement in practice?* J Pediatr Gastroenterol Nutr. 2014; 59: 600-3.

http://dx.doi.org/10.1097/MPG.0000000000000490
PMid:25061719

205. Elfstrom P, Sundstrom J, Ludvigsson JF. *Systematic review with meta-analysis: associations between coeliac disease and type 1 diabetes.* Aliment Pharmacol Ther. 2014; 40: 1123-32.

http://dx.doi.org/10.1111/apt.12973
PMid:25270960

206. Liu E, Lee HS, Aronsson CA, Hagopian WA, Koletzko S, Rewers MJ et al. *Risk of pediatric celiac disease according to HLA haplotype and country.* N Engl J Med. 2014; 371: 42-9.

http://dx.doi.org/10.1056/NEJMoa1313977
PMid:24988556 PMCid:PMC4163840

207. Kagnoff MF. *AGA Institute Medical Position Statement on the Diagnosis and Management of Celiac Disease.* Gastroenterology. 2006; 131: 1977-80.

http://dx.doi.org/10.1053/j.gastro.2006.10.003
PMid:17087935 PMCid:PMC2842922

208. Kordonouri O, Dieterich W, Schuppan D, Webert G, Müller C, Sarioglu N et al. *Autoantibodies to tissue transglutaminase are sensitive serological parameters for detecting silent coeliac disease in patients with Type 1 diabetes mellitus.* Diabet Med. 2000; 17: 441-4.

http://dx.doi.org/10.1046/j.1464-5491.2000.00291.x
PMid:10975212

209. Schober E, Bittmann B, Granditsch G, Huber WD. Hüppe A, Jäger A et al. *Screening by anti-endomysium antibody for celiac disease in diabetic children and adolescents in Austria.* J Pediatr Gastroenterol Nutr. 2000; 30: 391-6.

http://dx.doi.org/10.1097/00005176-200004000-00008
PMid:10776949

210. Ch'ng CL, Biswas M, Benton A, Jones MK, Kingham JG. *Prospective screening for coeliac disease in patients with Graves' hyperthyroidism using anti-gliadin and tissue transglutaminase antibodies.* Clin Endocrinol (Oxf). 2005; 62: 303-6.

http://dx.doi.org/10.1111/j.1365-2265.2005.02214.x
PMid:15730411

211. Ch'ng CL, Jones MK, Kingham JG. *Celiac disease and autoimmune thyroid disease.* Clin Med Res. 2007; 5: 184-92.

http://dx.doi.org/10.3121/cmr.2007.738
PMid:18056028 PMCid:PMC2111403

212. Hadithi M, de Boer H, Meijer J, Willekens F, Kerckhaert JA, Heijmans R et al. *Coeliac disease in Dutch patients with Hashimoto's thyroiditis and vice versa.* World J Gastroenterol. 2007; 13: 1715-22.

http://dx.doi.org/10.3748/wjg.v13.i11.1715
PMid:17461476 PMCid:PMC4146952

213. Spadaccino AC, Basso D, Chiarelli S, Albergoni MP, D'Odorico A, Plebani M et al. *Celiac disease in North Italian patients with autoimmune thyroid diseases.* Autoimmunity. 2008; 41: 116-21.

http://dx.doi.org/10.1080/08916930701620209
PMid:18176874

214. Collin P, Reunala T, Pukkala E, Laippala P, Keyriläinen O et al. *Coeliac disease-associated disorders and survival.* Gut. 1994; 35: 1215-8.

http://dx.doi.org/10.1136/gut.35.9.1215
PMid:7959226 PMCid:PMC1375696

215. Ansaldi N, Palmas T, Corrias A, Barbato M, D'Altiglia MR, Campanozzi A et al. *Autoimmune thyroid disease and celiac disease in children.* J Pediatr Gastroenterol Nutr. 2003; 37: 63-6.

http://dx.doi.org/10.1097/00005176-200307000-00010
PMid:12827007

216. Meloni A, Mandas C, Jores RD et al. *Prevalence of autoimmune thyroiditis in children with celiac disease and effect of gluten withdrawal.* J Pediatr. 2009; 155: 51-5.

217. Elfstrom P, Montgomery SM, Kampe O, Ekbom A, Ludvigsson JF. *Risk of thyroid disease in individuals with celiac disease.* J Clin Endocrinol Metab. 2008; 93: 3915-21.

http://dx.doi.org/10.1210/jc.2008-0798
PMid:18611971

218. Mainardi E, Montanelli A, Dotti M, Nano R, Moscato G. *Thyroid-related autoantibodies and celiac disease: a role for a gluten-free diet?* J Clin Gastroenterol. 2002; 35: 245-8.

http://dx.doi.org/10.1097/00004836-200209000-00009
PMid:12192201

219. Metso S, Hyytia-Ilmonen H, Kaukinen K, Huhtala H, Jaatinen P, Salmi J et al. *Gluten-free diet and autoimmune thyroiditis in patients with celiac disease. A prospective controlled study.* Scand J Gastroenterol. 2012; 47: 43-8.

http://dx.doi.org/10.3109/00365521.2011.639084
PMid:22126672

220. Ventura A, Neri E, Ughi C, Leopaldi A, Città A, Not T. *Gluten-dependent diabetes-related and thyroid-related autoantibodies in patients with celiac disease.* J Pediatr. 2000; 137: 263-5.

http://dx.doi.org/10.1067/mpd.2000.107160
PMid:10931424

221. Sategna-Guidetti C, Volta U, Ciacci C, Usai P, Carlino A, De Franceschi L et al. *Prevalence of thyroid disorders in untreated adult celiac disease patients and effect of gluten withdrawal: an Italian multicenter study.* Am J Gastroenterol. 2001; 96: 751-7.

http://dx.doi.org/10.1111/j.1572-0241.2001.03617.x
PMid:11280546

222. Diamanti A, Ferretti F, Guglielmi R, Panetta F, Colistro F, Cappa M et al. *Thyroid autoimmunity in children with coeliac disease: a prospective survey.* Arch Dis Child. 2011; 96: 1038-41.

http://dx.doi.org/10.1136/archdischild-2011-300595
PMid:21862521

223. Aggarwal S, Lebwohl B, Green PH. *Screening for celiac disease in average-risk and high-risk populations.* Therap Adv Gastroenterol. 2012; 5: 37-47.

http://dx.doi.org/10.1177/1756283X11417038
PMid:22282707 PMCid:PMC3263981

224. Naiyer AJ, Shah J, Hernandez L, Kim SY, Ciaccio EJ, Cheng J et al. *Tissue transglutaminase antibodies in individuals with celiac disease bind to thyroid follicles and extracellular matrix and may contribute to thyroid dysfunction.* Thyroid. 2008; 18: 1171-8.
http://dx.doi.org/10.1089/thy.2008.0110
PMid:19014325

225. Galli G, Esposito G, Lahner E, Pilozzi E, Corleto VD, Di Giulio E et al. *Histological recovery and gluten-free diet adherence: a prospective 1-year follow-up study of adult patients with coeliac disease.* Aliment Pharmacol Ther. 2014; 40: 639-47.
http://dx.doi.org/10.1111/apt.12893
PMid:25066096

226. Hall NJ, Rubin G, Charnock A. *Systematic review: adherence to a gluten-free diet in adult patients with coeliac disease.* Aliment Pharmacol Ther. 2009; 30: 315-30.
http://dx.doi.org/10.1111/j.1365-2036.2009.04053.x
PMid:19485977

227. Hall NJ, Rubin GP, Charnock A. *Intentional and inadvertent non-adherence in adult coeliac disease. A cross-sectional survey.* Appetite. 2013; 68: 56-62.
http://dx.doi.org/10.1016/j.appet.2013.04.016
PMid:23623778

228. Faulkner-Hogg KB, Selby WS, Loblay RH. *Dietary analysis in symptomatic patients with coeliac disease on a gluten-free diet: the role of trace amounts of gluten and non-gluten food intolerances.* Scand J Gastroenterol. 1999; 34: 784-9.
http://dx.doi.org/10.1080/003655299750025714
PMid:10499479

229. Hollon JR, Cureton PA, Martin ML, Puppa EL, Fasno A et al. *Trace gluten contamination may play a role in mucosal and clinical recovery in a subgroup of diet-adherent non-responsive celiac disease patients.* BMC Gastroenterol. 2013; 13: 40.
http://dx.doi.org/10.1186/1471-230X-13-40
PMid:23448408 PMCid:PMC3598839

230. Biagi F, Corazza GR. *Mortality in celiac disease. Nature reviews.* Gastroenterology & hepatology. 2010; 7: 158-62.
http://dx.doi.org/10.1038/nrgastro.2010.2
PMid:20125093

231. Grainge MJ, West J, Card TR, Holmes GK. *Causes of death in people with celiac disease spanning the pre- and post-serology era: a population-based cohort study from Derby, UK.* Am J Gastroenterol. 2011; 106: 933-9.
http://dx.doi.org/10.1038/ajg.2010.506
PMid:21245833

232. West J, Logan RF, Smith CJ, Hubbard RB, Card TR. *Malignancy and mortality in people with coeliac disease: population based cohort study.* BMJ. 2004; 329: 716-9.

http://dx.doi.org/10.1136/bmj.38169.486701.7C
PMid:15269095 PMCid:PMC518895

233. Askling J, Linet M, Gridley G, Halstensen TS, Ekström K, Ekbom A. *Cancer incidence in a population-based cohort of individuals hospitalized with celiac disease or dermatitis herpetiformis.* Gastroenterology. 2002; 123: 1428-35.

http://dx.doi.org/10.1053/gast.2002.36585
PMid:12404215

234. Elfstrom P, Granath F, Ekstrom SK, Montgomery SM, Askling J, Ekbom A et al. *Risk of lymphoproliferative malignancy in relation to small intestinal histopathology among patients with celiac disease.* J Natl.Cancer Inst. 2011; 103: 436-44.

http://dx.doi.org/10.1093/jnci/djq564
PMid:21289299

235. Ilus T, Kaukinen K, Virta LJ, Pukkala E, Collin P. *Incidence of Malignancies in Diagnosed Celiac Patients: A Population-based Estimate.* Am J Gastroenterol. 2014.

http://dx.doi.org/10.1038/ajg.2014.194
PMid:25047399

236. Tio M, Cox MR, Eslick GD. *Meta-analysis: coeliac disease and the risk of all-cause mortality, any malignancy and lymphoid malignancy.* Aliment Pharmacol Ther. 2012; 35: 540-51.

http://dx.doi.org/10.1111/j.1365-2036.2011.04972.x
PMid:22239821

237. Ludvigsson JF. *Mortality and malignancy in celiac disease.* Gastrointest Endosc Clin N Am. 2012; 22: 705-22.

http://dx.doi.org/10.1016/j.giec.2012.07.005
PMid:23083988

238. Baecklund E, Iliadou A, Askling J, Ekbom A, Backlin C, Granath F et al. *Association of chronic inflammation, not its treatment, with increased lymphoma risk in rheumatoid arthritis.* Arthritis Rheum. 2006; 54: 692-701.

http://dx.doi.org/10.1002/art.21675
PMid:16508929

239. Holmes GK, Prior P, Lane MR, Pope D, Allan RN. *Malignancy in coeliac disease-effect of a gluten free diet.* Gut. 1989; 30: 333-8.

http://dx.doi.org/10.1136/gut.30.3.333
PMid:2707633 PMCid:PMC1378455

240. Ludvigsson JF, Lebwohl B, Rubio-Tapia A, Murray JA; Green PH, Ekbom A et al. *Does celiac disease influence survival in lymphoproliferative malignancy?* Eur J Epidemiol. 2013.

http://dx.doi.org/10.1007/s10654-013-9789-8
PMCid:PMC3886816

241. Zouhairi ME, Venner A, Charabaty A, Psihvaian MJ. *Small bowel adenocarcinoma.* Curr Treat Options Oncol. 2008; 9: 388-99.

http://dx.doi.org/10.1007/s11864-009-0098-0
PMid:19365735

242. *CASE RECORDS of the Massachusetts General Hospital: case 44362.* N Engl J Med. 1958; 259: 491-5.

http://dx.doi.org/10.1056/NEJM195809042591011
PMid:13578092

243. Benhammane H, El M'rabet F Z, Idrissi Serhouchni K, El yousfi M, Charif I, Toughray I et al. *Small bowel adenocarcinoma complicating coeliac disease: a report of three cases and the literature review.* Case Rep Oncol Med. 2012: 935183.

http://dx.doi.org/10.1155/2012/935183

244. Grainge MJ, West J, Solaymani-Dodaran M, Card TR, Logan RF. *The long-term risk of malignancy following a diagnosis of coeliac disease or dermatitis herpetiformis: a cohort study.* Alimentary pharmacology & therapeutics. 2012; 35: 730-9.

http://dx.doi.org/10.1111/j.1365-2036.2012.04998.x
PMid:22288441

245. Viljamaa M, Kaukinen K, Pukkala E, Hervonen K, Reunala T, Collin P. *Malignancies and mortality in patients with coeliac disease and dermatitis herpetiformis: 30-year population-based study.* Digestive and liver disease: official journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver. 2006; 38: 374-80.

http://dx.doi.org/10.1016/j.dld.2006.03.002
PMid:16627018

246. Dickey W. Colon neoplasia co-existing with coeliac disease in older patients: coincidental, probably; important, certainly. Scand J Gastroenterol 2002; 37: 1054-1056.

http://dx.doi.org/10.1080/003655202320378257
PMid:12374231

247. Collin P, Pukkala E, Reunala T. *Malignancy and survival in dermatitis herpetiformis: a comparison with coeliac disease.* Gut. 1996; 38: 528-30.

http://dx.doi.org/10.1136/gut.38.4.528
PMid:8707082 PMCid:PMC1383109

248. Landgren AM, Landgren O, Gridley G, Dores GM, Linet MS, Morton LM. *Autoimmune disease and subsequent risk of developing alimentary tract cancers among 4.5 million US male veterans.* Cancer. 2011; 117: 1163-71.

http://dx.doi.org/10.1002/cncr.25524
PMid:21381009 PMCid:PMC3052786

249. Freeman HJ. *Lymphoproliferative and intestinal malignancies in 214 patients with biopsy-defined celiac disease.* Journal of clinical gastroenterology. 2004; 38: 429-34.

http://dx.doi.org/10.1097/00004836-200405000-00008
PMid:15100523

250. Gonzalez R, Pereyra L, Mohaidle A, Mella JM, Fischer C, Medrano MA et al. *Celiac disease and risk of colorectal neoplasia.* Acta Gastroenterol Latinoam. 2012; 42: 87-91.

PMid:22876709

251. Pereyra L, Gonzalez R, Mohaidle A, Fischer C, Mella JM, Panigadi GN et al. *Risk of colorectal neoplasia in patients with celiac disease: a multicenter study.* J Crohns.Colitis. 2013; 7: e672-e677.

http://dx.doi.org/10.1016/j.crohns.2013.06.005
PMid:23845233

252. Volta U, Vincentini O, Quintarelli F, Felli C, Silano M, Collaborating Centres of the Italian Registry of the Complications of Celiac Disease. *Low risk of colon cancer in patients with celiac disease.* Scand J Gastroenterol. 2014; 49: 564-8.

http://dx.doi.org/10.3109/00365521.2014.893012
PMid:24621303

253. Hopper AD, Leeds JS, Hurlstone DP, et al. *Are lower gastrointestinal investigations necessary in patients with coeliac disease?* Eur.J Gastroenterol Hepatol. 2005; 17: 617-21.

http://dx.doi.org/10.1097/00042737-200506000-00005

254. Card TR, West J, Holmes GK. *Risk of malignancy in diagnosed coeliac disease: a 24-year prospective, population-based, cohort study.* Aliment Pharmacol Ther. 2004; 20: 769-75.

http://dx.doi.org/10.1111/j.1365-2036.2004.02177.x
PMid:15379837

255. Goldacre MJ, Wotton CJ, Seagroatt V, Yeates D. *Cancers and immune related diseases associated with Down's syndrome: a record linkage study.* Arch Dis Child. 2004; 89: 1014-7.

http://dx.doi.org/10.1136/adc.2003.046219
PMid:15499053 PMCid:PMC1719725

256. Ludvigsson JF, Lebwohl B, Rubio-Tapia A, Murray JA, Green PH, Ekbom A. *Risk of lymphoproliferative malignancy in celiac patients with a family history of lymphoproliferative malignancy.* J Gastroenterol. 2013.

http://dx.doi.org/10.1007/s00535-013-0757-6
PMid:23440554 PMCid:PMC3664649

257. Soni S, Badawy SZ. *Celiac disease and its effect on human reproduction: a review.* J Reprod Med. 2010; 55: 3-8.
PMid:20337200

258. Ferguson R, Holmes GK, Cooke WT. *Coeliac disease, fertility, and pregnancy.* 1982; 17: 65-8.

259. Santonicola A, Iovino P, Cappello C, Capone P, Andreozzi P, Ciacci C. *From menarche to menopause: the fertile life span of celiac women.* Menopause. 2011; 18: 1125-30.
http://dx.doi.org/10.1097/gme.0b013e3182188421
PMid:21646922

260. Ludvigsson JF, West J, Ekbom A, Stephansson O. *Reduced risk of breast, endometrial and ovarian cancer in women with celiac disease.* Int J Cancer. 2012; 131: E244-50.
http://dx.doi.org/10.1002/ijc.26454
PMid:21953605

261. Barker JM, Liu E. *Celiac disease: pathophysiology, clinical manifestations, and associated autoimmune conditions.* Adv Pediatr. 2008; 55: 349-65.
http://dx.doi.org/10.1016/j.yapd.2008.07.001
PMCid:PMC2775561

262. Volta U, Vincentini O, Silano M. *Papillary cancer of thyroid in celiac disease.* J Clin Gastroenterol. 2011; 45: e44-e46.
http://dx.doi.org/10.1097/MCG.0b013e3181ea11cb
PMid:20697293

263. Kent L, McBride R, McConnell R, Neugut AI, Bhagat G, Green PH. *Increased risk of papillary thyroid cancer in celiac disease.* Dig Dis Sci. 2006; 51: 1875-7.
http://dx.doi.org/10.1007/s10620-006-9240-z
PMid:16957996

264. Ludvigsson JF, Lebwohl B, Kampe O, Murray JA, Green PH, Ekbom A. *Risk of thyroid cancer in a nationwide cohort of patients with biopsy-verified celiac disease.* Thyroid. 2013; 23: 971-6.
http://dx.doi.org/10.1089/thy.2012.0306
PMid:23281890 PMCid:PMC3752514

265. Harris OD, Cooke WT, Thompson H, Waterhouse JAH. *Malignancy in adult coeliac disease and idiopathic steatorrhoea.* Am J Med. 1967; 42: 899-912.
http://dx.doi.org/10.1016/0002-9343(67)90071-X

266. O'Brien CJ, Saverymuttu S, Hodgson HJ, Evans DJ. *Coeliac disease, adenocarcinoma of jejunum and in situ squamous carcinoma of oesophagus.* J Clin Pathol. 1983; 36: 62-7.
http://dx.doi.org/10.1136/jcp.36.1.62
PMid:6822678 PMCid:PMC498106

267. Silano M, Volta U, Mecchia AM, Dessi M, Di Benedetto R, De Vicenzi M et al. *Delayed diagnosis of coeliac disease increases cancer risk.* BMC Gastroenterol. 2007; 7: 8.

http://dx.doi.org/10.1186/1471-230X-7-8
PMid:17349035 PMCid:PMC2140155

Chapter 11

Follow-Up of CD Patient: Is Mucosal Recovery a Goal of Therapy?

Santiago Vivas, Laura Arias, Luis Vaquero

Gastroenterology Service, University Hospital of León, Biomedicine Institute, León University, Spain.

svivasa@gmail.com, luisvaqueroayala@gmail.com, lariasrodriguez@hotmail.com

Doi: http://dx.doi.org/10.3926/oms.259

How to cite this chapter

Vivas S, Arias L, Vaquero L. *Follow-Up of CD Patient: Is Mucosal Recovery a Goal of Therapy?* In Arranz E, Fernández-Bañares F, Rosell CM, Rodrigo L, Peña AS, editors. *Advances in the Understanding of Gluten Related Pathology and the Evolution of Gluten-Free Foods*. Barcelona, Spain: OmniaScience; 2015. p. 409-423.

Abstract

The main aim of this chapter is to give a comprehensive guide to the follow-up of patients with CD. The compliance with a strict gluten-free diet (GFD) is the main goal in the management. Patients must be trained in the GFD and the benefits obtained from a strict adherence. There are several methods to assess the compliance of the diet and they are summarized in the text: interviews with a dietitian or a doctor, structured surveys, serology, histology and gluten detection in the feces. Furthermore, CD patients must be included into a periodical follow-up made by a physician (general or gastroenterologist) qualified on the management of CD. Periodical visits include: clinical assessment, laboratory test (detection of nutritional deficiencies and CD serology) and other test in selected cases (bone densitometry and hyposplenism detection).

The evaluation of the duodenal mucosa recovery throughout the follow-up may be important to identify those patients who require a closer monitoring to detect nutritional deficiencies or complications associated to the persistence of mucosal atrophy.

Keywords

Celiac disease, management, gluten-free diet, duodenal biopsy.

1. Introduction

Patients diagnosed with celiac disease (CD) have a permanent intolerance to the gluten contained in their diet. Removal of gluten is associated with clinical and histological improvements, while poor adherence to a gluten-free diet (GFD) is associated with lower quality of life and higher risk of CD related symptoms and complications[1,2]. However, there are two key points that patients and physicians may have to take into account for the follow-up of CD patients:

Compliance with a strict GFD is a very hard and demanding daily task. Patients need as much dietary information as possible, and also clear advice from physicians. It is estimated that less than 50% of CD patients follow a strict GFD, mainly in the adult CD population. Better dietary compliance is achieved in the pediatric population when the disease is diagnosed in early childhood[3].

The second key point is the variability in the follow-up practices among physicians and the inadequate or absence of management after the diagnosis[4]. The lack of information in non-referral populations and the variability in the guidelines may be the main reason for these inadequate practices.

The main aim of this chapter is to give a comprehensive guide to the CD follow-up with a discussion of the leading goals of management.

2. Gluten-Free Diet

2.1. Importance of a Strict GFD Compliance

The tolerance of gluten in the diet is highly variable among patients. While someone present symptoms with small amount of gluten in the diet, others can tolerate routine transgressions[5]. Furthermore, some patients are diagnosed on the basis of screening approach and they have no symptoms to improve when gluten is avoided, making more difficult the compliance with the diet[6].

The first target of the physician following CD diagnosis is to explain to the patient the importance and benefits of strictly avoiding gluten in the daily diet. The patient must understand that no transgression is allowed to avoid complications and to achieve a similar quality of life and life expectancy than that of the general population. Strict GFD is associated with a decrease in the risk of developing lymphoproliferative disease in CD, which is the worst complication and with very poor prognosis[7].

The physicians involved in the management of CD must take into account that GFD compliance is the cornerstone of therapy. They must be able to adequately explain this concept to the patient. It is not clear who should perform the follow-up to investigate adherence to GFD: gastroenterologist, primary care physician or an expert dietitian[8]. Medical follow-up by primary care physicians or gastroenterologist may be similar in terms of rates of adherence to GFD[9]. The available evidence suggests that consultation with a dietitian may be useful when gluten contamination is suspected. However, follow-up by a dietitian and a doctor together may not be better than the care provide by either alone[10]. The final decision will depend both on the availability of an expert dietitian in the different centers and on the relationship between gastroenterology departments and primary care centers.

Patient associations or support groups can provide important care to achieve adequate dietary compliance. These associations offer detailed information about the importance of a strict GFD and answer questions related to gluten-free foods and cooking recipes. They also organize meetings where patients can share information about the disease and the compliance with diet[11].

2.2. Monitoring Adherence to the GFD

Gluten-free diet compliance may be assessed by several methods (Table 1). Dietary compliance assessed via interviews by a skilled dietitian is probably the best method. While some patients will only need consultation with their physician to achieve strict adherence to the GFD, others will require a multidisciplinary approach to assess GFD compliance.

Table 1. Methods proposed to monitor the adherence to GFD.

Interviews with a skilled dietitian
Consultation with the doctor
Structured surveys
Decrease of serological markers
Improve of villous atrophy
Detection of gluten peptides in feces

Resolution of symptoms may not be an accurate method to assess GFD adherence at the physician consult. On the other hand, persistence of symptoms is associated in most of the cases to continuous gluten ingestion[12]. Moreover, there are other issues different from gluten ingestion that may contribute to the perseverance of symptoms (see previous chapter). Structured short surveys have been employed as an alternative to dietitian consultation for quick assessment of GFD adherence. Questionnaires are easy and quickly to fill in the clinic. Their correlation with the antibody levels and duodenal biopsy appears be high and useful in the follow-up. However, they may be validated in different countries and clinical context before their widespread use[13].

Serologic levels of antibodies employed for CD diagnosis are gluten-dependent: a decrease is expected within months of strict GFD, a gluten challenge increase their values and the persistence of elevated levels suggest a lack of adherence to GFD[14]. Periodical testing for deaminated gliadin IgA and/or tissue-transglutaminase IgA antibodies may be useful for monitoring GFD compliance[15]. However, the normalization of these antibodies' titers does not identify minor dietary transgressions, and their usefulness may only be for predicting non-adherence but not for assessing strict adherence. Diagnosis of CD in adults is actually common in the absence of positive

antibodies (5-16% of biopsy confirmed CD) and serology is useless if antibody levels are not elevated before the start of the GFD[16].

Small bowel histology is the definitive way of assessing the healing of the mucosa. Villous atrophy recovery confirms that strict GFD is followed independently of serological titers or symptoms[1]. Intestinal biopsies in the follow-up may be important in adults where villous atrophy persists despite absence of symptoms and negative serology[17].

A novel method to monitor GFD compliance was recently described. This method can detect the presence of immunodominant gluten peptides in human feces based on the use of the anti-gliadin 33-mer G12 antibody. This antibody is able to detect small amounts of ingested gluten and would represent a quantitative method to assess gluten intake in CD patients. However, ongoing studies will clarify their role in CD management[18].

3. What Should We Test?

3.1. Clinical Assessment

Follow-up visits serve to check the improvement of initial symptoms or the manifestation of newly developed ones. The presence of gastrointestinal symptoms similar to those presented by patients complaining irritable bowel syndrome is common in patients with CD. The persistence or new onset of symptoms may be investigated as related to CD or as another entity. Furthermore, clinicians may be vigilant for symptoms associated with serious intestinal complications: unexplained fever, weight loss, severe diarrhea or signs of malnutrition[19]. Body weight and height in children may reflect adequate nutritional requirements and a correct absorption in the small intestine.

Autoimmune diseases are frequently associated with CD and they can develop at any time during the follow-up. Physicians must be aware of autoimmune and other related diseases associated with CD so to investigate them at the follow-up visits[20].

It is important to screen first-degree relatives and other relatives especially if they have some clinical symptoms. The index case must be informed about this family risk and recommend the screening of relatives[6].

3.2. Laboratory Tests

Laboratory test are important to recognize nutritional deficiencies and the development of associated diseases or complications. Physicians should check on the intestinal absorption status. The basic laboratory panel to analyze previous to each visit may include: full blood count, ferritin, vitamin B12, folate, calcium, alkaline phosphatase, thyroid-stimulatin hormone and thyroid hormone, glucose, aspartate and alanine aminotransferases and antibodies against deaminated gliadin IgA or tissue-transglutaminase IgA[21].

3.3. Other Tests

Decrease in the bone mineral density is probably due to vitamin D deficiency. However, the risk of fracture in CD patients is unclear and the predictive value of bone densitometry is not enough to identify individuals at high-risk of fracture. It seems reasonable to perform bone densitometry to those adult CD patients at high-risk situations that include post-menopausal women, men >55 years and those with known osteopenia before the diagnosis of CD[22]. Further studies are required to identify the efficacy and cost-effectiveness to perform bone densitometry to all the adult CD patients at diagnosis and to identify the follow-up frequency of performing this analysis[23].

Children may have reduced bone mass at the time of diagnosis. However, they are more likely than adults to have fully restored bone mass after 6-12 months of a GFD. Bone densitometry is not generally required in newly diagnosed pediatric patients with uncomplicated CD. In children, special attention to assure normal growth and development is recommended[24].

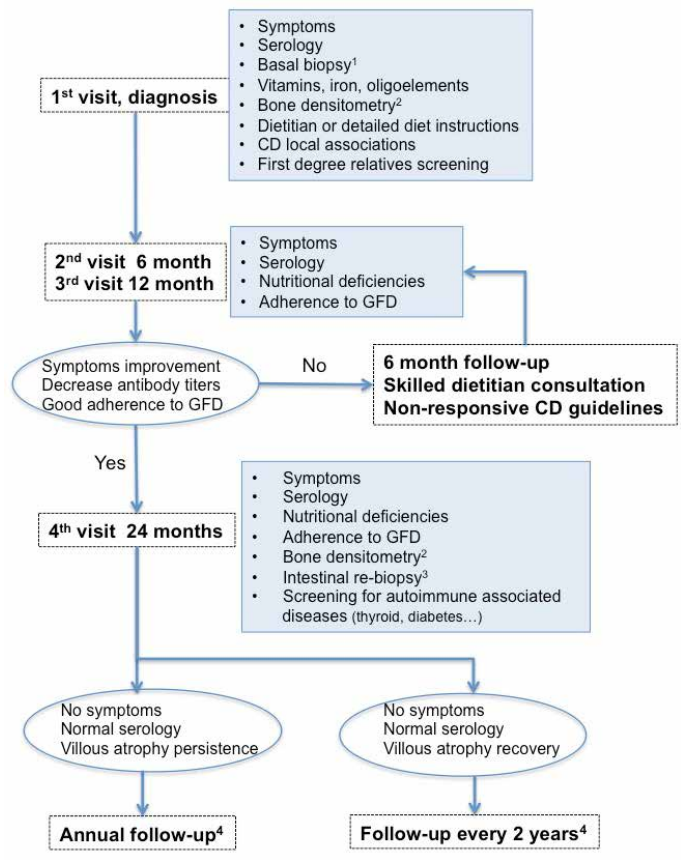
Hyposplenism may affect more than one-third of CD adult patients, while it is not a complication in pediatric patients. The incidence of hyposplenism

correlates with the duration of pre-exposure to gluten and it is higher in those with concomitant autoimmune disorders or pre-malignant conditions[25]. Based on this associated factors, the splenic function may be determined in a selected group of adult CD patients: older patients at diagnosis, concomitant autoimmune or premalignant disorders, and previous history of major infections or thromboembolism. As a diagnostic tool, pitted red cell counting remains an accurate, quantitative and inexpensive method[26]. Protein-conjugate vaccines should be recommended in patients with major hyposplenism, defined by a pitted red cells value higher than 10% and/or and IgM memory B cell frequency lower than 10%.

4. How Often Should We Test?

An algorithm that shows an approach to the monitoring and scheduled visits is shown in Figure 1. After the first visit we have established the diagnosis with a basal biopsy, nutritional status and bone mineralization in high-risk subjects. The second and third visits may be done at 6 months intervals and we must check the following items: symptoms, decrease of basal antibody titers, nutritional deficiencies and the grade of adherence to GFD.

After de first year of diagnosis the patient may experience one of the following situations: (i) symptoms persistence, (ii) elevated antibody titers or (iii) bad adherence to GFD. In these cases, the follow-up may continue at 6 months intervals and with the consultation of a skilled dietitian to ensure a strict GFD. When CD patient continue in this situation physicians must take into account the possibility of a non-responsive CD and follow the applicable guidelines.



(1) Basal biopsy is not always necessary in children. (2) In selected cases explained in the text. (3) Control biopsy in the follow-up may be useful in adult CD. (4) Monitoring in children may be performed annually until complete growth.

Figure 1. An algorithm for a suggest approach to the monitoring of celiac disease.

Those patients that remain without symptoms, decreased antibody titers and good adherence to GFD at one year after diagnosis, may be revised at 24 months. At this time, in adult CD, duodenal biopsy may be offered to the patient to assess duodenal atrophy recovery. So, in the case of persistence of mucosal atrophy, the interval of follow-up may be annual to rule out nutritional deficiencies and to check for other complications related to CD.

However, if the duodenal mucosa shows normal architecture, the follow-up visits may be delayed, and intervals of visits scheduled every two years.

Pediatric CD may be followed with the same scheme than adults. However, bone densitometry and follow-up biopsy would be done only in selected cases. The children with good adherence to GFD and normal antibodies levels would probably be followed yearly instead of every two years. The main reason for this shorter interval is the need for an early recognition of conditions associated to pediatric CD and specially to assure normal growth and development.

5. Biopsy Control: Is Mucosal Recovery a Goal of Therapy?

As statement in the latest EPSGHAN criteria, CD children diagnosed with CD do not need a histological re-evaluation on a GFD[27]. Thus, follow-up biopsy is not recommended as a routine in children, and may be offered only to those children with non-responsive CD.

Celiac disease shows several differences between children and adults that may be taken into account in the follow-up of the disease. A large number of patients in the adult age are asymptomatic or minimally symptomatic at presentation. These cannot be followed up using symptom relief as the main determinant of clinical response. Other adult patients are diagnosed with normal antibody titers showing histological abnormalities in the duodenal biopsy. In these "seronegative" subjects, serology is not useful to assess gluten adherence or to predict mucosal healing. Finally, histological recovery is achieved in most of children but is variable in adults where complete histological recovery is reported in less than 50% of the cases[17,28].

The American College of Gastroenterology recently published guidelines include the recommendation that it is reasonable to do a follow-up biopsy in adults after two years of starting a GFD in order to assess mucosal healing, but it is not recommended as routine in children[1]. The British Society of Gastroenterology guidelines are less categorical and suggest that there is little

evidence to address whether clinical outcomes are significantly altered as a result of re-biopsy. Furthermore, the British guidelines highlight the lack of data about the cost-benefit analysis of repeated biopsy, and their final recommendation is that follow-up biopsies are not mandatory if the patient is asymptomatic on a GFD and has no other features that suggest an increased risk of complication[29].

In Figure 1 we can see that a great benefit of re-biopsy on GFD is the stratification of patients with CD in two groups: those suitable for less strict controls when mucosal recovery is achieved and those requiring more intensive clinical management when the atrophy persists in duodenal mucosa. It is clear that the persistence of villous atrophy is associated with CD complications and adverse outcomes. Even the persistence on GFD of mild forms of enteropathy (Marsh I or duodenal lymphocytosis) may be associated with nutritional deficiencies or complications[30]. As the median time to mucosal recovery has been reported as two to three years, the control biopsy may be offered to adult patients at this time (Figure 1)[31].

Patients with villous atrophy persistency may require closer clinical supervision, and strict GFD compliance is mandatory for them. Subsequent re-biopsies may be offered when there is no evidence of gluten contamination in the diet. There is less evidence for duodenal re-biopsy in those cases with persistent mild forms of enterophaty where other causes different from gluten could be responsible (mainly the *Helicobacter pylori* infection and the NSAID ingestion)[32].

6. Conclusions

The compliance with a strict GFD is the cornerstone of CD management. Patients must be followed-up along their lives by a health-care practitioner with knowledge of CD, and in some cases with the support of a skilled dietitian. Duodenal biopsy in the follow-up is a useful practice in adult CD to assess mucosal recovery and would be helpful to detect those individuals at-risk for complications.

References

1. Rubio-Tapia A, Hill ID, Kelly CP, Calderwood AH, Murray JA, American College of G. *ACG clinical guidelines: diagnosis and management of celiac disease.* Am J Gastroenterol. 2013; 108: 656-76; quiz 77.
   http://dx.doi.org/10.1038/ajg.2013.79
   PMid:23609613 PMCid:PMC3706994
2. Hall NJ, Rubin G, Charnock A. *Systematic review: adherence to a gluten-free diet in adult patients with coeliac disease.* Aliment Pharmacol Ther. 2009; 30: 315-30.
   http://dx.doi.org/10.1111/j.1365-2036.2009.04053.x
   PMid:19485977
3. Hogberg L, Grodzinsky E, Stenhammar L. *Better dietary compliance in patients with coeliac disease diagnosed in early childhood.* Scand J Gastroenterol. 2003; 38: 751-4.
   http://dx.doi.org/10.1080/00365520310003318
   PMid:12889562
4. Herman ML, Rubio-Tapia A, Lahr BD, Larson JJ, Van Dyke CT, Murray JA. *Patients with celiac disease are not followed up adequately.* Clin Gastroenterol Hepatol. 2012; 10: 893-9 e1.
5. Murray JA, Watson T, Clearman B, Mitros F. *Effect of a gluten-free diet on gastrointestinal symptoms in celiac disease.* Am J Clin Nutr. 2004; 79: 669-73.
6. Vaquero L, Caminero A, Nunez A, Hernando M, Iglesias C, Casqueiro J et al. *Coeliac disease screening in first-degree relatives on the basis of biopsy and genetic risk.* Eur J Gastroenterol Hepatol. 2014; 26: 263-7.
   http://dx.doi.org/10.1097/MEG.0000000000000020
   PMid:24300305
7. Ludvigsson JF. *Mortality and malignancy in celiac disease.* Gastrointest Endosc Clin N Am. 2012; 22: 705-22.
   http://dx.doi.org/10.1016/j.giec.2012.07.005
   PMid:23083988
8. Haines ML, Anderson RP, Gibson PR. *Systematic review: The evidence base for long-term management of coeliac disease.* Aliment Pharmacol Ther. 2008; 28: 1042-66.
   http://dx.doi.org/10.1111/j.1365-2036.2008.03820.x
   PMid:18671779
9. Kurppa K, Lauronen O, Collin P, Ukkola A, Laurila K, Huhtala H et al. *Factors Associated with Dietary Adherence in Celiac Disease: A Nationwide Study.* Digestion. 2012; 86: 309-14.
   http://dx.doi.org/10.1159/000341416
   PMid:23095439

10. Simpson S, Thompson T. *Nutrition assessment in celiac disease.* Gastrointest Endosc Clin N Am. 2012; 22: 797-809.

http://dx.doi.org/10.1016/j.giec.2012.07.010
PMid:23083994

11. Leffler DA, Edwards-George J, Dennis M, Schuppan D, Cook F, Franko DL et al. *Factors that influence adherence to a gluten-free diet in adults with celiac disease.* Dig Dis Sci. 2008; 53: 1573-81.

http://dx.doi.org/10.1007/s10620-007-0055-3
PMid:17990115 PMCid:PMC3756800

12. Ciacci C, Cirillo M, Cavallaro R, Mazzacca G. *Long-term follow-up of celiac adults on gluten-free diet: prevalence and correlates of intestinal damage.* Digestion. 2002; 66: 178-85.

http://dx.doi.org/10.1159/000066757
PMid:12481164

13. Biagi F, Bianchi PI, Marchese A, Trotta L, Vattiato C, Balduzzi D et al. *A score that verifies adherence to a gluten-free diet: a cross-sectional, multicentre validation in real clinical life.* Br J Nutr. 2012; 108: 1884-8.

http://dx.doi.org/10.1017/S0007114511007367
PMid:22321199

14. Nachman F, Sugai E, Vazquez H, Gonzalez A, Andrenacci P, Niveloni S et al. *Serological tests for celiac disease as indicators of long-term compliance with the gluten-free diet.* Eur J Gastroenterol Hepatol. 2011; 23: 473-80.

http://dx.doi.org/10.1097/MEG.0b013e328346e0f1

15. Bai JC, Fried M, Corazza GR, Schuppan D, Farthing M, Catassi C et al. *World Gastroenterology Organisation global guidelines on celiac disease.* J Clin Gastroenterol. 2013; 47: 121-6.

http://dx.doi.org/10.1097/MCG.0b013e31827a6f83
PMid:23314668

16. Lewis NR, Scott BB. *Meta-analysis: deamidated gliadin peptide antibody and tissue transglutaminase antibody compared as screening tests for coeliac disease.* Aliment Pharmacol Ther. 2010; 31: 73-81.

http://dx.doi.org/10.1111/j.1365-2036.2009.04110.x
PMid:19664074

17. Lebwohl B, Murray JA, Rubio-Tapia A, Green PH, Ludvigsson JF. *Predictors of persistent villous atrophy in coeliac disease: a population-based study.* Aliment Pharmacol Ther. 2014; 39: 488-95.

http://dx.doi.org/10.1111/apt.12621
PMid:24428688 PMCid:PMC4012428

18. Comino I, Real A, Vivas S, Siglez MA, Caminero A, Nistal E et al. *Monitoring of gluten-free diet compliance in celiac patients by assessment of gliadin 33-mer equivalent epitopes in feces.* Am J Clin Nutr. 2012; 95: 670-7.
http://dx.doi.org/10.3945/ajcn.111.026708
PMid:22258271 PMCid:PMC3278243

19. Dewar DH, Donnelly SC, McLaughlin SD, Johnson MW, Ellis HJ, Ciclitira PJ. *Celiac disease: management of persistent symptoms in patients on a gluten-free diet.* World J Gastroenterol. 2012; 18: 1348-56.
http://dx.doi.org/10.3748/wjg.v18.i12.1348
PMid:22493548 PMCid:PMC3319961

20. Lauret E, Rodrigo L. *Celiac disease and autoimmune-associated conditions.* Biomed Res Int. 2013; 127589.
http://dx.doi.org/10.1155/2013/127589

21. Rostom A, Murray JA, Kagnoff MF. *American Gastroenterological Association (AGA) Institute technical review on the diagnosis and management of celiac disease.* Gastroenterology. 2006; 131: 1981-2002.
http://dx.doi.org/10.1053/j.gastro.2006.10.004
PMid:17087937

22. Lewis NR, Scott BB. *Should patients with coeliac disease have their bone mineral density measured?* Eur J Gastroenterol Hepatol. 2005; 17: 1065-70.
http://dx.doi.org/10.1097/00042737-200510000-00009
PMid:16148551

23. West J, Logan RF, Card TR, Smith C, Hubbard R. *Fracture risk in people with celiac disease: a population-based cohort study.* Gastroenterology. 2003; 125: 429-36.
http://dx.doi.org/10.1016/S0016-5085(03)00891-6

24. Ribes-Koninckx C, Mearin ML, Korponay-Szabo IR, Shamir R, Husby S, Ventura A et al. *Coeliac disease diagnosis: ESPGHAN 1990 criteria or need for a change? Results of a questionnaire.* J Pediatr Gastroenterol Nutr. 2012; 54: 15-9.
http://dx.doi.org/10.1097/MPG.0b013e31822a00bb
PMid:21716133

25. Thomas HJ, Wotton CJ, Yeates D, Ahmad T, Jewell DP, Goldacre MJ. *Pneumococcal infection in patients with coeliac disease.* Eur J Gastroenterol Hepatol. 2008; 20: 624-8.
http://dx.doi.org/10.1097/MEG.0b013e3282f45764
PMid:18679063

26. Di Sabatino A, Brunetti L, Carnevale Maffe G, Giuffrida P, Corazza GR. *Is it worth investigating splenic function in patients with celiac disease?* World J Gastroenterol. 2013; 19: 2313-8.
http://dx.doi.org/10.3748/wjg.v19.i15.2313
PMid:23613624 PMCid:PMC3631982

27. Husby S, Koletzko S, Korponay-Szabo IR, Mearin ML, Phillips A, Shamir R et al. *European Society for Pediatric Gastroenterology, Hepatology, and Nutrition guidelines for the diagnosis of coeliac disease.* J Pediatr Gastroenterol Nutr. 2012; 54: 136-60.

http://dx.doi.org/10.1097/MPG.0b013e31821a23d0
PMid:22197856

28. Sharkey LM, Corbett G, Currie E, Lee J, Sweeney N, Woodward JM. *Optimising delivery of care in coeliac disease - comparison of the benefits of repeat biopsy and serological follow-up.* Aliment Pharmacol Ther. 2013; 38: 1278-91.

http://dx.doi.org/10.1111/apt.12510
PMid:24117503

29. Ludvigsson JF, Bai JC, Biagi F, Card TR, Ciacci C, Ciclitira PJ et al. *Diagnosis and management of adult coeliac disease: guidelines from the British Society of Gastroenterology.* Gut. 2014; 63: 1210-28.

http://dx.doi.org/10.1136/gutjnl-2013-306578
PMid:24917550 PMCid:PMC4112432

30. Kurppa K, Collin P, Sievanen H, Huhtala H, Maki M, Kaukinen K. *Gastrointestinal symptoms, quality of life and bone mineral density in mild enteropathic coeliac disease: a prospective clinical trial.* Scand J Gastroenterol. 2010; 45: 305-14.

http://dx.doi.org/10.3109/00365520903555879
PMid:20059405

31. Rubio-Tapia A, Rahim MW, See JA, Lahr BD, Wu TT, Murray JA. *Mucosal recovery and mortality in adults with celiac disease after treatment with a gluten-free diet.* Am J Gastroenterol. 2010; 105: 1412-20.

http://dx.doi.org/10.1038/ajg.2010.10
PMid:20145607 PMCid:PMC2881171

32. Rosinach M, Esteve M, Gonzalez C, Temino R, Marine M, Monzon H et al. *Lymphocytic duodenosis: aetiology and long-term response to specific treatment.* Dig Liver Dis. 2012; 44: 643-8.

http://dx.doi.org/10.1016/j.dld.2012.03.006
PMid:22497904

Chapter 12

Quality of Life and Psychological Distress in the Patient with CD

Claudia Herrera de Guise, Francesc Casellas

Digestive System Research Unit, Hospital Universitari Vall d'Hebron, Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (Ciberehd), Barcelona, Spain.

cherreradeguise@gmail.com, fcasellas@vhebron.net

Doi: http://dx.doi.org/10.3926/oms.260

How to cite this chapter

Herrera de Guise C, Casellas F. *Quality of Life and Psychological Distress in the Patient with CD.* In Arranz E, Fernández-Bañares F, Rosell CM, Rodrigo L, Peña AS, editors. *Advances in the Understanding of Gluten Related Pathology and the Evolution of Gluten-Free Foods*. Barcelona, Spain: OmniaScience; 2015. p. 425-440.

Abstract

Celiac disease (CD) is a chronic disorder that can impact patients in many ways including their health-related quality of life (HRQOL). There are several factors that can affect HRQOL in CD patients; from manifestations of the disease to the compliance with a gluten-free-diet. Furthermore, there has been a beneficial response to treatment with a gluten-free diet. Measuring HRQOL in celiac disease offers important advantages, not only for healthcare providers and caregivers but also for patients. The purpose of focusing on HRQOL is to go beyond the presence and severity of symptoms of disease or side-effects of treatment, examining how patients perceive and experience these manifestations in their daily lives. We describe the instruments to measure HRQOL in CD patients and recent studies that evaluate the impact that CD has on patient's HRQOL.

Keywords

Celiac disease, health-related quality of life, questionnaires for HRQOL in CD.

1. Introduction

Celiac disease (CD) is an immune-based reaction to dietary gluten that primarily affects the small intestine in those with a genetic predisposition and resolves with exclusion of gluten from the diet. Over the last 50 years there has been a substantial increase in the prevalence of CD and an increase in the rate of diagnosis in the last 10 years[1]. CD is a chronic disorder that can impact patients in many ways including their health-related quality of life (HRQOL). In recent years the health-related quality of life has become an important issue in this context, and most studies carried out so far have shown impaired quality of life in untreated patients compared with healthy controls. Furthermore, there has generally been a beneficial response to treatment with a gluten-free diet[2].

The purpose of focusing on HRQOL is to go beyond the presence and severity of symptoms of disease or side-effects of treatment, examining how patients perceive and experience these manifestations in their daily lives[3].

2. Definition of Quality of Life

There is no universal agreement on the definition of 'quality of life'. 'Quality of life' is a term, which has been applied to various disciplines, such as politics, economics and religion. However, this term has been used mainly in medical studies. Quality of life as applied to medicine is more specifically known as HRQOL or 'subjective health status'. Despite the lack of universal consensus on a definition most researchers agree that quality of life is a subjective, multidimensional and dynamic concept[4,5]. HRQOL represents a subjective appraisal of an individual's perceptions, beliefs, feelings and expectations. Therefore, the person's own appraisal of his or her health and well-being is a key factor in quality of life studies[6]. Quality of life construct is made up of a number of domains or dimensions; physical, social and psychological[7]. Lastly, quality of life is dynamic, since it varies over time and it depends on changes within the patient and the patient's surroundings[5].

HRQL can be formally defined as: "The extent to which one's usual or expected physical, emotional and social well-being are affected by a medical condition or its treatment"[8].

3. Benefits of Utilization of HRQOL Measurement

Measuring HRQOL in celiac disease offers important advantages, not only for healthcare providers and caregivers but also for patients. Information regarding the impact of a medical condition on quality of life can used to capture changes in clinical status before, during and after treatment[4]. It can also aid in planning tools for clinical care and for treatment decision-making, and can be used as a predictor of the outcome of treatment[9]. Measuring HRQOL is also useful for patients because it allows them to explain the impact of disease in other dimensions of their life.

4. HRQOL Measurement in Celiac Disease

The instruments mainly used to measure HRQOL are questionnaires. These instruments are classified as generic or disease specific, according to the target population addressed. Generic instruments can be administered to the normal population or to any patient, with any disease. These instruments are used to describe the general impact of chronic diseases on patients' health and to compare the HRQOL of patient groups across different diseases. The generic questionnaires that are most used in celiac disease are shown in Table 1. The Short Form Health Survey (SF-36) is mainly used as a generic instrument in gastroenterology. The SF-36 is a brief (36-item), comprehensive measure of general health status originally developed for use in the Medical Outcomes Study[10]. It was designed for use in clinical practice and research, evaluation of health policy, and general population surveys. The SF-36 is currently the most widely used health status measure, particularly in the gastroenterology literature. There are abbreviated versions of this instrument, the SF-20 and SF-12, although their reliability and validity are slightly lower than for the

SF-36[11]. The EuroQol five-dimensional (EQ-5D) questionnaire is used world-wide as a patient-reported outcome instrument for the measurement and valuation of health. Developed by the EuroQol Group[12], this instrument measures health in five dimensions; mobility, self-care, usual activities, pain/discomfort, and anxiety/depression with three levels of severity in each dimension; no problems, some/moderate problems, and extreme problems/unable to perform the activity. Eypasch et al. developed the gastrointestinal quality of life index (GIQLI) to measure HRQOL in multiple gastrointestinal diseases[13]. It contains 36 items, scored on a five point Likert scale (range 0-144). It evaluates the past two weeks.

Generic questionnaires may not focus adequately on the area of interest for a specific patient or disease, and may lack the sensitivity to detect important changes in status over time. Disease-specific instruments have the potential for increased responsiveness and as they address specific diseases they are considerably more sensitive to the effects of interventions and time trends in a specific disease condition[14]. There are few disease-specific HRQOL instruments designed for CD. The Celiac Disease Questionnaire (CDQ) was developed and validated by Haüser et al.[15]. This instrument evaluates 4 domains with 7 items each: emotional and social problems, disease-related worries, and gastrointestinal symptoms, in the last two weeks. The CDQ discriminates in all subscales patients with CD-associated diseases from patients without CD-associated diseases. The CDQ has also been validated in Italy[16].

The celiac disease quality of life survey (CD-QOL) is a reliable and valid celiac disease specific instrument developed by Dorn et al.[17]. It includes 20 questions across four clinically relevant subscales (Limitations, Dysphoria, Health Concerns, and Inadequate Treatment). The instrument assesses the respondent's feelings to particular celiac disease-associated symptoms over the previous 30 days. The questions consist of a five-point Likert scale labeled 1 through 5, where 1 is not at all and 5 is a great deal. For analysis, the responses are reverse coded and totaled. A higher score, with a maximum value of 100, may mean a higher quality of life and a decreased degree of celiac disease symptoms.

Table 1. Generic instruments for evaluation of the HRQOL in CD patients

Instrument	Items	No of items	Categories or domains
SF-36	Questions	36	Physical functioning Bodily pain Role limitations-physical Social functioning General mental health Role limitations–emotional Vitality (energy/fatigue) General health perception
EQ-5D	Statements VAS*	5 + VAS	Mobility Self-care Usual activities Pain/discomfort Anxiety/depression
GIQLI	Questions	36	GI symptoms Physical function Social function Emotional function Subjective treatment assessment
TACQOL (children)	Questions Scales	56	Pain and symptoms (body) Basic motor functioning (motor) Autonomy (auto) Cognitive functioning (cognition) Social functioning (social) Global positive emotional functioning (emo-pos) Global negative emotional functioning (emo-neg)

VAS: visual analogue scale

Table 2. CD-specific instruments for evaluation of the HRQOL in CD patients.

Instrument	Items	No of items	Categories or domains
CDQOL	Statements	20	Limitations Dysphoria Health Concerns Inadequate Treatment
CDQ	Questions	28	GI symptoms Emotional problems Social problems Disease-related worries
CDDUX (children)	Questions	12	Communication Diet Having CD

The CDQOL differs from the CDQ in that the latter focuses on both physical and psychological symptoms, as well as impairments in daily function, while the former employs a needs-based model that is more proximate to the attitudes and perceptions of individuals with CD that relate to meeting the basic needs of the condition[18]. The needs-based model postulates that life gains its quality from the ability of the individual to satisfy his or her needs. Quality of life is high when these needs are fulfilled and low when few needs are satisfied[19]. Needs-based measures are more sensitive to changes over time[20]. The CDQOL has also been translated and validated in Spain[21].

Two instruments are used to assess the quality of life in children with celiac disease. The generic Health related quality of life measure for children (TACQOL) is an instrument applicable to children in the age group of 6-15 years[22]. It evaluates seven domains of HRQOL: pain and symptoms (body), basic motor functioning (motor), autonomy (auto), cognitive functioning (cognition), social functioning (social), global positive emotional functioning (emo-pos) and global negative emotional functioning (emo-neg). Items are scored 0 for no health status problem, 1 for a health status problem without

negative emotional responses and 2 for a health status problem with negative emotional responses.

The Disease-specific HRQOL Questionnaire for Children with CD (CDDUX) is an instrument designed for CD patients ages 8 to 18 years[23]. The CDDUX includes 12 items across 3 subscales: ''Communication'', ''Diet'', and ''Having CD''. The CDDUX has also been translated and validated in Argentina[24].

The difference between the TACQOL and the CDDUX is that the latter is disease-specific, therefore it elicits information about aspects of life that are influenced by CD. These specific aspects may be evaluated by the children as negative, but this does not mean that their perception of their generic QOL is negative as well.

5. Impact of Celiac Disease in Patient's HRQOL

Health-related quality of life (HRQOL) as an aspect of living with CD has been studied frequently[25-30]. The HRQOL of CD patients' is reduced compared to that of the general population. Factors that affect HRQOL in CD patients can be related to the manifestations of the disease itself, the compliance with a gluten-free-diet (GFD) or even the timing of diagnosis.

Symptomatic, untreated CD patients have a markedly reduced QOL compared to the general population[26-29]. One study using EQ-5D collected retrospective data concluded that the HRQOL before CD diagnosis is quantitatively similar to that of stroke patients[25]. A multicenter, cross-sectional prospective study found that the HRQOL of untreated, recently diagnosed CD patients is significantly impaired, on almost all of the dimensions and on the overall score of both the EQ-5D and the GIQLI[26]. Female patients have a tendency to do less well during the course of a GFD in some studies[31,32], but not in others[33].

Impact of the timing of diagnosis of CD and the HRQOL has also been evaluated. Often in adult patients, the diagnostic process for CD is often very late and can generate health complications that would be avoided by earlier

diagnosis. A recent large cross-sectional study found that long duration and severity of symptoms predisposed CD patients to persistency of symptoms and a reduced quality of life[34]. Screen-detected patients have shown a better HRQOL than symptom-detected patients. Paavola et al. studied a cohort of 466 screen-detected and symptom-detected CD patients[35]. The authors found that QOL of screen-detected CD patients was comparable to non-celiac controls. This was not affected by a GFD. Fatigue is a symptom that many CD patients complain about and it can impair HRQOL. In a study evaluating fatigue in CD, fatigue-related problems and intensity of fatigue were higher in untreated CD patients, and fatigue was inversely correlated with patients' perception of health[36].

5.1. Gluten-Free-Diet and Quality of Life

Currently, a lifelong GFD is the only treatment for CD. A GFD requires a radical and lifelong change in daily habits. GFD can be troublesome, expensive and socially restrictive, which makes for difficulties in adherence and it can impact patients' QOL. Changing life-long dietary patterns can be laborious and compliance with a GFD varies from 42% to 91% depending on the method of assessment[37], however up to 50% of patients do not strictly adhere with the diet (either voluntary or involuntary) and will develop an active symptomatology[38]. Nevertheless, in symptomatic CD patients the GFD results in rapid recovery from symptoms and improvement in HRQOL[30,31,35,39-41], and adherence to GFD also allows progressive restoration of HRQOL perception[33]. It seems that this improvement is maintained long-term, and strictly compliant patients can have comparable long-term HRQOL to healthy people[26,42-44]. A longitudinal study that reported long-term data for patients followed for 4 years from diagnosis found long-term deterioration of HRQOL in patients who were poor-compliers with a GFD[45]. An interesting study by Barratt et al. evaluated the perceived degree of difficulty following a GFD among 225 CD patients[46]. The authors found a reduction in HRQOL among patients who had a higher perceived difficulty to follow the GFD.

Studies of the effect of GFD on HRQOL of asymptomatic, screen-detected CD have shown that it either remains the same as healthy controls[43,44] or it improves[29].

6. Conclusion

Celiac disease involves permanent changes in different aspects in the life of patients leading to an impairment of their quality of life.

The global attention to the person with celiac disease should be directed towards improvement of physical symptoms and minimize the psychosocial impact of the disease.

References

1. Rubio-Tapia A, Hill ID, Kelly CP, Calderwood AH, Murray JA, American College of G. *ACG clinical guidelines: diagnosis and management of celiac disease. The American journal of gastroenterology.* 2013; 108(5): 656-76; quiz 77.

   http://dx.doi.org/10.1038/ajg.2013.79
   PMid:23609613 PMCid:PMC3706994
2. Kurppa K, Collin P, Maki M, Kaukinen K. *Celiac disease and health-related quality of life.* Expert review of gastroenterology & hepatology. 2011; 5(1): 83-90.

   http://dx.doi.org/10.1586/egh.10.81
   PMid:21309674
3. Kerr GD. *Quality of Life - a personal view.* Scandinavian journal of gastroenterology. 1993; 199 Suppl: 14-5.

   http://dx.doi.org/10.3109/00365529309098348
4. Glise H, Wiklund I. *Health-related quality of life and gastrointestinal disease.* Journal of gastroenterology and hepatology. 2002; 17 Suppl: S72-84.

   http://dx.doi.org/10.1046/j.1440-1746.17.s1.6.x
   PMid:12000595
5. Camilleri-Brennan J, Steele RJ. *Measurement of quality of life in surgery.* Journal of the Royal College of Surgeons of Edinburgh. 1999; 44(4): 252-9.

   PMid:10453149
6. Testa MA, Simonson DC. *Assessment of quality-of-life outcomes.* The New England journal of medicine. 1996; 334(13): 835-40.

   http://dx.doi.org/10.1056/NEJM199603283341306
   PMid:8596551
7. Cella D, Riley W, Stone A, Rothrock N, Reeve B, Yount S et al. *The Patient-Reported Outcomes Measurement Information System (PROMIS) developed and tested its first wave of adult self-reported health outcome item banks: 2005-2008.* Journal of clinical epidemiology. 2010; 63(11): 1179-94.

   http://dx.doi.org/10.1016/j.jclinepi.2010.04.011
   PMid:20685078 PMCid:PMC2965562
8. Schipper H. *Guidelines and caveats for quality of life measurement in clinical practice and research.* Oncology. 1990; 4(5): 51-7; discussion 70.

   PMid:2143410
9. Reck M, Thatcher N, Smit EF, Lorigan P, Szutowicz-Zielinska E, Liepa AM et al. *Baseline quality of life and performance status as prognostic factors in patients with extensive-stage disease small cell lung cancer treated with pemetrexed plus carboplatin vs. etoposide plus carboplatin.* Lung cancer. 2012; 78(3): 276-81.

   http://dx.doi.org/10.1016/j.lungcan.2012.09.002
   PMid:23043970

10. Ware JE, Jr., Sherbourne CD. *The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection.* Medical care. 1992; 30(6): 473-83.
http://dx.doi.org/10.1097/00005650-199206000-00002
PMid:1593914

11. Stewart AL, Hays RD, Ware JE, Jr. *The MOS short-form general health survey. Reliability and validity in a patient population.* Medical care. 1988; 26(7): 724-35.
http://dx.doi.org/10.1097/00005650-198807000-00007
PMid:3393032

12. Rabin R, Gudex C, Selai C, Herdman M. *From translation to version management: a history and review of methods for the cultural adaptation of the EuroQol five-dimensional questionnaire.* Value in health: the journal of the International Society for Pharmacoeconomics and Outcomes Research. 2014; 17(1): 70-6.
http://dx.doi.org/10.1016/j.jval.2013.10.006
PMid:24438719

13. Eypasch E, Williams JI, Wood-Dauphinee S, Ure BM, Schmulling C, Neugebauer E et al. *Gastrointestinal Quality of Life Index: development, validation and application of a new instrument.* The British journal of surgery. 1995; 82(2): 216-22.
http://dx.doi.org/10.1002/bjs.1800820229
PMid:7749697

14. Guyatt GH, Feeny DH, Patrick DL. *Measuring health-related quality of life.* Annals of internal medicine. 1993; 118(8): 622-9.
http://dx.doi.org/10.7326/0003-4819-118-8-199304150-00009
PMid:8452328

15. Hauser W, Gold J, Stallmach A, Caspary WF, Stein J. *Development and validation of the Celiac Disease Questionnaire (CDQ), a disease-specific health-related quality of life measure for adult patients with celiac disease.* Journal of clinical gastroenterology. 2007; 41(2): 157-66.
http://dx.doi.org/10.1097/01.mcg.0000225516.05666.4e
PMid:17245214

16. Marchese A, Klersy C, Biagi F, Balduzzi D, Bianchi PI, Trotta L et al. *Quality of life in coeliac patients: Italian validation of a coeliac questionnaire.* European journal of internal medicine. 2013; 24(1): 87-91.
http://dx.doi.org/10.1016/j.ejim.2012.09.015
PMid:23102568

17. Dorn SD, Hernandez L, Minaya MT, Morris CB, Hu Y, Leserman J et al. *The development and validation of a new coeliac disease quality of life survey (CD-QOL).* Alimentary pharmacology & therapeutics. 2010; 31(6): 666-75.
http://dx.doi.org/10.1111/j.1365-2036.2009.04220.x
PMid:20015103

18. Hunt SM, McKenna SP. *The QLDS: a scale for the measurement of quality of life in depression.* Health policy. 1992; 22(3): 307-19.

http://dx.doi.org/10.1016/0168-8510(92)90004-U

19. Acquadro C, Conway K, Hareendran A, Aaronson N, European Regulatory I, Quality of Life Assessment G. *Literature review of methods to translate health-related quality of life questionnaires for use in multinational clinical trials.* Value in health: the journal of the International Society for Pharmacoeconomics and Outcomes Research. 2008; 11(3): 509-21.

http://dx.doi.org/10.1111/j.1524-4733.2007.00292.x
PMid:18179659

20. Doward LC, McKenna SP, Meads DM. *Effectiveness of needs-based quality of life instruments.* Value in health: the journal of the International Society for Pharmacoeconomics and Outcomes Research. 2004; 7 Suppl 1: S35-8.

21. Casellas F, Rodrigo L, Molina-Infante J, Vivas S, Lucendo AJ, Rosinach M et al. *Transcultural adaptation and validation of the Celiac Disease Quality of Life (CD-QOL) survey, a specific questionnaire to measure quality of life in patients with celiac disease.* Revista espanola de enfermedades digestivas: organo oficial de la Sociedad Espanola de Patologia Digestiva. 2014; 105(10): 585-93.

http://dx.doi.org/10.4321/S1130-01082013001000003

22. Verrips GH, Vogels T, Koopman HM, Theunissen NC, Kamphuis RP, Wit JM et al. *Measuring health-related quality of life in a child population.* Eur J Public Health. 1999; 9(3): 188-93.

http://dx.doi.org/10.1093/eurpub/9.3.188

23. van Doorn RK, Winkler LM, Zwinderman KH, Mearin ML, Koopman HM. *CDDUX: a disease-specific health-related quality-of-life questionnaire for children with celiac disease.* Journal of pediatric gastroenterology and nutrition. 2008; 47(2): 147-52.

http://dx.doi.org/10.1097/MPG.0b013e31815ef87d
PMid:18664865

24. Pico M, Spirito MF, Roizen M. *Quality of life in children and adolescents with celiac disease: Argentinian version of the specific questionnaire CDDUX.* Acta gastroenterologica Latinoamericana. 2012; 42(1): 12-9.

PMid:22616492

25. Gray AM, Papanicolas IN. *Impact of symptoms on quality of life before and after diagnosis of coeliac disease: results from a UK population survey.* BMC health services research. 2010; 10: 105.

http://dx.doi.org/10.1186/1472-6963-10-105
PMid:20423498 PMCid:PMC2907763

26. Casellas F, Rodrigo L, Vivancos JL, Riestra S, Pantiga C, Baudet JS et al. *Factors that impact health-related quality of life in adults with celiac disease: a multicenter study.* World journal of gastroenterology: WJG. 2008; 14(1): 46-52.

http://dx.doi.org/10.3748/wjg.14.46
PMid:18176960 PMCid:PMC2673390

27. Green PHR, Stavropoulos SN, Panagi SG, Goldstein SL, McMahon DJ, Absan H et al. *Characteristics of adult celiac disease in the USA: results of a national survey.* The American journal of gastroenterology. 2001; 96(1): 126-31.
http://dx.doi.org/10.1111/j.1572-0241.2001.03462.x
PMid:11197241

28. Lee AR, Ng DL, Diamond B, Ciaccio EJ, Green PH. *Living with coeliac disease: survey results from the U.S.A.* Journal of human nutrition and dietetics: the official journal of the British Dietetic Association. 2012; 25(3): 233-8.
http://dx.doi.org/10.1111/j.1365-277X.2012.01236.x
PMid:22364496

29. Ciacci C, D'Agate C, De Rosa A, Franzese C, Errichiello S, Gasperi V et al. *Self-rated quality of life in celiac disease.* Digestive diseases and sciences. 2003; 48(11): 2216-20.
http://dx.doi.org/10.1023/B:DDAS.0000004530.11738.a2
PMid:14705832

30. Ukkola A, Maki M, Kurppa K, Collin P, Huhtala H, Kekkonen L et al. *Diet improves perception of health and well-being in symptomatic, but not asymptomatic, patients with celiac disease.* Clinical gastroenterology and hepatology: the official clinical practice journal of the American Gastroenterological Association. 2011; 9(2): 118-23.
http://dx.doi.org/10.1016/j.cgh.2010.10.011
PMid:21029791

31. Hallert C, Granno C, Hulten S, Midhagen G, Strom M, Svensson H et al. *Living with coeliac disease: controlled study of the burden of illness.* Scandinavian journal of gastroenterology. 2002; 37(1): 39-42.
http://dx.doi.org/10.1080/003655202753387338
PMid:11843033

32. Roos S, Wilhelmsson S, Hallert C. *Swedish women with coeliac disease in remission use more health care services than other women: a controlled study.* Scandinavian journal of gastroenterology. 2011; 46(1): 13-9.
http://dx.doi.org/10.3109/00365521.2010.516448
PMid:20809768

33. Tontini GE, Rondonotti E, Saladino V, Saibeni S, de Franchis R, Vecchi M. *Impact of gluten withdrawal on health-related quality of life in celiac subjects: an observational case-control study.* Digestion. 2010; 82(4): 221-8.
http://dx.doi.org/10.1159/000265549
PMid:20588037

34. Paarlahti P, Kurppa K, Ukkola A, Collin P, Huhtala H, Maki M et al. *Predictors of persistent symptoms and reduced quality of life in treated coeliac disease patients: a large cross-sectional study.* BMC gastroenterology. 2013; 13: 75.
http://dx.doi.org/10.1186/1471-230X-13-75
PMid:23631482 PMCid:PMC3651340

35. Paavola A, Kurppa K, Ukkola A, Collin P, Lahdeaho ML, Huhtala H et al. *Gastrointestinal symptoms and quality of life in screen-detected celiac disease.* Digestive and liver disease: official journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver. 2012; 44(10): 814-8.

http://dx.doi.org/10.1016/j.dld.2012.04.019
PMid:22673312

36. Casellas Jorda F, Lopez Vivancos J. *Fatigue as a determinant of health in patients with celiac disease.* Journal of clinical gastroenterology. 2010; 44(6): 423-7.

PMid:19935081

37. Black JL, Orfila C. *Impact of coeliac disease on dietary habits and quality of life.* Journal of human nutrition and dietetics: the official journal of the British Dietetic Association. 2011; 24(6): 582-7.

http://dx.doi.org/10.1111/j.1365-277X.2011.01170.x
PMid:21615555

38. Lanzini A, Lanzarotto F, Villanacci V, Mora A, Bertolazzi S, Turini D et al. *Complete recovery of intestinal mucosa occurs very rarely in adult coeliac patients despite adherence to gluten-free diet.* Alimentary pharmacology & therapeutics. 2009; 29(12): 1299-308.

http://dx.doi.org/10.1111/j.1365-2036.2009.03992.x
PMid:19302264

39. Zarkadas M, Cranney A, Case S, Molloy M, Switzer C, Graham ID et al. *The impact of a gluten-free diet on adults with coeliac disease: results of a national survey.* Journal of human nutrition and dietetics: the official journal of the British Dietetic Association. 2006; 19(1): 41-9.

http://dx.doi.org/10.1111/j.1365-277X.2006.00659.x
PMid:16448474

40. Roos S, Karner A, Hallert C. *Psychological well-being of adult coeliac patients treated for 10 years.* Digestive and liver disease: official journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver. 2006; 38(3): 177-80.

http://dx.doi.org/10.1016/j.dld.2006.01.004
PMid:16461026

41. Norstrom F, Lindholm L, Sandstrom O, Nordyke K, Ivarsson A. *Delay to celiac disease diagnosis and its implications for health-related quality of life.* BMC gastroenterology. 2011; 11: 118.

http://dx.doi.org/10.1186/1471-230X-11-118
PMid:22060243 PMCid:PMC3233515

42. Nordyke K, Norstrom F, Lindholm L, Stenlund H, Rosen A, Ivarsson A. *Health-related quality of life in adolescents with screening-detected celiac disease, before and one year after diagnosis and initiation of gluten-free diet, a prospective nested case-referent study.* BMC public health. 2013; 13: 142.

http://dx.doi.org/10.1186/1471-2458-13-142
PMid:23414483 PMCid:PMC3585471

43. Nachman F, Maurino E, Vazquez H, Sfoggia C, Gonzalez A, Gonzalez V et al. *Quality of life in celiac disease patients: prospective analysis on the importance of clinical severity at diagnosis and the impact of treatment.* Digestive and liver disease: official journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver. 2009; 41(1): 15-25.

http://dx.doi.org/10.1016/j.dld.2008.05.011
PMid:18602354

44. Johnston SD, Rodgers C, Watson RG. *Quality of life in screen-detected and typical coeliac disease and the effect of excluding dietary gluten.* European journal of gastroenterology & hepatology. 2004; 16(12): 1281-6.

http://dx.doi.org/10.1097/00042737-200412000-00008

45. Nachman F, del Campo MP, Gonzalez A, Corzo L, Vazquez H, Sfoggia C et al. *Long-term deterioration of quality of life in adult patients with celiac disease is associated with treatment noncompliance.* Digestive and liver disease: official journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver. 2010; 42(10): 685-91.

http://dx.doi.org/10.1016/j.dld.2010.03.004
PMid:20399159

46. Barratt SM, Leeds JS, Sanders DS. *Quality of life in Coeliac Disease is determined by perceived degree of difficulty adhering to a gluten-free diet, not the level of dietary adherence ultimately achieved.* Journal of gastrointestinal and liver diseases: JGLD. 2011; 20(3): 241-5.

PMid:21961090

Chapter 13

Non-Celiac Gluten Sensitivity

Javier Molina-Infante[1], Santos Santolaria[2], Fernando Fernández-Bañares[3,4]

[1] Department of Gastroenterology. Hospital San Pedro de Alcantara, Caceres, Spain.

[2] Department of Gastroenterology. Hospital San Jorge, Huesca, Spain.

[3] Department of Gastroenterology. Hospital Universitario Mutua Terrassa, Barcelona, Spain.

[4] CIBERehd, Spain.

xavi_molina@hotmail.com, ssantolariap@gmail.com, ffbanares@mutuaterrassa.es

Doi: http://dx.doi.org/10.3926/oms.261

How to cite this chapter

Molina-Infante J, Santolaria S, Fernández-Bañares F. *Non-Celiac Gluten Sensitivity*. In Arranz E, Fernández-Bañares F, Rosell CM, Rodrigo L, Peña AS, editors. *Advances in the Understanding of Gluten Related Pathology and the Evolution of Gluten-Free Foods*. Barcelona, Spain: OmniaScience; 2015. p. 441-462.

Abstract

Non-celiac gluten sensitivity (NCGS) is a resurfaced emerging disorder characterized by intestinal and extra-intestinal symptoms related to the ingestion of gluten-containing food in subjects not affected with either celiac disease (CD) or wheat allergy. Despite lacking solid epidemiological data, its prevalence has been estimated five to ten-times higher than that of CD and sells from gluten-free food market have rocketed three-fold lately. Unlike CD, NCGS seems to be associated with activation of the innate immune response. NCGS remains a diagnosis of exclusion of CD, due to the absence of diagnostic specific biomarkers. Evolving evidence has pointed the possibility of a relevant proportion of NGCS in literature actually suffering from overlooked minor forms of CD, the so-called "celiac lite" disease. The efficacy of a gluten-free diet for NCGS is controversial and other components in wheat, specially low-fermentable, poorly-absorbed, short-chain carbohydrates have been lately postulated as major contributors to symptoms, instead of gluten. This review updates evidence on epidemiology, pathophysiology, diagnosis and dietary interventions in NCGS, stressing the need of thorough screening for CD before a diagnosis of NCGS is given, considering that natural history and dietary restriction for both entities are radically different.

Keywords

Non-celiac gluten sensitivity, celiac disease, FODMAP, gluten-free diet, wheat, irritable bowel syndrome.

Abbreviations

CD: celiac disease,

FODMAPs: Fermentable Oligosaccharides, Disaccharides, Monosaccharides And Polyols,

GFD: gluten-free diet,

HLA-DQ2/DQ8: human leukocyte antigen DQ2/DQ8,

LE: lymphocytic enteritis,

NCGS: non-celiac gluten sensitivity.

1. Introduction

Non-celiac gluten sensitivity (NCGS) was originally described in 1976 and 1978[1,2] and the first series dates back to 1980[3], but only since 2010 a rapidly increasing number of papers have called our attention to an apparently novel syndrome entity, which has challenged physicians and researchers involved in gluten-related disorders. NCGS is characterized by intestinal and extraintestinal symptoms related to the ingestion of gluten-containing food, in subjects that are not affected with either celiac disease (CD) or wheat allergy. NGCS currently lacks diagnostic criteria and remains mostly a diagnosis of exclusion of CD. Additionally, many aspects of epidemiology, pathophysiology, clinical spectrum, and treatment are still unclear. In spite of these limitations, NCGS has been reported to presumably affect up to 5-10% of western population and gluten-free foods among non-celiac patients have grown in popularity[4]. As a matter of fact, sells from gluten-free food US market rocketed three-fold from 2006 to 2010 and another three-fold increase is expected by 2015[5]. A recent report revealed that about a third of U.S. adults (the highest percentage ever) expressed their willingness of avoiding gluten from their diets[6]. Therefore, NCGS has definitely settled down among gluten related disorders as a clinical, social and economical relevant entity.

The proposed diagnostic criteria for NCGS are displayed in Table 1[7-9], whereas the main pathogenic and clinical differences between CD and NCGS are summarized in Table 2[8]. The present review aims to critically overview available evidence on NCGS, focusing on epidemiology, adequate distinction of CD before a diagnosis of NCGS, pathogenesis and the efficacy of different dietary interventions for NCGS patients.

Table 1. Current proposed diagnostic criteria for NCGS[7-9].

1. Gluten ingestion elicits the rapid occurrence of intestinal and extraintestinal symptoms, which rapidly disappear after gluten withdrawal and recur upon reintroduction of gluten. 2. Specific IgE to gluten and wheat and skin prick tests results are negative (*exclusion of wheat allergy*). 3. Celiac disease serology (IgA endomysial antibodies, IgA tissue transglutaminase antibodies, IgG deamidated gliadin antibodies) results are negative and no villous atrophy is found on duodenal histology (*exclusion of CD*).
Observations: – HLA-DQ2 and/or HLA-DQ8 positive in 40-50% of NCGS patients. – Normal mucosa or increase in the number of intraepithelial lymphocytes can be found at histopathology. – Antigliadin antibodies (mainly of IgG class) are positive in about 50% of NCGS patients.

Table 2. Pathogenetic, clinical and prognostic differences between CD and NCGS[8-11].

	Celiac disease (CD)	**Non celiac gluten sensitivity (NCGS)**
Interval between exposure to gluten and onset of symptoms	Week to years	Hours to days
Pathogenesis	Adaptative immunity	Innate immunity
HLA	HLA-DQ2/DQ8 positive in 97% of cases	HLA-DQ2/DQ8 positive in 40-50% of cases
Symptoms	Gastrointestinal and extraintestinal symptoms; undistinguishable from NCGS and wheat allergy	Gastrointestinal and extraintestinal symptoms; undistinguishable from CD and wheat allergy
Autoantibodies *(including IgA endomysial and tissue transglutaminase antibodies)*	Almost always present*	Always absent

	Celiac disease (CD)	Non celiac gluten sensitivity (NCGS)
Histopathology	Villous atrophy almost always present**	Villous atrophy always absent
Natural history	Coexisting conditions Long-term complications	Absence of coexisting conditions and long-term complications
Gluten-free diet (GFD)	A strict GFD modifies the natural history of the disease	A strict GFD does not seem mandatory on account of its natural history

***** According to ESPGHAN updated guidelines[10], CD antibodies are not detectable in the blood of all patients with CD; in seronegative cases for anti-TG2, EMA, and anti-DGP but with severe symptoms and a strong clinical suspicion of CD, small intestinal biopsies and HLA-DQ testing are recommended. According to Catassi and Fassano´s diagnostic rules[11], response to a GFD supporting a diagnosis of CD could be assessed histologically in patients with seronegativity.
****** According to ESPGHAN updated guidelines[10], LE without villous atrophy might be specific for CD upon high count of $\gamma\delta$ cells (or $\gamma\delta$/CD3 ratio) in immunohistochemical assessment of biopsies or the presence of IgA anti-TG2 intestinal deposits. According to Catassi and Fassano´s diagnostic rules[11], celiac enteropathy in the small intestine biopsy could be LE without villous atrophy associated with IgA subepithelial deposits.

2. Epidemiology

The overall prevalence of NCGS in the general population is still unknown, mainly because many patients are currently self-diagnosed and start a gluten-free diet (GFD) without medical advice or consultation. Besides, NCGS lacks diagnostic biomarkers. Despite no solid epidemiological study on NCGS is available, it has been reported to be five to ten times more common than CD[4,9]. Recent studies have shown variable rates of self-reported NCGS (0.55% in the USA[12], 5% in children in New Zealand[13], 13% in adults in UK[14]). In patients with self-reported gluten sensitivity, gluten avoidance is associated with improvement of nonspecific behavioral and gastrointestinal complaints[13,15]. However, the vast majority of the NCGS children involved in one of the aforementioned studies were not tested for CD nor underwent an intestinal biopsy[16]. In the study from the UK, 7% of patients were reclassified

as having CD during the study[14], whereas a more recent study conducted in Australia pointed out that just over 1 in 4 self-reporting improvement on a GFD fulfilled strict criteria for a diagnosis of NCGS[17]. Furthermore, initiation of a GFD without adequate exclusion of CD occurred in 62% of patients. As such, an inadequate exclusion of CD in patients with self-reported improvement on a GFD (not performing HLA genotyping and serology combined with small bowel biopsy if positive haplotypes), might lead to an overestimation of both the prevalence of NCGS and the response to a GFD in patients with NCGS.

Although risk factors for NCGS have not yet been identified, the disorder seems to be more common in females and in young/middle age adults. The prevalence of NCGS in children is still unknown, although the first series has been recently described[18].

3. Clinical Picture and Natural History

NCGS is characterized by symptoms that usually occur soon after gluten ingestion, disappear with gluten withdrawal and relapse following gluten challenge, within hours or few days. The "classical" presentation of NCGS is a combination of irritable bowel syndrome-like symptoms, including abdominal pain, bloating, bowel habit abnormalities (either diarrhea or constipation), and systemic manifestations such as "foggy mind", headache, fatigue, joint and muscle pain, leg or arm numbness, dermatitis (eczema or skin rash), depression[7-9]. It is also quite common that many NCGS patients self-report the causal relationship between the ingestion of gluten-containing food and worsening of symptoms. In children, extra-intestinal manifestations seem to be less frequent, the most common symptom being tiredness[18].

No familiar aggregation or major complication of untreated NCGS has so far been described, especially malabsorption-related and autoimmune comorbidities[7-9]. Interestingly, several studies have reported a remarkable prevalence of malabsorption symptoms, familiar history of CD and

autoimmune disorders among NCGS patients[3,19-24]. Yet again, concerns arise about the possibility of having labelled CD patients as having NCGS.

4. NCGS: How Many Patients are Really Suffering "C*eliac-lite*" Disease?

Two major and consistent criteria supporting NCGS and ruling out CD have been negative CD disease serology (including IgA endomysial antibodies, IgA tissue transglutaminase antibodies and IgG deamidated gliadin peptide antibodies) and the absence of villous atrophy on duodenal biopsies[7-9]. Nonetheless, it is accepted that NGCS patients do not have villous atrophy but might have an increased number of intraepithelial duodenal lymphocytes (>25 intraepithelial lymphocytes/100EC), i.e, lymphocytic enteritis (LE), which represent Marsh 1 lesions in the Marsh-Oberhuber histological classification for CD[7-9]. LE is a non-specific histological lesion which may be associated not only to CD, but also to *Helicobacter pylori* infection, small bowel bacterial overgrowth or use of anti-inflammatory drugs. However, the most frequent cause of LE in patients with positive HLA-DQ2/DQ8 after exhaustive diagnostic work-up has been CD, ranging from 16% to 43%[25-28]. Furthermore, seronegative CD is more common in patients without villous atrophy, but Marsh 1 patients may have similar clinical manifestations than those with villous atrophy[29,30] and may show similar clinical-histological remission and reversal of haematological or biochemical disturbances on a GFD[31,32].

As such, it would be important to make a clear distinction between CD and NCGS in patients with gluten-dependent symptoms, especially upon the absence of autoantibodies and/or villous atrophy. In this regard, consensus guidelines from the European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) state that a high count of $\gamma\delta$ cells (or $\gamma\delta$/CD3 ratio) in immunohistochemical assessment of biopsies or the presence of IgA anti-TG2 intestinal deposits might be specific for CD in patients with LE[10]. Similarly, Catassi and Fasano nicely published simplified rules for

diagnostic criteria of CD, accepting that celiac enteropathy in the small intestine biopsy could be LE associated with IgA subepithelial deposits, whereas response to a GFD supporting a diagnosis of CD could be assessed histologically in patients with seronegativity[11].

In an upcoming review on adequate exclusion of CD in NCGS patients[33], our group has found significant methodological flaws in available literature regarding thorough diagnostic efforts to rule out CD before giving a diagnosis of NCGS, in agreement with the aforementioned Australian survey[17]. Among 1561 NCGS evaluated patients, HLA haplotypes could not be linked to histology (normal or LE) in 1123 patients. Furthermore, 20% of patients were reclassified as CD in three studies evaluating advanced diagnostic techniques in 189 NCGS patients combining LE and HLADQ2/DQ8 haplotypes.

Overall, evolving evidence is suggesting that a subset of patients with NCGS may actually belong to the spectrum of CD, specifically some patients with negative antibodies and without villous atrophy, which some authors have so-called "celiac lite" disease[34]. There are two studies which might be prime example for this train of thought. In the first one, conducted in Germany, the authors thoroughly evaluated 102 patients with diarrhea-type IBS in whom CD had been precluded through negative serology and absence of villous atrophy[35]. Thirty five percent of patients were HLADQ2 positive, 23% had LE and notably, 30% had CD-associated antibodies in duodenal aspirate. Those HLADQ2 and intestinal antibody-positive IBS patients significantly improved on a GFD and were likely celiac patients. The second study, from Italy, showed 70 adult NCGS patients who were identified through a double-blind randomized placebo-controlled wheat trial[20]. All patients were seronegative and had no villous atrophy, but 94% NCGS patients had LE, 75% CD haplotypes and 30% positive anti-endomysium antibodies in the supernatant of biopsy culture[36]. The authors further admitted that these latter 30% of NCGS patients could actually suffer from CD[37]. Therefore, the inclusion of patients with positive HLA-DQ2/DQ8 and LE as having NCGS, in the absence of adequate efforts to exclude CD, will always cast doubt on potential misdiagnosis of *"celiac lite"* disease. In this

respect, the importance of misdiagnosing NCGS in CD patients relies not only on the possibility of a CD patient following a non strict GFD, but also on overestimating response to a GFD in NCGS patients.

5. Pathogenesis

The pathophysiology of NCGS is not fully understood yet. Several pioneers studies suggested an important role of the intestinal innate immune system in NCGS, unlike CD, which is triggered by an adaptive immune response[38,39]. However, more recent studies have posed the possibility of NCGS being a mixed disease, with an activation of both innate and adaptative immunity[40,41].

Over the last 3 years, we have witnessed a progressive weakening of an unquestioned dogma, such as gluten-related proteins being the cause for NCGS. As a matter of fact, wheat has multiple constituents, so discussion of NCGS cannot be divorced from considering the role of other components in wheat as potential responsible for NCGS[42]. The two major components of wheat, quantitatively speaking, are carbohydrates (71 g/100 mg) and proteins (12.6 g/100 g). Dietary carbohydrates can be classified into sugars, oligosaccharides and polysaccharides based on their degree of polymerisation. A discrete group of carbohydrates are described as 'fermentable' owing to their availability for fermentation in the colon, which is either due to the absence, or reduced concentration, of suitable hydrolase enzymes for digestion (for example, lactase deficiency), or in the case of monosaccharides because of incomplete absorption in the small intestine. These short-chain fermentable carbohydrates (termed FODMAPs *"Fermentable Oligosaccharides, Disaccharides, Monosaccharides And Polyols"*) are known to induce gastrointestinal symptoms (abdominal pain, flatulence and diarrhoea) through their effects on luminal water handling and colonic gas production[43]. Indeed, emerging evidence is highlighting the efficacy of the low-FODMAP diet for irritable bowel-syndrome symptoms[43]. Regarding improvement of NCGS on a GFD, the withdrawal of gluten may inadvertently be reducing the ingestion of

fructans, the main carbohydrate in wheat, that could actually be the offending agent.

Apart from carbohydrates, other potential culprits in wheat grain for GI symptoms have been postulated, such as non-gluten proteins (α-amylase/trypsin inhibitors), which have recently been suggested to induce intestinal inflammation, polyphenols or wheat germ agglutinin[42].

Several studies have addressed different hypothesis to explain symptom production after wheat ingestion in NCGS patients[44-50], which are summarized in Table 3.

Table 3. Putative pathogenic mechanisms to explain symptoms in NCGS patients after wheat ingestion.

Effects of gliadin in intestinal mucosa
• Increase of epithelial permeability with alteration in protein expression of components of the tight junction (zonulin)[44] • Activation of the innate immune response determined as IL-15 production (in vitro studies) and increased number of intraepithelial lymphocytes[45] • Induction of apoptosis, increase of oxidative stress and inhibition of epithelial cells growth (in vitro studies)[46] • Enhance of cytokine production by peripheral blood mononuclear cells, independent of DQ-status and induction of basophil activation (in vitro studies)[44] • Stimulation of cholinergic nervous system secondary to acetylcholine release by the myenteric plexus (animal studies)[47]
Fructans and undigested gluten proteins
• Carbohydrates present in wheat, such as fructans, are poorly absorbed and may produce gastrointestinal symptoms[48] • Fermentation of undigested gluten protein by sulphate-reducing bacteria can produce hydrogen sulphide and ammonia. Such gases might have a local effect of luminal distension and systemic effects (tiredness)[49] • Other gluten proteins, including alpha-amylase/trypsin inhibitors, and even yeast, could also play a role as triggers of the innate immune response[50]

6. Gluten-free Diet for NCGS: is it Gluten or Carbohydrate Restriction the Key?

So far today, four placebo-controlled dietary interventions in patients with presumptive NCGS have been published[20,51-53]. Carroccio *et al* reported that NCGS patients could be selected on the basis of a double-blind placebo-controlled gluten challenge[20]. In this regard, 276 out of 920 (30%) IBS patients symptom-free on a GFD were considered as NCGS. Compared to placebo, wheat induced significantly more symptoms in patients categorized as NCGS. Nevertheless, it is important to emphasize, as aforementioned, that 30% of NCGS patients in this trial had HLA haplotypes, LE and EmA positive in culture medium of biopsy, so most likely 1 out of 3 NCGS patients included in this trial had actually CD, as admitted by the own authors[37]. The other three trials have been performed by the same Australian group with conflicting results[51-53]. On a first gluten *vs.* placebo rechallenge trial, patients who received gluten challenge had more abdominal symptoms than those on placebo[51]; however, in a second trial with a crossover design, there were no differences between, high-gluten, low-gluten or placebo challenge[52]. In the run-in period of this latter study, patients received a diet low in fermentable oligosaccharides, disaccharides, monosaccharides and polyols (FODMAPs) while maintaining the GFD. Noteworthy, patients whom had been included in the study due to symptom improvement on a GFD, showed a further significant clinical improvement on this run-in period[51]. Furthermore, high-gluten challenge did not worsen abdominal symptoms in a third trial, but NCGS patients showed high depression scores when compared to placebo, but interestingly not to high-whey[53]. In these three trials, CD was excluded on the basis of a HLA-DQ2 and DQ8 negative genetic study or a normal duodenal histology (Marsh 0) on patients HLA-DQ2/8 positive.

A recent study published in abstract form evaluating the efficacy of a GFD and afterwards a low FODMAP diet in patients given a diagnosis of NCGS[54], disclosed that the proportion of NCGS patients responsive to carbohydrate restriction outnumbered that of patients responding to a GFD. Overall, evolving evidence suggests there might be different patients lumped together

under the NCGS term: "celiac lite" patients, NCGS patients (an entity mediated through activation of the innate immunity) and patients intolerant to FODMAPs (carbohydrate intolerance).

7. Conclusions

NCGS is an emerging novel entity overlapping with CD and irritable bowel syndrome, lacking diagnostic criteria or biomarkers. This novel concept has been adopted by public far more readily than the medical scientific community (the ratio for the number of Google vs. PubMed citations for the terminology non-celiac gluten sensitivity was 4,598:1)[55]. In fact, epidemiology, diagnosis and the efficacy of a GFD are largely surrounded by controversy. Concerns about labelling minor forms of CD disease as NCGS have lately arisen, since both diseases have radically different levels of dietary restriction and prognosis if untreated. We currently now that gluten withdrawal may definitely provide clinical benefit to a subset of non-celiac patients, but possibly fermentable carbohydrate (FODMAP) restriction during a GFD may play a major role in symptom improvement. Now, more than ever, we need to separate the wheat from the chaff regarding NCGS and upcoming research will probably shed more light on all of these questions[56].

8. Epidemiology

- The overall prevalence of NCGS in the general population is still unknown, mainly due to lack of diagnostic markers, besides many patients are currently self-diagnosed and start a gluten-free diet (GFD) without medical advice or consultation.
- The growing market for gluten-free foods, which rocketed three-fold during the last 5 years, makes it even harder to decipher whether NCGS is a medical insight or a fad.

9. Natural History

- Unlike CD, no familiar aggregation, coexisting conditions (malabsorption and nutrition deficiencies, auto-immune disorders) and long-term complications have been described for NCGS.
- A diagnosis of NCGS in patients with gluten-dependent symptoms and familiar history of CD, malabsorption signs/symptoms or auto-immune diseases will always cast doubt on the possibility of these patients actually belonging to the spectrum of CD ("celiac-lite" disease).
- The importance of misdiagnosing NCGS in CD patients relies not only on the possibility of a CD patient following a non strict GFD, but also on overestimating response to a GFD in NCGS.

10. Diagnosis

- Gastrointestinal and extraintestinal symptoms in NCGS are indistinguishable from those present in CD.
- NCGS lacks diagnostic biomarkers, so it still remains a diagnosis of exclusion of CD
- Emerging evidence is pointing out inadequate exclusion of CD in a remarkable proportion of NCGS patients.
- Seronegativity and/or absence of villous atrophy may not positively rule out CD in patients with gluten-dependent symptoms

11. Pathogenesis

- Unlike CD, NCGS is mainly driven by activation of innate immunity
- Currently, gluten is not believed to be the only culprit component in wheat for NCGS
- Other wheat components, specially fructans as fermentable carbohydrates, have been postulated as a potential explanation for

symptoms after wheat consumption. These pathogenic pathways do not activate innate immunity but have to do with carbohydrate colonic fermentation.

12. Therapy

- Currently, no solid evidence support a GFD for NCGS patients.
- Compared to a GFD, recent randomized double-blind trials have shown a higher efficacy of a low FODMAP diet for NCGS patients.
- On account of variable responses to different dietary intervention, emerging evidence is posing the possibility of different patients lumped together under the NCGS term: "celiac lite" patients, NCGS patients (an entity mediated through activation of the innate immunity) and patients intolerant to FODMAPs (carbohydrate intolerance).

References

1. Cooper BT, Holmes GK, Ferguson R, Thompson RA, Cooke WT. *Proceedings: chronic diarrhea and gluten sensitivity.* Gut. 1976; 17: 398.
   PMid:1278762
2. Ellis A, Linaker BD. *Non-coeliac gluten sensitivity?* Lancet. 1978; 1: 1358-9.
   http://dx.doi.org/10.1016/S0140-6736(78)92427-3
3. Cooper BT, Holmes GK, Ferguson R, Thompson RA, Allan RN, Cooke WT. *Gluten-sensitive diarrhea without evidence of celiac disease.* Gastroenterology. 1980; 79: 801-6.
   PMid:7419003
4. Beck M. *Clues to gluten sensitivity.* Wall Street J 2011. http://online.wsj.com/article/SB10001424052748704893604576200393522456636.html. Accessed: June 2014.
5. *Gluten-free foods and beverages in the U.S.* 3rd edition. Rockville, MD: Packaged Facts; 2011. http://www.packagedfacts.com/Gluten-Free-Foods-2710664. Accessed: June 2014.
6. *Percentage of U.S. Adults Trying to Cut Down or Avoid Gluten in Their Diets Reaches New High in 2013, Reports NPD.* https://www.npd.com/wps/portal/npd/us/news/press-releases/percentage-of-us-adults-trying-to-cut-down-or-avoid-gluten-in-their-diets-reaches-new-high-in-2013-reports-npd/. Accessed: June 2014.
7. Volta U, De Giorgio R. *New understanding of gluten sensitivity.* Nat Rev Gastroenterol Hepatol. 2012; 9: 295-9.
   http://dx.doi.org/10.1038/nrgastro.2012.15
   PMid:22371218
8. Catassi C, Fasano A. *Clinical practice: Celiac disease.* N Eng J Med. 2012; 267: 2419-26.
9. Catassi C, Bai JC, Bonaz B, Bouma G, Calabro A, Carroccio A et al. *Non-celiac gluten sensitivity: the new frontier of gluten related disorders.* Nutrients. 2013; 5: 3839-3853.
   http://dx.doi.org/10.3390/nu5103839
   PMid:24077239 PMCid:PMC3820047
10. Husby S, Koletzko S, Korponay-Szabo IR, Mearin ML, Phillips A, Shamir R et al. *European Society for Pediatric Gastroenterology, Hepatology, and Nutrition guidelines for the diagnosis of coeliac disease.* J Pediatr Gastroenterol Nutr. 2012; 54: 136-60.
    http://dx.doi.org/10.1097/MPG.0b013e31821a23d0
    PMid:22197856

11. Catassi C, Fasano A. *Celiac disease diagnosis: simple rules are better than complicated algorithms.* Am J Med. 2010; 123: 691-3.

http://dx.doi.org/10.1016/j.amjmed.2010.02.019
PMid:20670718

12. Digiacomo DV, Tennyson CA, Green PH, Demmer RT. *Prevalence of gluten-free diet adherence among individuals without celiac disease in the USA: results from the Continuous National Health and Nutrition Examination Survey 2009-2010.* Scand J Gastroenterol. 2013; 48: 921-5.

http://dx.doi.org/10.3109/00365521.2013.809598
PMid:23834276

13. Tanpowpong P, Ingham TR, Lampshire PK, Kirchberg FF, Epton MJ, Crane J et al. *Coeliac disease and gluten avoidance in New Zealand children.* Arch Dis Child. 2012; 9: 12-6.

http://dx.doi.org/10.1136/archdischild-2011-300248
PMid:22075107

14. Aziz I, Lewis NR, Hadjivassiliou M, Winfield SN, Rugg N, Kelsall A et al. *A UK study assessing the population prevalence of self-reported gluten sensitivity and referral characteristics to secondary care.* Eur J Gastroenterol Hepatol. 2014; 26: 33-9.

http://dx.doi.org/10.1097/01.meg.0000435546.87251.f7
PMid:24216570

15. Coburn JA, Vande Voort JL, Lahr BD, Van Dyke CT, Kroning CM, Wu TT et al. *Human leukocyte antigen genetics and clinical features of self-treated patients on a gluten-free.* J Clin Gastroenterol. 2013; 47: 828-33.

http://dx.doi.org/10.1097/MCG.0b013e31828f531c
PMid:23632357 PMCid:PMC3735773

16. Tanpowpong P, Broder-Fingert S, Katz AJ, Camargo CA Jr. *Predictors of gluten avoidance and implementation of a gluten-free diet in children and adolescents without confirmed celiac disease.* J Pediatr. 2012; 161: 471-5.

http://dx.doi.org/10.1016/j.jpeds.2012.02.049
PMid:22484356

17. Biesiekierski JR, Mewnham ED, Shepherd SJ, Muir JG, Gibson PR. *Characterization of adults with a self-diagnosis of nonceliac gluten sensitivity.* Nutr Clin Pract. 2014; 29: 504-9.

PMid: 24740495

18. Francavilla R, Cristofori F, Castellaneta S, Polloni C, Albano V, Dellatte S et al. *Clinical, serological, and histologic features of gluten sensitivity in children.* J Pediatr. 2014; 164: 463-7.

http://dx.doi.org/10.1016/j.jpeds.2013.10.007
PMid:24252792

19. Massari S, Liso M, De Santis L, Mazzei F, Carlone A, Mauro S et al. *Occurrence of nonceliac gluten sensitivity in patients with allergic disease.* Int Arch Allergy Immunol. 2011; 155: 389-94.
http://dx.doi.org/10.1159/000321196
PMid:21346369

20. Carroccio A, Mansueto P, Iacono G, Soresi M, D'Alcamo A, Cavataio F et al. *Non-celiac wheat sensitivity diagnosed by double-blind placebo-controlled challenge: exploring a new clinical entity.* Am J Gastroenterol. 2012; 107: 1898-906.
http://dx.doi.org/10.1038/ajg.2012.236
PMid:22825366

21. Volta U, Tovoli F, Cicola R, Parisi C, Fabbri A, Piscaglia M et al. *Serological tests in gluten sensitivity (nonceliac gluten intolerance).* J Clin Gastroenterol. 2012; 46: 680-5.
http://dx.doi.org/10.1097/MCG.0b013e3182372541
PMid:22138844

22. Aziz I, Lewis NR, Hadjivassiliou M, Winfield SN, Rugg N, Kelsall A et al. *A UK study assessing the population prevalence of self-reported gluten sensitivity and referral characteristics to secondary care.* Eur J Gastroenterol Hepatol. 2014; 26: 33-9.
http://dx.doi.org/10.1097/01.meg.0000435546.87251.f7
PMid:24216570

23. Isasi C, Colmenero I, Casco F, Tejerina E, Fernandez N, Serrano-Vela JI et al. *Fibromyalgia and non-celiac gluten sensitivity: a description with remission of fibromyalgia.* Rheumatol Int. 2014; 34: 1607-12.
http://dx.doi.org/10.1007/s00296-014-2990-6
PMid:24728027 PMCid:PMC4209093

24. Kabbani TA, Vanga RR, Leffler DA, Villafuerte-Galvez J, Pallav K, Hansen J et al. *Celiac Disease or Non-Celiac Gluten Sensitivity? An Approach to Clinical Differential Diagnosis.* Am J Gastroenterol. 2014; 109: 741-6.
http://dx.doi.org/10.1038/ajg.2014.41
PMid:24619056

25. Van de Voort JL, Murray JA, Lahr BD, Van Dyke CT, Kroning CM, Moore SB et al. *Lymphocytic duodenosis and the spectrum of celiac disease.* Am J Gastroenterol. 2009; 104: 142-8.
http://dx.doi.org/10.1038/ajg.2008.7
PMid:19098862 PMCid:PMC2608723

26. Aziz I, Evans KE, Hopper AD, Smillie DM, Sanders DS. *A prospective study into the aetiology of lymphocytic duodenosis.* Aliment Pharmacol Ther. 2010; 32: 1392-7.
http://dx.doi.org/10.1111/j.1365-2036.2010.04477.x
PMid:21050242

27. Rosinach M, Esteve M, González C, Temiño R, Mariné M, Monzón H et al. *Lymphocytic duodenosis: aetiology and long-term response to specific treatment.* Dig Liver Dis. 2012; 44: 643-8.

http://dx.doi.org/10.1016/j.dld.2012.03.006
PMid:22497904

28. Santolaria S, Dominguez M, Alcedo J, Abascal M, Garcia-Prats MD, Marigil M et al. *Lymphocytic duodenosis: etiological study and clinical presentations.* Gastroenterol Hepatol. 2013; 36: 565-73.

http://dx.doi.org/10.1016/j.gastrohep.2013.06.003
PMid:24007857

29. Esteve M, Rosinach M, Fernandez-Banares F, Farre C, Salas A, Alsina M et al. *Spectrum of gluten-sensitive enteropathy in first-degree relatives of patients with coeliac disease: clinical relevance of lymphocytic enteritis.* Gut. 2006; 55: 1739-45.

http://dx.doi.org/10.1136/gut.2006.095299
PMid:16709658 PMCid:PMC1856469

30. Zanini B, Caselani F, Magni A, Turini D, Ferraresi A, Lanzarotto F, Villanacci V, Carabellese N, Ricci C, Lanzini A. *Celiac disease with mild enteropathy is not mild disease.* Clin Gastroenterol Hepatol. 2013; 11: 253-8.

http://dx.doi.org/10.1016/j.cgh.2012.09.027
PMid:23022697

31. Kurppa K, Collin P, Viljamaa M, Haimila K, Saavalainen P, Partanen J et al. *Diagnosing Mild Enteropathy Celiac Disease: A Randomized, Controlled Clinical Study.* Gastroenterology. 2009; 136: 816-23.

http://dx.doi.org/10.1053/j.gastro.2008.11.040
PMid:19111551

32. Tursi A, Brandimarte G. *The symptomatic and histologic response to a gluten-free diet in patients with borderline enteropathy.* J Clin Gastroenterol. 2003; 36: 13-7.

http://dx.doi.org/10.1097/00004836-200301000-00006
PMid:12488700

33. Molina-Infante J, Santolaria S, Sanders DS, Fernandez-Bañares F. *Systematic review: noncoeliac gluten sensitivity.* Aliment Pharmacol Ther. 2015; 41: 807-20.

http://dx.doi.org/10.1111/apt.13155
PMid:25753138

34. Husby S, Murray JA. *Gluten sensitivity: celiac lite versus celiac like.* J Pediatr. 2014; 164: 436-8.

http://dx.doi.org/10.1016/j.jpeds.2013.11.024
PMid:24411520

35. Wahnschaffe U, Ullrich R, Riecken EO, Schulzke JD. *Celiac disease-like abnormalities in a subgroup of patients with irritable bowel syndrome.* Gastroenterology. 2001; 121: 1329-38.
http://dx.doi.org/10.1053/gast.2001.29572
PMid:11729112

36. Molina-Infante J, Santolaria S, Fernandez-Bañares F, Montoro M, Esteve M. *Lymphocytic enteropathy, HLA DQ2/DQ8 genotype and wheat-dependent symptoms: non-celiac wheat hypersensitivy or Marsh I celiac disease?* Am J Gastroenterol. 2013; 108: 451.
http://dx.doi.org/10.1038/ajg.2012.433
PMid:23459052

37. Carroccio A, Mansueto P. *Response to Molina-Infante et al.* Am J Gastroenterol. 2013; 108: 451-2.
http://dx.doi.org/10.1038/ajg.2012.435
PMid:23459051

38. Sapone A, Lammers KM, Mazzarella G, Mikhailenko I, Carteni M, Casolaro V et al. *Differential mucosal IL-17 expression in two gliadin-induced disorders: gluten sensitivity and the autoimmune enteropathy celiac disease.* Int Arch Allergy Immunol. 2010; 152: 75-80.
http://dx.doi.org/10.1159/000260087
PMid:19940509 PMCid:PMC2956008

39. Sapone A, Lammers KM, Casolaro V, Cammarota M, Giuliano MT, De Rosa M et al. *Divergence of gut permeability and mucosal immune gene expression in two gluten-associated conditions: celiac disease and gluten sensitivity.* BMC Med. 2011; 9: 23.
http://dx.doi.org/10.1186/1741-7015-9-23
PMid:21392369 PMCid:PMC3065425

40. Brottveit M, Beitnes AC, Tollefsen S, Bratlie JE, Jahnsen FL, Johansen FE et al. *Mucosal cytokine response after short-term gluten challenge in celiac disease and non-celiac gluten sensitivity.* Am J Gastroenterol. 2013; 108: 842-50.
http://dx.doi.org/10.1038/ajg.2013.91
PMid:23588237

41. Bucci C, Zingone F, Russo I, Morra I, Tortora R, Pogna N et al. *Gliadin Does Not Induce Mucosal Inflammation or Basophil Activation in Patients with Non-Celiac Gluten Sensitivity.* Clin Gastroenterol Hepatol. 2013; 11: 1294-9.
http://dx.doi.org/10.1016/j.cgh.2013.04.022
PMid:23639603

42. Biesiekierski JR, Muir JG, Gibson PR. *Is Gluten a Cause of Gastrointestinal Symptoms in People Without Celiac Disease?* Curr Allergy Asthma Rep. 2013; 13: 631-8.
http://dx.doi.org/10.1007/s11882-013-0386-4
PMid:24026574

43. Staudacher HM, Irving MN, Lme MC, Whelan K. *Mechanisms and efficacy of dietary FODMAP restriction in IBS.* Nat Rev Gastroenterol Hepatol. 2014; 11: 256-66.

http://dx.doi.org/10.1038/nrgastro.2013.259
PMid:24445613

44. Vazquez-Roque MI, Camilleri M, Smyrk T, Murray JA, Marietta E, O'Neill J et al. *A controlled trial of gluten-free diet in patients with irritable bowel syndrome-diarrhea: effects on bowel frequency and intestinal function.* Gastroenterology. 2013; 144: 903-11.

http://dx.doi.org/10.1053/j.gastro.2013.01.049
PMid:23357715 PMCid:PMC3633663

45. Bernardo D, Garrote JA, Fernandez-Salazar L, Riestra S, Arranz E. *Is gliadin really safe for non-coeliac individuals? Production of interleukin 15 in biopsy culture from non-coeliac individuals challenged with gliadin peptides.* Gut. 2007; 56: 889-90.

http://dx.doi.org/10.1136/gut.2006.118265
PMid:17519496 PMCid:PMC1954879

46. Beckett CG, Dell'Olio D, Shidrawi RG, Rosen-Bronson S, Ciclitira PJ. *Gluten-induced nitric oxide and pro-inflammatory cytokine release by cultured coeliac small intestinal biopsies.* Eur J Gastroenterol Hepatol. 1999; 11: 529-35.

http://dx.doi.org/10.1097/00042737-199905000-00011
PMid:10755257

47. Verdu EF, Huang X, Natividad J, Lu J, Blennerhassett PA, David CS et al. *Gliadin-dependent neuromuscular and epithelial secretory responses in gluten-sensitive HLA-DQ8 transgenic mice.* Am J Physiol Gastrointest Liver Physiol. 2008; 294: G217-25.

http://dx.doi.org/10.1152/ajpgi.00225.2007
PMid:18006603

48. Fernandez-Banares F, Esteve M, Viver JM. *Fructose-sorbitol malabsorption.* Curr Gastroenterol Rep. 2009; 11: 368-74.

http://dx.doi.org/10.1007/s11894-009-0056-9
PMid:19765364

49. Bernardo D, Garrote JA, Arranz E. *Are non-celiac disease gluten-intolerant patients innate immunity responders to gluten?* Am J Gastroenterol. 2011; 106: 2201; author reply 2201-2.

http://dx.doi.org/10.1038/ajg.2011.297
PMid:22138947

50. Junker Y, Zeissig S, Kim SJ, Barisani D, Wieser H, Leffler DA et al. *Wheat amylase trypsin inhibitors drive intestinal inflammation via activation of toll-like receptor 4.* J Exp Med. 2012; 209: 2395-408.

http://dx.doi.org/10.1084/jem.20102660
PMid:23209313 PMCid:PMC3526354

51. Biesiekierski JR, Newnham ED, Irving PM, Barrett JS, Haines M, Doecke JD et al. *Gluten causes gastrointestinal symptoms in subjects without celiac disease: a double-blind randomized placebo-controlled trial.* Am J Gastroenterol. 2011; 106: 508-14.

http://dx.doi.org/10.1038/ajg.2010.487
PMid:21224837

52. Biesiekierski JR, Peters SL, Newnham ED, Rosella O, Muir JG, Gibson PR. *No effects of gluten in patients with self-reported non-celiac gluten sensitivity after dietary reduction of fermentable, poorly absorbed, short-chain carbohydrates.* Gastroenterology. 2013; 145: 320-8.

http://dx.doi.org/10.1053/j.gastro.2013.04.051
PMid:23648697

53. Peters SL, Biesiekierski JR, Yelland GW, Muir JG, Gibson PR. *Randomised clinical trial: gluten may cause depression in subjects with non-coeliac gluten sensitivity: an exploratory randomised clinical study.* Aliment Pharmacol Ther. 2014; 39: 1104-12.

http://dx.doi.org/10.1053/j.gastro.2013.04.051
PMid:23648697

54. Zanini B, Marrullo M, Ricci C, Lanzarotto F, Lanzini A. *Non Celiac Gluten Sensitivity (NCGS) Is Outnumbered by FODMAPs Sensitivity in Patients Spontaneously Adhering to Gluten Free Diet (GFD): A Two Stage Double Blind Prospective Study.* Gastroenterology. 2014; 146(Suppl 1): S-348.

http://dx.doi.org/10.1016/S0016-5085(14)61258-0

55. Di Sabatino A, Corazza GR. *Nonceliac gluten sensitivity: sense or sensibility?* Ann Intern Med. 2012; 156: 309-11.

http://dx.doi.org/10.7326/0003-4819-156-4-201202210-00010
PMid:22351716

56. Sanders DS, Aziz IM. *Non-celiac wheat sensitivity: separating the wheat from the chat!* Am J Gastroenterol. 2012; 107: 1908-12.

http://dx.doi.org/10.1038/ajg.2012.344
PMid:23211856

Chapter 14

Wheat as an Allergen: Baker's Asthma, Food and Wheat Pollen Allergy

Alicia Armentia[1], Eduardo Arranz[2], José Antonio Garrote[2,3], Javier Santos[4]

[1] Allergy Unit. Rio Hortega University Hospital. Valladolid. Spain.

[2] IBGM. University of Valladolid-CSIC. Valladolid. Spain.

[3] Clinical Laboratory Department. Rio Hortega University Hospital. Valladolid. Spain.

[4] Gastroenterology Department. Rio Hortega University Hospital. Valladolid. Spain.

aliciaarmentia@gmail.com, earranz@med.uva.es, jagarrote@saludcastillayleon.es, javiersantos_88@hotmail.com

Doi: http://dx.doi.org/10.3926/oms.262

How to cite this chapter

Armentia A, Arranz E, Garrote JA, Santos J. *Wheat as an Allergen: Baker's Asthma, Food and Wheat Pollen Allergy*. In Arranz E, Fernández-Bañares F, Rosell CM, Rodrigo L, Peña AS, editors. *Advances in the Understanding of Gluten Related Pathology and the Evolution of Gluten-Free Foods*. Barcelona, Spain: OmniaScience; 2015. p. 463-488.

Abstract

Food incompatibilities affect approximately 20% of the population and can be caused by allergy. Many plant proteins act as sensitizing agents in humans upon repeated exposure. Wheat is a prominent allergen source and is one of the causes of baker's asthma, food and pollen allergy. On the basis of differential solubility, wheat grain proteins have been classified as salt-soluble albumins and gluten fraction or prolamins, which include gliadins and glutenins. Both proteins sources have been implicated in the development of wheat hypersensitivity.

During the past years we have purified and characterized several proteins from wheat, barley and rye, which are associated with flour allergy. These allergens have a potential role as a biological defense against the insect infestation of the grain.

Until recently gluten intolerance has been has been considered to be typical of celiac disease and wheat allergy. In the last the last few years, new digestive syndromes has been described. A new syndrome has been named non-celiac gluten sensitivity (NCGS) and eosinophilic esophagitis can be also due to wheat ingestion.

The introduction of microarray techniques featuring a large panel of purified allergens has been a major advance in the diagnosis of allergic diseases. However, this technique has been hardly applied to the diagnosis and characterization of patients with occupational asthma due to wheat allergy.

Here, we described these investigations, the most important pathologies associated with wheat, their prevention and treatment.

Keywords

Baker's asthma, wheat allergy, wheat pollen allergy, non-celiac gluten sensitivity, Eosinophilic esophagitis, lipid transfer protein, Wheat alpha amylase inhibitors, microarrays, component resolved diagnosis.

Abbreviations

IgE: antibodies E,

IgG_4: antibodies IgG_4,

IL10: Interleukin 10,

ATG: Anti-transglutaminase antibody,

AGA: Anti-gliadin antibody,

ARA: Antireticulin antibody,

AEA: anti-endomisyal antibody,

LTP: Non-specific lipid transfer protein,

CM3: Wheat tetrameric alpha amylase inhibitor subunit,

ELISA: Enzymo-linked-immuno-assay,

NCGS: non-celiac gluten sensitivity,

EoE: Eosinophilic oesophagitis,

LTP: Lipid transfer proteins,

Tri a a 19: Wheat LTP.

This manuscript is dedicated to professor Raphaël Panzani, in memoriam.

Raphaël Panzani was born August 1921 in Marseille. He received his MD degree at the age of 25. He became interested in allergy at the beginning of his long career. In 1966 he received a Fullbright scholarship award in recognition for his distinguished work on asthma. Raphaël pursued his research investigations, in close collaboration with many of his colleagues, until the very end of his life with great devotion and boundless enthusiasm. Raphaël was fond of nature, expert on Roman culture and in history and literature. He identified with Shakespeare and Dante and embraced the philosophy of Seneca. He was a great sportsman and an exemplary family man. For all of us who knew him, we will miss him immensely and will always remember him as a friend and a scientific role model.

1. Introduction

1.1. Allergy to Wheat and Related Diseases

Wheat is a potent allergen source and is one of the causes of baker's asthma, food and pollen allergy[1]. The prevalence of wheat flour allergy is increasing, ranging from 2 to 3.6%, depending on diagnostic methods and geographic areas[2]. Wheat is also one of the most frequent allergenic foods associated with food-dependent exercise-induced anaphylaxis[3]. Another type of wheat IgE-mediated hypersensitivity is baker's asthma, an important occupational disease, caused by inhalation of wheat and other cereals flours[4] (Figure 1). In our data base of 22.726 allergic patients, baker's asthma caused 8.20% of allergic asthma and the 52.5% of the occupational asthma diagnosed, but we works in an area were the jobs related with cereals are very important. Nowadays, the prevalence of wheat pollen allergy is not known although the sensitization to grass pollen is estimated in 38.6% in United Kingdom and 33.6% in EEUU[1].

Common symptoms of food wheat allergy can begin within a few minutes after eating, or they can start a few hours after. Symptoms often involve the skin and include reactions such as rashes, swelling around the mouth, hives, and eczema. Also, symptoms can typically involve the intestines and might include diarrhea, vomiting, nausea, indigestion, and stomach and abdominal cramps.

Figure 1. Baker's asthma is an important occupational disease, caused by inhalation of wheat and other cereals flours.

The most severe allergy response, anaphylaxis, is a severe reaction involving major body systems.

Conventional medical advice in dealing with food related allergy is to avoid the substance people are sensitive to (Table 1). But since wheat is as important element in all diets, avoidance becomes a major ordeal. This type of avoidance diet severely limits the selection of foods. Wheat is quite difficult to avoid. Aside from being an ingredient in many foods, it is a substance that is also present in medical tablets. It is also used as a glaze and thickener, a stabilizer, a bulking agent, an emulsifier, a binder, and a starch. Is very useful to have information on wheat substitutes (Table 2), and ever read labels (Table 3).

Table 1. General guidelines for wheat allergy.

Foods	Allowed	Not allowed
Beverages	Coffee, tea, fruit juices, decaffeinated coffee, carbonated beverages, all milks, cocoa	Cereal beverages, coffee substitutes Beverages made from wheat products: beer, ale, root beer Instant chocolate drink mixes
Breads & Cereals	Ry-Krisp, rice wafers Pure corn, rice, arrowroot, barley, potato, or rye bread made without wheat flour or wheat products Cornmeal, cornstarch, soybean flour, barley flour, oat flour, rice flour, potato starch, arrowroot flour Oatmeal, cream of rice, puffed rice, or other cereals made from pure corn, oats, or rice to which no wheat has been added	Whole wheat, enriched, or white bread, rolls, or bread crumbs Graham or gluten bread Donuts, sweet rolls, muffins, french toast, waffles, pancakes, dumplings, bread stuffing, rusk, popovers Prepared mixes for pancakes, waffles, biscuits, breads, and rolls Cornbread, potato, or soybean bread unless made without wheat flour or wheat products Cereals made from farina, wheat, or those with wheat products or malt added Pretzels, crackers
Desserts	Custards, Bavarian creams Oatmeal, arrowroot, rice, or rye cookies made without wheat products Cornstarch, tapioca, or rice puddings Water or fruit ices, meringues, gelatin	Cakes, pastries, commercial frosting, icing, ice cream, sherbet, ice cream cones Cookies, prepared mixes, or packaged pudding containing wheat flour Graham crackers, donuts

Foods	Allowed	Not allowed
Eggs	Eggs prepared any way without wheat products	Souffles or creamed eggs made with wheat products
Fats	Butter, margarine, animal, or vegetable fats and oils, cream Salad dressings or gravy prepared without wheat flour or products	Any salad dressing thickened or gravy with wheat flour or products
Fruit	All fresh, canned, dried, or frozen fruits and fruit juices	Strained fruits with added cereals
Meat, Fish, Poultry	Baked, broiled, boiled, roasted or fried: beef, veal, pork, ham, chicken, turkey, lamb, or fish "All meat" wieners or luncheon meats prepared without wheat flour fillers or wheat products	All breaded or floured meats, meats containing filler such as meatloaf, frankfurters, sausage, luncheon meats, bologna, or prepared meat patties
Milk & Milk Products	Milk, buttermilk, yogurt, cheese, some cottage cheese	Malted milk, milk drink containing powdered wheat cereal or products Cottage cheese with modified starch or other wheat containing ingredients
Potatoes & Substitutes	White and sweet potatoes Rice	Scalloped potatoes Noodles, spaghetti, macaroni, and other pasta products at semolina
Soup	Clear bouillon, consommé, or broth Homemade soups made without wheat products	Cream soups unless made without wheat flour Soups with noodles, alphabets, dumplings, or spaghetti Soup thickened with wheat flour
Sweets	Corn syrup, honey, jams, jellies, molasses, sugar	Chocolates, chocolate candy containing malt, candy with cereal extract
Vegetables	All fresh, frozen, or canned vegetables, and vegetable juices	Vegetables combined with wheat products Breaded or floured vegetables
Miscellaneous	Salt, chili powder, condiments, flavoring extracts, herbs, nuts, olives, pickles, popcorn, peanut butter	Malt products, Worcestershire sauce, gravies thickened with wheat flour Monosodium glutamate (MSG), meat tenderizers containing MSG, prepared oriental food seasoned with MSG, soy sauce

A wheat allergy is an abnormal response of the body to the protein found in wheat. Wheat products are found in many foods. In order to avoid foods that contain wheat, it is important to read food labels.

Table 2. Information for using wheat substitutes.

1-cup wheat flour equals:
• 1 cup rye meal • 1 to 1 1/4 cups rye flour • 1 cup potato flour • 1 1/3 cups rolled oats or oat flour • 1/2 cup potato four plus 1/2 cup rye flour • 5/8 cup potato starch • 5/8 cup rice flour plus 1/3 cup rye flour

Table 3. Other possible sources of wheat or wheat products.

How to read a label for a wheat-free diet	
Be sure to avoid foods that contain any of the following ingredients:	
• bread crumbs • bran • cereal extract • couscous • cracker meal • enriched flour • farina • gluten • graham flour • high gluten flour	• high protein flour • spelt • vital gluten • wheat bran • wheat germ • wheat gluten • wheat malt • wheat starch • whole wheat flour
Ingredients that may indicate the presence of wheat protein include the following:	
• gelatinized starch • hydrolyzed vegetable protein • kamut • modified food starch • modified starch	• natural flavoring • soy sauce • starch vegetable • gum • vegetable starch

In this chapter we review the allergenic power among wheat proteins, the changes in allergenic properties of wheat induce by heat and industrial processing, the allergenic cross-reactivity between cereals, pollens and other vegetal foods, the possible reason why patients with baker's asthma due to wheat tolerate wheat flour ingestion, the relationship between wheat allergy and grass pollen asthma, the relationship between cereals in diet and allergic digestive symptoms and the mechanisms of immune tolerance to cereals.

Finally we summarized the new trends in diagnosis (component resolved diagnosis) and therapy of wheat allergy.

2. Allergenic Power Of Wheat Proteins

On the basis of differential solubility, wheat grain proteins have been classified[5]:

Water/salt-soluble albumins and globulins, mainly structural proteins and metabolically enzymes, such as α-amylases and their inhibitors and they are implicated in allergy respiratory symptoms.

The water/salt insoluble gliadins and glutenins, together known as prolamins or gluten, are the major storage proteins of the wheat grain, associated to other clinical expression of allergy.

Among salt soluble proteins, members of the α-amylase inhibitor family seem to be the most important allergens responsible for baker's asthma[6,7]. These allergens have a potential role as a biological defense against the insect infestation of the grain (Figure 2). They have also been described as wheat food allergens[8,9]. Other salt-soluble proteins, such as peroxidase and non-specific lipid transfer protein (LTP), have been implicated in allergy to wheat, both by inhalation and ingestion[9-11]. Gliadins are involved mainly in IgE-mediated reactions to ingested wheat[9-12] and recently in baker's asthma as well[13]. Wheat allergy can cause only digestive symptoms in children and adults although the real prevalence has not been published yet. These patients may be misdiagnosed as suffering from irritable bowel syndrome (IBS).

In celiac disease, limited information is available regarding cereal allergens responsible for allergic reactions, although both diseases can affect at the same patient[14,15]. Celiac disease is a lifelong intolerance to the gluten found in wheat, barley and rye, genetically determined as in allergic diseases. Of the patients with celiac disease 95% are human leucocyte antigen (HLA-DQ2 or HLA-DQ8 positive). Characteristically, the jejunal mucosa becomes damaged by a T-cell-mediated autoimmune response that is thought to be initiated by a 33-mer peptide fragment in A2 gliadin, and patients with this disorder have raised levels of anti-endomysium (AEA) and tissue transglutaminase antibodies (tTG) in blood samples. This disease is the major diagnosable food intolerance and, with the event of a simple blood test for case finding, prevalence rates are thought to be approximately 1:100[15].

Figure 2. Members of the α-inhibitor family of wheat are proteases with have a potential role as a biological defence against the insect infestation of the grain. In the figure Eurygaster austriaca *(left) and* Tenebrio mollitor *(right). The first is a frequent pest of wheat and the second parasite the barley.*

The allergenic reactivity of ingested and inhaled cereal allergens in allergic and celiac people was recently studied by our group. Allergic sensitisation IgE mediated to cereals may be observed in celiac children. Inhalation and ingestion routes causing cereal allergy seem to involve similar allergens, but,

in celiac disease specific response to the α-amylase inhibitor CM3 may be important[15].

There is no doubt that the intestinal mucosa can be involved in food allergy. However, food allergy-induce ulcerative colitis has been questioned[16]. Specific IgEs to foods are more frequent in patients with inflammatory bowel disease than in healthy subjects, but this is probably due to a greater absorption of antigens. Patients with positive colic intramucosal provocation tests with food, have been cured by exclusion of the offending food. Moreover, the specific treatment of ulcerative colitis was suspended[17].

Until gluten intolerance has been believed to be typical of celiac disease and wheat allergy the last few years, new digestive syndromes has been described. Two new syndromes have been named non-celiac gluten sensitivity (NCGS) and eosinophilic esophagitis (EE) due to wheat ingestion. NCGS has been included in the new list of gluten-related disorders published in 2012[18-21]. In other chapter of this book Molina-Infante and cols. review different aspects of epidemiology, diagnosis and dietary interventions in NCGS.

Recent evidence shows that a personal history of food allergy in infancy, coexistent atopy, positive for immunoglobulin G (IgG) antigliadin antibodies and flow cytometric basophil activation test, with wheat and duodenal and/or ileum-colon intraepithelial and lamina propria eosinophil counts, could be useful to identify a subgroup of NCGS patients with characteristics pointing to food allergy[20]. Nevertheless, we require a better understanding of the clinical presentation of NCGS as well as on its pathogenesis, epidemiology, and management.

On the other hand, the role of wheat in conditions like IBS is not clear. A paradigm shift has led to focus on the relationship between diet, that restricts a group of short-chain carbohydrates known collectively as fermentable oligosaccharides, disaccharides, monosaccharides and polyols (FODMAPs), and the pathophysiological mechanisms in IBS such as effects on intestinal microbiota, inflammation, motility, permeability and visceral hypersensitivity. Carroccio et al carried out in vitro basophil activation tests (BAT) for gluten- and cow's milk protein sensitivity in IBS-like patients. The BAT based on

CD63 detection on whole blood samples did not work in the diagnosis of food hypersensitivity diagnosis and showed a significant lower sensitivity, specificity and diagnostic accuracy that the assay based on separated leukocytes[21].

EE is a disorder characterized by esophageal dysfunction and, histologically, by eosinophilic inflammation. Although treatment, which include dilatation, oral corticosteroids and restrictive diets, is often effective, choosing the foods to be eliminated from the diet is difficult. Actual treatment includes proton pump inhibitor therapy and the Six-Food- Elimination Diet (SFDE) that include wheat. This empirical elimination sometimes is effective, but requires multiple control endoscopies and can significantly hinder quality of life. A definitive aetiological diagnosis would be fundamental in determining the specific allergens which cause eosinophilic inflammation of the oesophageal mucosa and which foods should be avoided[22]. Very recently, component resolved diagnostic by microarray allergen assay have been applied in detecting allergens that might be involved in the inflammatory process. The predominant allergens in EE patients were pollen enzymes and among foods allergens lipid transfer proteins (LTP) of peach and nuts. LTP from wheat Tria 19, were detected in only few patients[23].

3. Changes in Allergenic Properties of Wheat Induced By Heat and Industrial Processing

As we comment before, salt-soluble proteins from wheat flour have been described as main allergens associated with both baker's asthma and food allergy. However, most studies have used raw flour as starting material, thus not considering potential changes in allergenic properties induced by the heat treatment and other industrial processing to produce wheat-derived foodstuffs. Salt extracts from different commercial wheat-derived products were obtained and their allergenic properties investigated by IgE-immunedetection, ELISA assays, and skin prick test (Figure 3)[24]. The IgE-binding capacity of salt-soluble proteins from commercial breads and

cooked pastas was reduced around 50% compared with that of raw flour, the reduction being less dramatic in no-ncooked pastas and biscuits. Several wheat-derived foodstuffs showed major IgE-binding components of 20 and 35 kDa, identified as avenin-like and globulin proteins, respectively. These proteins, as well as most flour and bread salt-soluble proteins, were hydrolyzed when subjected to simulated gastrointestinal digestion. However, the digested products still exhibited a residual IgE-binding capacity in SDS-PAGE- immunedetection.

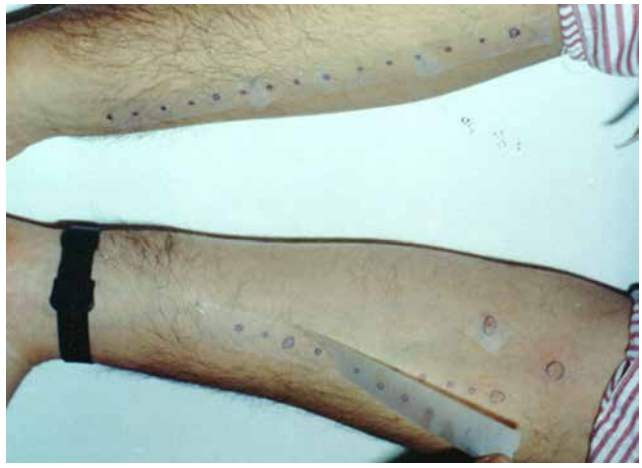


Figure 3. Prick tests with purified proteins from wheat.

Therefore, processing of wheat flour to obtain derived foodstuffs decreases the IgE binding-capacity of the major salt-soluble wheat proteins. Moreover, simulated gastric fluid digestion further inactivates some heat-resistant IgE-binding proteins[25].

4. Allergen Cross-Reactivity Between Cereals, Pollen and Other Vegetal Foods

Many plant proteins, particularly those found in foods and pollen, are known to act as sensitizing agents in humans upon repeated exposure. Among the cereal flour proteins involved in asthmatic reactions, those members of the alpha-amylase inhibitor family which are glycosylated, polypeptides, BMAI-1, BTAI-CMb*, and WTAI-CM16* are particularly reactive both in vivo and in vitro. These major glycoprotein allergens carry a single asparagine-linked complex glycan that contains both beta 1-->2 xylose and alpha 1-->3 fucose. These residues (xilose and fucose) are key IgE-binding epitopes and largely responsible for the allergenicity of these and unrelated proteins from plants and insects (Figure 3). Our results[26], suggested that the involvement of xylose- and fucose-containing complex glycans in allergenic responses may have been underestimated previously; these glycans provide a structural basis to help explain the cross-reactivities often observed between pollen, vegetable food, and insect allergens.

Baker's asthma is a frequent occupational allergic disorder mainly caused by inhalation of cereal flours. Lipid transfer proteins (LTPs) constitute a family of plant food panallergens, but their role as inhalant and wheat allergens is still unclear. We sought to explore the involvement of wheat LTPs in baker's asthma caused by wheat flour sensitization[27]: Forty patients with occupational asthma caused by wheat flour inhalation were studied. Wheat LTP, Tri a 14, was purified by using a 2-step chromatographic protocol and characterized by N-terminal amino acid sequencing and 3-dimensional modeling. Its reactivity was confirmed by means of IgE immunedetection, ELISA and ELISA-inhibition assays, and skin prick tests. Specific IgE to Tri a 14 was found in 60% of 40 individual sera from patients with baker's asthma, and the purified allergen elicited positive skin prick test reactions in 62% of 24 of these patients. Tri a 14 and peach LTP, Pru p 3, showed a sequence identity of 45%, but the low cross-reactivity between both allergens detected in several individual sera reflected great differences in their 3-dimensional IgE-binding regions.

Wheat LTP is a major inhalant allergen associated with baker's asthma caused by wheat flour sensitization. Poor cross-reactivity with its peach homolog was found in some patients. LTPs can be considered relevant inhalant allergens linked to respiratory disorders. LTP from wheat (Tri a 14) can be used as a helpful tool for the diagnosis of baker's asthma.

Peach non-specific lipid transfer protein (Pru p 3; nsLTP) has been characterized as the major food allergen in the adult Mediterranean population. Its wheat homologous protein, Tri a 14 has a relevant inhalant allergen in occupational baker's asthma. Different sensitization patterns to these allergens have been found in patients with this latter disorder[28]. Cross-reactivity between grass-pollen, cereals flours and fruits belong to Rosaceae family (Figure 4) are very common in patients allergic to pollen. Sensitization only to a LTP from peach can be associated with more severe symptoms like anaphylaxis.

Figure 4. Cross-reactivity between grass-pollen, cereals flours and fruits belong to Rosaceae family are very common in patients allergic to pollen.

5. Why Patients With Baker's Asthma Due to Wheat Tolerate Wheat Flour Ingestion?

Wheat is a potent allergen source and is one of the causes of baker's asthma, food and pollen allergy. Recently, we have performed a study on pollen sensitization in our area, where cereal crops are very important[30]. The clinical data from 19718 patients reviewed showed that grass pollen was the main source of clinical symptoms (6369 patients, 32.30% of asthmatics). However, wheat and cereal crop pollen showed very low prevalence. On the other hand, patients with wheat flour allergy after ingestion and/or with baker's asthma were not sensitized to wheat pollen, despite it containing some common allergens. In the same way, all our asthmatic bakers (135 patients) tolerated the ingestion of bread. The reason of these surprising observations was difficult to explain.

The most of patients with baker's asthma in different surveys[4,31] did not present wheat food allergy. A different via of sensitization (inhalation versus ingestion) and allergenic source (wheat flour versus wheat processed foodstuffs) could explain this fact, despite some wheat allergens, like α-amylase inhibitors or lipid transfer proteins are implicated in both types of allergy[7,8] and in some cases of celiac disease as we have demonstrated before[15].

We have shown already that the allergenicity of foods could be modified by heat and other treatment. Most of studies on wheat food allergy have been performed with raw flour, although raw wheat flour is rarely consumed. Thus, the effect of heat treatment during processing or cooking did not be taken into account on the IgE-binding capacity of potential wheat allergens. Recently, using extracts from wheat-derived foodstuffs (French bread, wholemeal tin loaf bread, toasted bread, pasta, biscuits, pizza, baby cereal food and breakfast cereals), we have found that this processing of these foods seems to decrease strongly the IgE binding capacity of the major salt-soluble proteins. Moreover, the simulated gastric fluid digestion could further inactivate some heat-resistant potential allergens[24]

On the other hand, bakers can usually eat bread and wheat-derived foodstuffs during all their life without problems. Their symptoms begin with the inhalation of wheat flour probably due to a change in target immune receptors. IgE sensitization to soy and wheat are classified as "primary" when it is generated by food ingestion and as "secondary" when it is a consequence of primary sensitization to cross-reacting pollen antigens via inhalation. In a German multi-centre longitudinal study, in which 1314 children were followed from birth to age 13, IgE sensitization to wheat and soy were so uncommon. In the early infancy, the type of sensitization was mostly primary, while they were secondary at school age[9]. In our patients, wheat flour sensitization did not seem secondary to wheat pollen inhalation. Perhaps, the cereal ingestion may be acting like an oral mechanism of tolerance, similarly to oral immunotherapy. In a study on tolerance mechanisms in response to antigens responsible for baker's, we found that the presence of higher levels of IgG_4, IL10 and sub-clinic grass-pollen sensitization may have helped to develop a kind of natural hyposensitization[32].

High pollen exposure is not always associated with more severe allergic conditions. Researches working on the relationship between diet and allergic asthma showed conflictive results[33-37]. Serological studies using micro-arrayed wheat seed and grass pollen allergens for the discrimination of baker's asthma, wheat-induced food allergy and grass pollen would be very useful[33]. We will explain the usefulness of component resolved diagnostic later.

6. Relationship Between Wheat Allergy and Asthma

We have previously demonstrated that allergy after ingestion or inhalation of cereals involves similar allergens in different ages[29]. The aim of a new study was to evaluate the allergenic reactivity of ingested and inhaled cereal allergens in different ages, in order to investigate if the response to different allergens would depend on the sensitization route.

We included 66 patients in three groups. Group 1: 40 children aged 3 to 6 months who suffered from diarrhea, vomiting, eczema or weight loss after the

introduction of cereal formula in their diet and in which a possibility of celiac disease was discarded. Group 2: 18 adults with food allergy due to cereals by prick tests, specific IgE and food challenge. Group 3: eight patients previously diagnosed as having baker's asthma. Sera pool samples were collected from each group of patients and IgE immunoblotting was performed.

We found an important sensitization to cereal in the 40 children. The most important allergens were wheat followed by barley and rye. Among the adults with cereal allergy, sensitization to other allergens was common, especially to *Lolium perenne* (rye grass) pollen. Immunodetection showed similar allergenic detection in the three groups.

In summary, clinically significant reactivity to cereal may be observed in early life. Inhalation and ingestion routes causing cereal allergy seem to involve similar allergens. Therefore, the possibility of cereal allergy after the introduction of cereal formula during the lactation period should not be underestimated. In order to investigate this possible risk factor, we performed another study.

The prevalence of asthma has increased from the last 30 years. The relationship between diet and asthma is an area of controversy that has never been fully evaluated. Attempts at dietary prevention of asthma have produced conflicting results.

We identified allergens from cereals that show cross-reactivity with proteins in grass pollen[7]. An early intake of cereals in the diet during early life might cause IgE sensitization to cereals. It was not known whether such sensitization predisposes the development of allergy to pollen. To test this hypothesis, a cross-sectional study and an observational case-control analysis of reviewed data were carried out on 16381 patients who had been admitted to our Allergy Unit during ten years. All the patients underwent allergy tests to identify asthma risk-factor. We demonstrated that grass-pollen asthma was associated with sensitization to cereals. The early introduction of cereals in the diet of children was found to be a risk factor for grass-pollen asthma (OR = 5.95; 95% CI 3.89-9.10). These findings documented the progression of allergic asthma during a decade in a large sample of people who were

influenced by similar environmental conditions and studied with the same diagnostic methods. This study represented the largest database of patients in which a common food is shown to be a risk factor for asthma.

7. Cereal Tolerance Mechanism and Treatment Possibilities

Although baker's asthma (BA) is among the most common occupational disease, the risk factors and immune features that may be important as predictors of tolerance or development of the disease are not completely understood. We try to study the evolution over time of antigenic reactivity on baker's asthma in Spain and in France (BA is the second most common reported cause of occupational asthma in France), in order to find differences in their allergenic response and evidence of protective or risks mechanisms against this disease[32].

Two groups of subjects were randomly selected. A group of bakers with asthma from Spain and a second group from France whose blood was taken 30 years ago: bakers with asthma, bakers without occupational respiratory allergy, and wives and children living close to the bakery. In all subjects skin tests were carried out with cereals, insects and the most common allergens in their area. Serum levels of specific IgE, IgG_4 and IL10 (implicated in tolerance mechanism) measurement were also determined. Spanish patients were mainly sensitized to cereal allergens and presented higher levels of IgE ($p < 0.001$). French patients are more often sensitized to insects and cereal pests than the Spanish ones: 5.26% versus 80% ($p < 0.005$). Symptom free or without aggravation at work subjects have higher specific IgG_4 and IL 10 levels than the others ($p < 0.01$). Antigens implicated in baker's asthma may change with time. The presence of higher levels of IgG_4, IL10 and diversity of sources of sensitization in French patients may have helped them to develop a kind of natural hypo-sensitization[32].

8. Medical Treatment for Wheat Related Allergy

Medical treatment for wheat related allergy as well as food allergy in general can include the following: Epinephrine - given for severe allergic reactions (anaphylaxis), antihistamine and corticosteroid.

Although treatment of wheat allergy is focused in avoidance measures, in bakers' asthma disease is possible specific immunotherapy. One hundred thirty-nine bakers and pastry cooks were included in a prevalence study of IgE-mediated hypersensitivity to wheat flour demonstrated by skin tests, specific IgE to wheat flour (RAST), and inhalation challenge[38]. From the sensitized workers, we selected 30 asthmatic patients. Twenty patients were treated with a standardized wheat flour extract, and ten with a placebo in a double-blind clinical trial. Before and after immunotherapy we performed tests in vivo (skin tests with wheat flour and methacholine tests), and in vitro (total IgE and specific IgE to wheat flour). We found substantial prevalence of wheat flour allergy (25.17% of workers), and a significant decrease ($p < 0.001$) in hyperresponsiveness to methacholine, skin sensitivity ($p = .002$), and specific IgE ($p < 0.005$) to wheat flour after 20 months of immunotherapy. There was also significant subjective improvement ($p < 0.001$). The placebo group showed no changes in these variables.

9. Diagnostic Usefulness of Component Resolved Diagnosis (Microarrays) in Wheat Hypersensitivity

The exposure to wheat proteins through different routes can trigger IgE-mediated allergic reactions affecting several populations and age groups worldwide. However, the current diagnosis of wheat allergy has several limitations. Regarding in vitro diagnosis (specific IgE assays), all known diagnostic approaches have shown poor predictability and specificity[1,2], which may be associated with insufficient purity of wheat extracts used or with the lack of inclusion of all major allergens in these extracts. In contrast, basophil

activation test are considered to be a reliable in vitro diagnostic technique although its use is not widely used in routine practice[21].

The introduction of microarray techniques featuring a large panel of purified allergens has been a major advance in the diagnosis of allergic diseases. However, this technique has been hardly applied to the diagnosis and characterization of patients with occupational asthma due to wheat allergy or BA.

Recently we tested the usefulness of microarrays in diagnosis of wheat allergy[39]. The aim of our study was to characterize the allergenic profiles of BA patients from three different regions in Spain. The pattern of recognition will be compared with subjects allergic to wheat by ingestion and with seasonal rhinitis patients. To this end, a panel of wheat allergens purified and pollen allergens from natural sources and printed on a protein microarray is used. Forty five patients from 3 regions in Spain (Madrid n = 17, Malaga n = 10, Valladolid n = 18) with confirmed diagnosis of BA, positive results to skin-prick test and bronchial challenge with wheat flour were recruited. Twelve wheat allergens (WDAI-0.19 and WDAI-0.53, WTAI-CM1, WTAI-CM2, WTAI-CM3, WTAI-CM16,WTAI-CM17, Tri a 14, profilin, ω-5-gliadin, Tri a Bd 36 and Tri a TLP) were purified. A group of subjects with seasonal rhinitis (SR, n = 41) and allergy to wheat through ingestion (wheat food allergy patients or WFA, n = 9) were also analyzed for comparison.

As results, WTAI-CM16 and Tri 14 were defined as the most prevalent allergens (54 and 45% on average, respectively) covering a total of 64% of the baker's asthma population. On the other hand, ω-5-gliadin and Tri a Bd36 were recognized by less than 10% of the baker´s population. Tri a 14 (wheat LTP) was exclusively recognized by BA patients only (45%, p = 0.0379) and not for WFA or SR patients.

We concluded that the highest prevalence of IgE binding was observed for WTAI-CM16 (54%) and Tri a 14 (45%), with 64% of patients with baker's asthma that recognized at least one of these markers. Tri a 14 seems to be exclusively recognized by BA patients.

In summary, the diagnosis in patients sensitized to wheat is difficult by the relationship between pollen and this allergens and the different expression of

the disease. The misdiagnosis is often a cause for unsuccessful specific immunotherapy and no necessary food avoidance. Epidemiological analysis by molecular component-resolved diagnosis is a new method which may elucidate the interaction between allergen exposure gradient and patient sensitization (Figure 5).

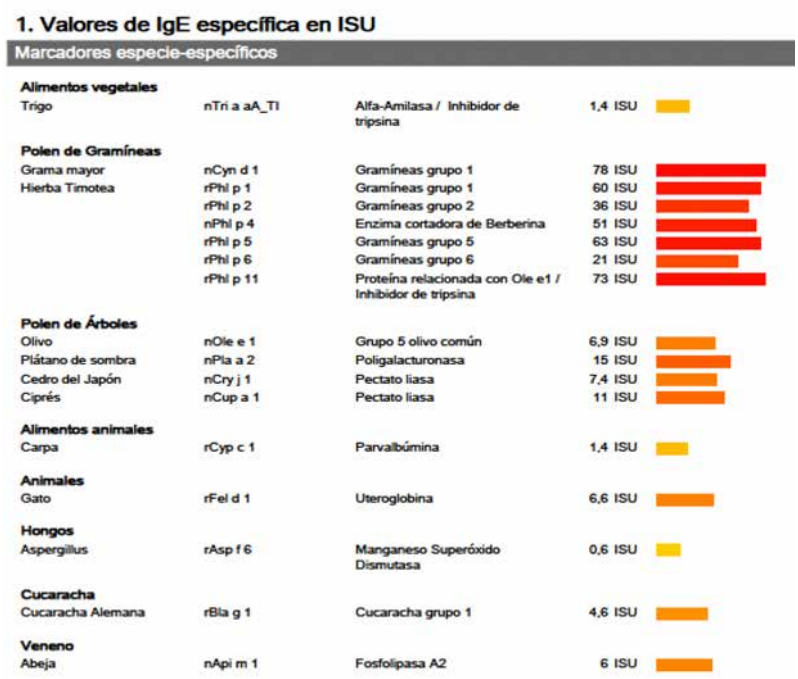


Figure 5. Array in a patient suffering from celiac disease.

References

1. Constantin C, Quirce S, Poorafshar M, Touraev A, Niggemann B, Mari A et al. *Micro-arrayed wheat seed and grass pollen allergens for component-resolved diagnosis.* Allergy. 2009.
   http://dx.doi.org/10.1111/j.1398-9995.2009.01955.x
   PMid:19210348
2. Zuidmeer L, Goldhahn K, Rona RJ, Gislason D, Madsen C, Summers C et al. *The prevalence of plant food allergies: a systematic review.* J Allergy Clin Immunol. 2008; 121(5): 1210-8.
   http://dx.doi.org/10.1016/j.jaci.2008.02.019
   PMid:18378288
3. Morita E, Kunie K, Matsuo H. *Food-dependent exercise induced anaphylaxis.* J Dermatol Sci. 2007; 47: 109-17.
   http://dx.doi.org/10.1016/j.jdermsci.2007.03.004
   PMid:17507204
4. Brant A. *Baker's asthma.* Curr Opin Allergy Clin Immunol. 2007; 7: 152-5.
   http://dx.doi.org/10.1097/ACI.0b013e328042ba77
   PMid:17351468
5. Palosuo K. *Update on wheat hypersensitivity.* Curr. Opin Allergy Clin Immunol. 2003; 3: 205-9.
   http://dx.doi.org/10.1097/00130832-200306000-00009
   PMid:12840704
6. Gómez L, Martín E, Hernández D, Sanchez-Monge R, Barber D, Del Pozo V et al. *Members of the alpha-amylase inhibitors family from wheat endosperm are major allergenss associated with baker's astma.* FEBS Lett. 1990; 261: 85-8.
   http://dx.doi.org/10.1016/0014-5793(90)80642-V
7. Armentia A, Sánchez-Monge R, Gómez L, Barber D, Salcedo G. *In vivo allergenic activities of eleven purified members of a major allergen family from wheat and barley flour.* Clin Exp Allergy. 1993; 23: 410-5.
   http://dx.doi.org/10.1111/j.1365-2222.1993.tb00347.x
   PMid:8334538
8. James JM, Sixbey JP, Helm RM, Bannon GA, Burks AW. *Wheat alpha-amylase inhibitor: a second route of allergic sensitization.* J Allergy Clin Immunol. 1997; 99: 239-3.
   http://dx.doi.org/10.1016/S0091-6749(97)70103-9

9. Pastorello EA, Farioli L, Conti A, Pravettoni V, Bonomi S, Iametti S et al. *Wheat IgE-mediated food allergy in European patients: alpha-amylase inhibitors, lipd transfer proteins and low-molecular-weight glutenins. Allergenic molecules recognized by double-blind placebo controlled food challege.* Int Arch Allergy Immunol. 2007; 144: 10-22.

http://dx.doi.org/10.1159/000102609
PMid:17496422

10. Sánchez-Monge R, García-Casado G, López-Otín C, Armentia A, Salcedo G. *Wheat flour peroxidase is a prominent allergen associated with baker's asthma.* Clin Exo Allergy. 1997; 27: 1130-7.

http://dx.doi.org/10.1111/j.1365-2222.1997.tb01149.x

11. Palacín A, Quirce S, Armentia A, Fernández-Nieto M, Pacios LF, Asensio T et al. *Wheat lipid transfer protein is a major allergen associated with baker's asthma.* J Allergy Clin Immunol. 2007; 120: 1132-8.

http://dx.doi.org/10.1016/j.jaci.2007.07.008
PMid:17716720

12. Sandiford CP, Tathman AS, Fido R, Welch A, Jones MG, Tee RD et al. *Identification of he major water/salt insoluble wheat proteins involved in cereal hypersensitivity.* Clin Exp Allergy. 1997, 27; 1120-9.

http://dx.doi.org/10.1111/j.1365-2222.1997.tb01148.x
PMid:9383251

13. Bittner C, Grassau B, Frenzel K, Baur X. *Identification of wheat gliadins as an allergen family related to baker's asthma.* J Allergy Clin Immunol. 2008; 121: 744-9.

http://dx.doi.org/10.1016/j.jaci.2007.09.051
PMid:18036646

14. Uvackova L, Skultety L, Bekesova S, McClain S, Hajduch M. *MS(E) based multiplex protein análisis quantified important allergenic proteins and detected relevanrt peptides carrying known epitopes in wheat grain extracts.* J Proteome Res. 2013. 12: 4862-9.

http://dx.doi.org/10.1021/pr400336f
PMid:24007624

15. Armentia A, Arranz E, Hernandez N, Garrote A, Panzani R, Blanco A. *Allergy after inhalation and ingestion of cereals involve different allergens in allergic and celiac disease.* Recent Pat Inflamm Allergy Drug Discov. 2008; 2: 47-57.

http://dx.doi.org/10.2174/187221308783399234
PMid:19075991

16. Bischoff SC, Mayer JH, Manns MP. *Allergy and the gut.* Int Arch Allergy Immunol. 2000; 121: 270-83.

http://dx.doi.org/10.1159/000024340
PMid:10828717

17. Moneret Vautrin DA, Sainte-Laudy J, Kanny G. *Ulcerative colitis possibly due to hyoersensiticity to wheat and egg.* Allergy. 2001: 56: 458-9.
http://dx.doi.org/10.1034/j.1398-9995.2001.056005458.x
PMid:11350318

18. Czaja-Bulsa G. *Non celiac gluten sensitivity- A new disease with gluten intolerance.* Clin Nutr. 2014; Aug 29. pii: S0261-5614 (14)00218-0.
http://dx.doi.org/10.1016/j.clnu.2014.08.012

19. Caio G, Volta U, Tovoli F, De Giorgio R. *Effect of gluten free diet on immune response to gliadin in patinets with non-celiac gluten sensitivity.* BMC Gastroenterol. 2014; 14: 26.
http://dx.doi.org/10.1186/1471-230X-14-26

20. Mansuelo P, Seidita A, D'Alcamo A, Carroccio A. *Non-celiac gluten sensitivity: literatote review.* J Am Coll Nutr. 2014; 33: 39-54.
http://dx.doi.org/10.1080/07315724.2014.869996
PMid:24533607

21. Carroccio A, Brusca I, Mansueto P, D'alcamo A, Barrale M, Soresi M et al. *A comparison between two different in vitro basophil activation tests for gluten- and cow's milk protein sensitivity in irritable bowel syndrome (IBS)-like patients.* Clin Chem Lab Med. 2013; 51: 1257-63.
http://dx.doi.org/10.1515/cclm-2012-0609
PMid:23183757

22. Molina-Infante J, Martín-Noguerol E, Alvarado-Arenas M, Porcel-Carreño SL, Jimenez-Timon S, Hernandez-Arbeiza FJ. *Selective elimination diet based on skin testing has suboptimal efficacy for adult eosinophilic oesophagitis.* J Allergy Clin Immunol. 2012; 130: 1200-2.
http://dx.doi.org/10.1016/j.jaci.2012.06.027
PMid:22867695

23. Armentia A, Martín S, Jesús B, Martín B, Sánchez A, Orcajo P et al. *Value f microarray allergen assay in the management of eosinophilic oesophagitis.* Allergol Immunophatol. 2015. In press.
http://dx.doi.org/10.1016/j.aller.2014.02.006
PMid:24961955

24. De Gregorio M, Armentia A, Diaz-Perales A, Dueñas-Laita A, Marín B, Salcedo G et al. *Salt-soluble proteins from wheat-derived foodstuffs show lower allergenic potency than those from raw flour.* J Agric Food Chem. 2009; 57: 3325-30.
http://dx.doi.org/10.1021/jf803475v
PMid:19275238

25. Kosti RI, Triga M, Tsabouri S, Priftis KN. *Food allergen selective thermal processing regimens may change oral tolerance in infancy.* Allergologia et immunopathologia. 2013; 41: 407-17.

http://dx.doi.org/10.1016/j.aller.2012.08.011
PMid:23253679

26. García-Casado G, Sánchez-Monge R, Chrispeels MJ, Armentia A, Salcedo G, Gómez L. *Role of complex asparagines-linked glycans in the allergenicity of plants glycoproteins.* Clin Exp Allergy. 2001; 31: 1250-5.

27. Palacín A, Quirce S, Armentia A, Fernández-Nieto M, Pacios LF, Asensio T et al. *Wheat lipid transfer protein is a major allergen associated with baker's asthma.* J Allergy Clin Immunol. 2007; 120: 1132-8.

http://dx.doi.org/10.1016/j.jaci.2007.07.008
PMid:17716720

28. Tordesillas L, Pacios LF, Palacín A, Quirce S, Armentia A, Barber D et al. *Molecular basis of allergen cross-reactivity: Non-specific lipid transfer proteins from wheat flour and peach fruit as models.* Molecular Immunology. 2009; 47: 534-40.

http://dx.doi.org/10.1016/j.molimm.2009.07.028
PMid:19846220

29. Armentia A, Rodriguez R, Callejo A, Martin-Esteban M, Martín-Santos JM, Salcedo G et al. *Allergy after ingestion or inhalation of cereals involves similar allergens in different ages.* Clin Exp Allergy. 2002; 32: 1216-22.

http://dx.doi.org/10.1046/j.1365-2745.2002.01456.x
PMid:12190662

30. Armentia A, Diaz-Perales A, Castrodeza J, Dueñas-Laita A, Palacín A, Fernández S. *Why can patients with baker's asthma tolerate wheat flour ingestion? Is wheat pollen allergy relevant?* Allergol Immunopathol. 2009; 37: 203-4.

http://dx.doi.org/10.1016/j.aller.2009.05.001
PMid:19775798

31. Baur X, Degens PO, Snader I. *Baker's asthma: still among the most frequent occupational respiratory disorders.* J Allergy Clin Immunol. 1998; 6863-70.

http://dx.doi.org/10.1016/S0091-6749(98)70337-9

32. Panzani R, Armentia A, Lobo R, Postigo I, MArtínez J, Arranz ML et al. *Tolerance mechanisms in esponse to antigens responsible for baker´s asthma in different exposed people.* J Asthma. 2008; 45: 333-8.

http://dx.doi.org/10.1080/02770900801939294
PMid:18446599

33. Barber D, de la Torre F, Feo F, Florido F, Guardia P, Moreno C et al. *Understanding patient sensitization profiles in complex pollen areas: a molecular epidemiological study.* Allergy. 2008; 63: 1550-8.

http://dx.doi.org/10.1111/j.1398-9995.2008.01807.x
PMid:18925892

34. Britton J, Pavord I, Richards K. *Dietary magnesium, lung function, wheezing and airway hyper-reactivity in a random adult sample.* Lancet. 1994; 344: 357-62.

http://dx.doi.org/10.1016/S0140-6736(94)91399-4

35. Grievink L, Smit HA, Ocke MC, van't Veer P. *Dietary intake of antioxidant pro-vitamins, respiratory symptoms and pulmonary function: The MORGEN-study.* Thorax. 1998; 53: 166-71.

http://dx.doi.org/10.1136/thx.53.3.166
PMid:9659349 PMCid:PMC1745167

36. de Luis DA, Armentia A, Aller R, Asensio A, Sedano E, Izaola O et al. *Dietary intake in patients with asthma: a case control study.* Nutrition. 2005; 21(3): 320-4.

http://dx.doi.org/10.1016/j.nut.2004.06.027
PMid:15797673

37. Troisi RJ, Willett WC, Weiss ST, Trichopoulos D, Rosner B. *A prospective study of diet and adult onset asthma.* Am J of Respir and Crit Care Medicine. 1995; 151: 1401-8.

http://dx.doi.org/10.1164/ajrccm.151.5.7735592
PMid:7735592

38. Armentia A, Martin-Santos JM, Quintero A, Fernández A, Barber D, Alonso E et al. *Bakers'asthma: prevalence and evaluation of immunotherapy with a wheat flour extract.* Ann Allergy. 1990; 65: 265-72.

PMid:2221484

39. Gómez C, Garrido M, Pereira C, Catarino M, Parro V, Armentia A et al. *Component resolved diagnosis of wheat flour allergy in baker's asthma.* J Allergy Clin Immunol. 2014 Apr 30. pii: S0091-6749(14)00437-0.

http://dx.doi.org/10.1016/j.jaci.2014.03.016

SECTION III: The evolution of gluten-free foods

Preface Section III

Cristina M. Rosell, PhD

Chapters 15 to 20

Evidence of the requirement of a gluten free diet dates back 60 years, when Dicke et al. (1953) pointed out the possible relationship between wheat gluten intake and celiac disease. However, gluten is not only present in wheat, some other cereals like rye, barley and oat, contain gluten when mixed with water. This information had great impact on food development due to the numerous foods and drinks that used those cereals in their production. Later (around 1970), gluten-free bread started to be produced, overcoming the technological restrictions that the absence of gluten provoked in the development of fermented cereal based foods. From that time an increasing consciousness has persisted, which prompted the development of gluten-free foodstuff, looking for tools to technologically replace the gluten giving sensory of accepted products.

Gluten is not just a great protein matrix, it is a protein with incomparable viscoelastic properties, because of that its replacement has been an enormous challenge during decades, and it is still a hot topic. Initially, only starches and hydrocolloids were considered but later on different tools have been developed for defining food recipes resembling the quality of gluten containing goods. In addition, it is necessary to understand where gluten is present and in which food processes it is really relevant its functionality, because this understanding will give us the required information for developing foods and drinks with sufficient scientific knowledge, taking advantage also of autochthonous gluten-free foodstuff. Nevertheless, in this scenario not only the sensorial quality must be consider, it is an essential requirement that those gluten-free foods provide the required nutrients' intake for those gluten free intolerants, contributing also to their wellbeing and healthy status.

The section Gluten-free foods within the book Advances in the understanding of gluten related pathology and trends of gluten-free foods is intended to give the most updated information about Gluten in the context of food development addressed to gluten intolerant population. Gluten-free foods section compiles six chapters authored by well-known worldwide scientists with a holistic approach covering from agronomic aspects to gluten-free food products and drinks of gluten containing cereals, gluten functionality, the alternatives that food technologist have available for making healthy and nutritious breads understanding the role of the ingredients and processes. Particularly, this section includes the following chapters i. Cereals taxonomy and the role of breeding on gluten intolerance; ii. Analytical tools for gluten detection: Policies and regulation; iii. Gluten functionality in food technology; iv. Alternatives for gluten replacement; v. Gluten-free autochthonous foodstuff; Gluten-free spirits and drinks; vi. Nutrition issues of gluten-free foodstuff. Up to date information, besides innovative aspects and emerging fields, have been identified, highlighting the importance of gluten management in the frame of Gluten related pathologies.

This book is intended to cover all aspects that could have an impact in the nutritional and health benefits of gluten intolerant populations. Its reading is essential for research scientists, dieticians, industrial bakers, consumers, food chemists, technologists, academics and regulatory authorities and to the general public interested in gluten and gluten-free products.

This Associate Editor would like to thank all contributors for their excellent and critical revision to show the state of the art of gluten-free foods and drinks from cereals to market and nutrition, without forgetting the analytical tools currently available to quantify gluten.

This Associate Editor would like also to thank Dr. Luis Rodrigo and Amado Salvador Peña, the Book Editors, for inviting me for composing and editing this book section.

Chapter 15

Cereals Taxonomy: The Role of Domestication and Breeding on Gluten Intolerance

María J. Giménez[1], Javier Gil-Humanes[2], Juan B. Alvarez[3], Francisco Barro[1]

[1] Instituto de Agricultura Sostenible, CSIC, Córdoba, Spain.

[2] Department of Genetics, Cell Biology, and Development and Center for Genome Engineering, University of Minnesota, Minneapolis, Minnesota, United States.

[3] Departamento de Genética, Escuela Técnica Superior de Ingeniería Agronómica y de Montes, Campus de Rabanales, Universidad de Córdoba, Córdoba, Spain.

mjga06@ias.csic.es, gilxx016@umn.edu, jb.alvarez@uco.es, fbarro@ias.csic.es

Doi: http://dx.doi.org/10.3926/oms.263

How to cite this chapter

Giménez MJ, Gil-Humanes J, Alvarez JB, Barro F. *Cereals Taxonomy: The Role of Domestication and Breeding on Gluten Intolerance*. In Arranz E, Fernández-Bañares F, Rosell CM, Rodrigo L, Peña AS, editors. *Advances in the Understanding of Gluten Related Pathology and the Evolution of Gluten-Free Foods*. Barcelona, Spain: OmniaScience; 2015. p. 493-526.

Abstract

Storage proteins of wheat, rye, barley and, to a lesser extent, oats contain epitopes responsible for triggering the celiac disease (CD). In recent decades an increased frequency of CD has been observed, and though the reasons for this increase are unclear, modern plant breeding has attracted criticism attributing to the new varieties a part of the responsibility in worsening the data of prevalence. Wheat is one of the most important crops worldwide, presenting both high adaptability to different environments and yields. The domestication of wheat is the result of a previous natural interspecific hybridization first between diploid, and then between diploid and tetraploid species that resulted in hexaploid wheat. The old farmers began to select the traits that were better adapted to the use. In the 20th century the wheat breeding had its great advance and modern varieties were developed. The gliadin-related genes, responsible for triggering CD, have no adaptive value and therefore, if the toxicity of wheat was increased during the process of domestication and breeding this would have been made unconsciously. During the process of natural hybridization the number of gliadin-related genes was increased. Bread wheat, rye, and *Ae. tauschii* have the highest number of CD epitopes per gene, and it seems that in bread wheat, this high number of epitopes is explained by the D genome from *Ae. tauschii*. During the process of domestication and breeding, the number of CD epitopes per gene did not increase and even decreased in some cases.

Keywords

Cereal domestication, wheat breeding, prolamins, gluten, immunotoxicity.

1. Introduction

The change in the human diet during the Neolithic Revolution has been associated with a general decline in health in some areas[1]. Celiac disease (CD) is one of diseases that emerged in that period[2,3] but despite of being known since ancient times, its history is relatively recent. The first references concerning the intake of certain foods may be harmful do not appear until the late nineteenth century, and the first breakthrough came after World War II, with the demonstration of the role of gluten as the agent responsible for triggering the disease[4]. In the past 60 years, the knowledge about CD has improved significantly, resulting in a better understanding of the disease pathogenesis, diagnosis, and therapy[5]. Progress in the understanding of the disease includes the recognition of its autoimmune nature, its genetic basis, and the identification of immunogenic gluten fragments causing CD in many patients[6].

The definition of the disease and the diagnostic criteria, have undergone changes as all questions concerning the CD have become clearer. Thus, the guidelines for the diagnosis of CD recommended by the European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN), first published in 1970, have been revised twice. As defined in the last guidelines of 2012[7], "CD is an immune-mediated systemic disorder elicited by gluten and related prolamins in genetically susceptible individuals and characterized by the presence of a variable combination of gluten-dependent clinical manifestations, CD-specific antibodies, HLA-DQ2 or HLA-DQ8 haplotypes, and enteropathy. CD-specific antibodies comprise autoantibodies against TG2, including endomysial antibodies (EMA), and antibodies against deamidated forms of gliadin peptides (DGP)".

The prevalence of CD is 1% of the general population in western countries but varies from country to country. However, an increased frequency of CD in recent decades has been observed, which can be partly attributed to the advent of serological testing and increased public awareness in some countries[8]. The reasons for this increase are unclear, but several hypotheses as

hygiene[9] and the rising consumption of cereals, especially wheat (or its derivatives)[10], have been proposed among others. The possible roles attributed to modern plant breeding in worsening figures of prevalence of CD are discussed in this chapter.

2. Taxonomy and Domestication of Cereals

The transition process from hunter-gathering to a sedentary, agriculture-based human society started around 12,000 years ago[11]. Cereals cultivation and their elaborated products have played an essential role in the development of human societies, and nowadays represent an important element in most of the different cultures. Archaeological evidences show that humans harvested the wild forms of cereals from natural stands before they started to deliberate, cultivate and domesticate cereals, which involved the selection and modification of important traits such as seed size and rachis stiffness in the first cultivated fields. The easy carry and storage, together with the high content in carbohydrates and proteins, are some of the characteristics associated to the first plants that were cultivated and domesticated. Cereals are the domestic variants of the species from the *Gramineae* family (*Poaceae* Barnhart). The paleobotanical records suggest that this family was generated about 50-70 million years ago (MYA). Within this family, the main species of agronomical interest are found in the three subfamilies: *Ehrhartoideae* Link (rice), *Panicoideae* Link (maize, sugar cane, and sorghum) and *Pooideae* Bentham (oat, wheat, rye, and barley), this last subfamily is formed by 15 tribes, being the tribes *Avenae* (oat) and *Triticeae* (barley, rye, and wheat) the most important (Figure 1). Although now, species of both tribes are named as cereals, phylogenetic relationships suggest that the separation between both groups began about 20 MYA[12]. The separation within the *Triticeae* tribe is more recent and has been established around two MYA; although a recent study suggests that these speciation events might have occurred along to the last ten million years[13].

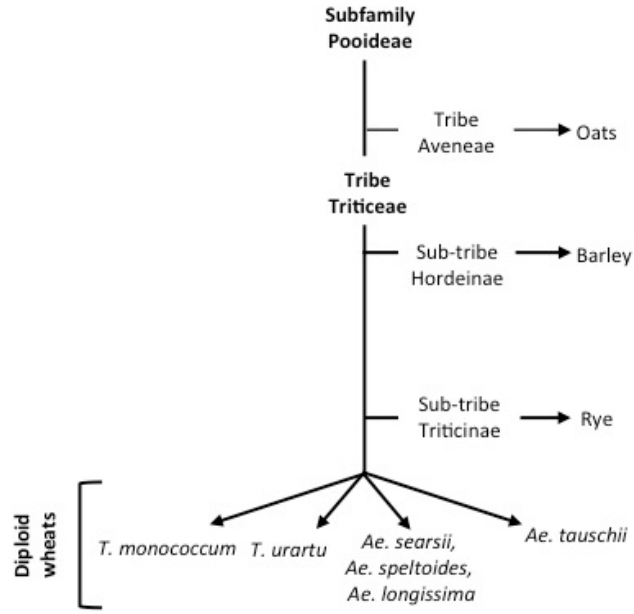


Figure 1. Origin and relationship of major cereals. Subfamily Pooideae *Bentham (oat, wheat, rye, and barley), comprised 15 tribes, being the tribes* Avenae *(oat) and* Triticeae *(barley, rye, and wheat) the most important.*

2.1. Oat

The genus *Avena* includes cultivated species with different ploidy level. Three cytogenetically independent stocks of *Avena* L. occurred during domestication (*A. sativa*, *A. strigosa*, and *A. abyssinica*), although only *A. sativa* became a principal cereal. Oats were probably evolved from weedy types that infected wheat and barley fields, and not under the domestication as a crop[14]. The hexaploid *A. sativa*, common oat (2n = 6× = 42), is widely cultivated, and its main uses are for human consumption (oat meals and rolled oats) and livestock feed. The wild relative of *A. sativa* is the hexaploid wild oat *A. sterilis*, whose first evidences of cultivation date ~11,400 years before present (BP), in which is thought to be a predomestication cultivation practice[15]. Besides, the first evidences of domesticated *A. sativa* cultivation have been found in Sacarovca, Moldavia (~7,600-7,400 BP).

2.2. Barley

Cultivated barley (*Hordeum vulgare*) (2n = 2× = 14; HH) constitutes one of the first domesticated crops, and one of the most important world crops. It evolved from its wild progenitor *H. spontaneum*, which was originated 5.5 MYA. Wild barley started to be harvested by humans as early as 50,000 years ago[16], and the main characters associated with its domestication were: non-brittle rachis, increased seed weight, six-rowed ears and naked seeds. The domestication of wild barley has been described in the literature to occur in several geographical locations, with at least three main centers of domestication: Fertile Crescent, Central Asia, and Tibet (reviewed in Greco et al[17]). However, molecular data obtained from wide collections of wild and cultivated barleys indicate a single origin for all modern varieties and landraces[18,19] consistent with a single domestication event, which probably took place in the Israel-Jordan area[17]. Compared to wheat, barley has an inferior staple and bread-making quality. However, it withstands drier conditions, poorer soils, and some salinity[14], which makes it an important crop in several areas. It is used for animal feed and for human consumption in soups, stews and barley bread, although its main use is for the production of beers (malt) and distilled beverages.

2.3. Rye

Domesticated rye belongs to the small genus *Secale* L. The domestication of this cereal has brought less attention than other cereals in the literature on the origin of agriculture since rye was not among the cereals that promoted the Agricultural Revolution. Some archaeological evidences found in the Euphrates valley in modern Syria indicate that the domestication of rye occurred around 11,500 BC[20], although as other cereals wild rye was cultivated long before its domestication. The wild progenitor of rye is thought to be *S. vavilovii*[14,21], which is fully inter-fertile in crosses with *S. cereale* and has been found in primary habitats[22]. Rye is particularly cultivated in Northern and Eastern Europe. It adapts well to acidic and sandy soils, and is

resistant to cold and dry conditions. The grain has a high content of proteins, and most of present world production is consumed in the form of bread[14].

2.4. Wheat

Wheat is one of the most important crops worldwide, and its extended cultivation is in part due to its high adaptability to different environments and its high yields, but also to the unique viscoelastic properties of wheat dough, which allow the entrapment of CO_2 during fermentation, enabling the preparation of leavened breads and other baked products. The domestication of wheat began around 10,000 years ago as part of the Agricultural Revolution, and it has been placed in the Near East, in the zone known as Fertile Crescent[23]. Wheat is a polyploidy complex formed by multiple species of different ploidy level, consequence of the merge of genomes from different species of the *Triticeae* tribe (Figure 2). Thus, diploid (2n = 2× = 14, AA), tetraploid (2n = 4× = 28, AABB), and hexaploid species (2n = 6× = 42, AABBDD) of wheat can be found.

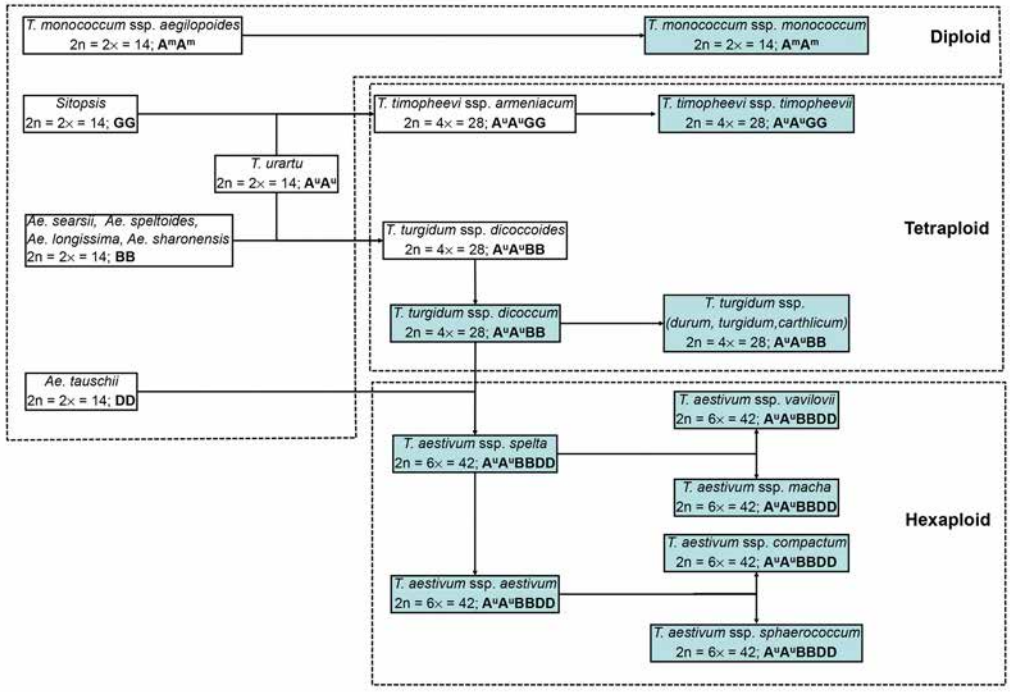


Figure 2. Wheat and its ancestral relatives. Hybridization events leading to tetraploid and hexaploid wheats. The species in gray are or have been cultivated.

The origin of the A and D genomes is well known. *T. urartu* Thum ex. Gandil (2n = 2× = 14, A^uA^u), a wild diploid specie, has been proposed as the donor of the A genome in polyploid species of wheat[24]. However, the origin of the B genome presents certain controversy. The currently accepted hypothesis suggests that *T. urartu* could have generated the wild tetraploid wheats mainly in two different events. On the one hand, by crossing with an *Aegilops* species (section *Sitopsis*), probably *Ae. speltoides* Tausch. (2n = 2× = 14, putative BB), and subsequent chromosome doubling it was generated wild emmer (*T. turgidum* ssp. *dicoccoides* Korn. ex Asch. & Graebner em. Thell., 2n = 4× = 28, A^uA^uBB), from which cultivated emmer (*T. turgidum* ssp. *dicoccum* Schrank Thell., 2n = 4× = 28, A^uA^uBB) was domesticated. The rest of the tetraploid wheats, including durum wheat (*T. turgidum* ssp. *durum* Desf. em. Husn.), as well as the hexaploid wheats derive from this species[25]. On the other hand, the crossing with some other species of the section

Sitopsis with *T. urartu* originated *T. timophevii* ssp. *armeniacum* Jakubz. em. Slageren (2n = 4× = 28, A^uA^uGG), which domesticated form (*T. timophevii* ssp. *timophevii*) is restricted to western Georgia. With respect to the D genome, several studies suggest that the donor of this genome is *Aegilops tauschii* Coss. (2n = 2× = 14, DD)[26,27], which after crossing with cultivated emmer (*Triticum turgidum* ssp. *dicoccum* Schrank em. Thell., 2n = 4× = 28, A^uA^uBB) and subsequent chromosome doubling, led to spelt (*T. aestivum* ssp. *spelta* L. em. Thell., 2n = 6× = 42, A^uA^uBBDD), putative ancestor of bread wheat (*T. aestivum* ssp. *aestivum* L. em. Thell.), the most important species of the genus *Triticum* today.

Despite the relative recent origin, wheat shows an enormous genetic variability that has allowed the development of around 25,000 different types[28]. Hexaploid bread wheat (AABBDD) represents ~95% of today's cultivated wheat, whereas durum wheat represents almost the other 5%. Cultivation of diploid wheats has been reduced to marginal lands.

3. Grain Storage Proteins of Cereals

Cereal grains contain relatively little protein compared to legume seeds, with about 10-12% dry weight. Storage proteins form approximately half of this protein, which can be included in four different fractions (albumins, globulins, prolamins and glutelins) according with their solubility. The gluten proteins of wheat classically fall into two of these fractions, with the alcohol-soluble gliadins being defined as prolamins and the alcohol-insoluble glutenins as glutelins. With exception of oats and rice, the main endosperm storage proteins in cereal grains are prolamins, which are so named because they present high content of proline and glutamine. On the contrary, in oats and rice the storage proteins are mainly globulins 11-12S, although the rice storage proteins have been classically classified as glutelins since they are not readily soluble in salt solutions[29].

In wheat, the prolamins are divided in two groups: gliadins and glutenins. The former are monomeric while the latter are polymeric. For this reason,

although both fractions are soluble in alcohol, the glutenins were originally classified as glutelin (alcohol-insoluble) because they have to be denatured by reducing agents (β–mercaptoethanol or dithiothreitol) to make them soluble in alcoholic solutions. Both protein groups are the major components of the gluten, which has been defined as "*the viscoelastic mass that remains after thoroughly washing out the starch from a dough*"[30]. This structure is the main responsible of the properties of the wheat flour that permit technological processes as bread making.

These proteins make up a complex mixture that can range between 50 components in hexaploid wheat and about 20 in diploid species[31]. Glutenins are classified into high molecular weight subunits (HMWGs) and low molecular weight subunits (LMWGs)[32]. The HMWGs, with molecular weights ranging between 80-140 kDa, are encoded by the *Glu-1* complex loci located on the long arm of each chromosome of group 1, called *Glu-A1*, *Glu-B1* and *Glu-D1*, respectively[33]. The LMWGs, meanwhile, have molecular weights between 30-50 kDa, and are encoded by the *Glu-A3*, *Glu-B3* and *Glu-D3* loci located on the short arm of group 1 chromosomes[34]. Gliadins are classified in *α/β*-, *γ*-and *ω*-gliadins, being synthesized also by genes on the short arm of group 1 chromosomes (*Gli-A1*, *Gli-B1* and *Gli-D1* loci that encode the *γ*- and *ω*-gliadins,) and group 6 chromosomes (*Gli-A2*, *Gli-B2* and *Gli-D2* loci that codes the *α*- and *β*-gliadins)[35]. Other minor loci of gliadins and glutenins have been also detected in the short arm of the group 1 chromosomes[36]. In a single bread wheat cultivar, the gluten proteins might be comprised of up to 45 different gliadins, 7 to 16 LMW-GS, and 3 to 6 HMW-GS. All those gluten proteins are synthesized and deposited in the starchy endosperm during grain development. Wieser[37] determined by reversed-phase HPLC, using a range of cultivated wheat species, that α-gliadins were predominant in most cases, followed by *γ*-gliadins and LMW-GS; *ω*-gliadins and HMW-GS were generally minor components.

The increased availability of detailed information on the molecular structures and genetics of the proteins present in glutenin and gliadin fractions has allowed them to be redefined into three groups, or families,

called sulfur-rich (S-rich), S-poor, and high molecular weight (HMW) prolamins. In wheat, the HMW prolamins are present only in glutenin polymers, while the S-rich prolamins are present as monomers (gliadins) and polymers (glutenins), and the S-poor predominantly (but not solely) as monomers[38].

Barley is a diploid species and therefore the genetics of storage proteins is much simpler than in hexaploid wheat. The prolamins of cultivated barley consist of S-rich B- and γ-hordein, S-poor C-hordein, and HMW D-hordein[39]. In cultivated barley all the hordein genes are linked in the short and long arms of chromosome 5, where they are organized in complex loci[40]. The C-hordeins are encoded in the *Hor 1* locus of the short arm of chromosome 5, whereas the D-hordeins are found in the *Hor 3* locus of the same chromosome[41].

All prolamin genes are intronless, and consist of several domains, being one of them a long repetitive domain formed by motifs with high content in proline and glutamine. The other domains present in these genes show high conservation of their nucleotide and amino acid sequences, suggesting that all these genes could derive from a unique ancestral gene[42]. However, gliadins and glutenins are not at the same amounts in the grain of cereals, their proportions can vary within a broad range and depends on both genotype (variety) and growing conditions (soil, climate, fertilization, etc.). The ratio gliadins to glutenins was examined in a range of cereals[43], and hexaploid common wheat showed the lowest ratio (1.5–3.1), followed by oats and spelt (1.7–3.3), barley (1.4–5.0), durum wheat and kamut (3.1–3.4), emmer wheat (3.5–7.6), rye (6.3–8.2), and einkorn wheat (4.0–13.9).

4. Wheat Breeding

In the Mediterranean region, wheat cultivation is linked to its flour transformation and consumption. The original consumption of the flour was in the form of porridge, since it does not require special conditions for its elaboration[44]. A more elaborated use is in the form of bread, whose first

written references go back to 4.6 thousand years ago (TYA); although, the archaeological findings indicate the possibility that it was already known in Babylon six TYA. However, the baking process was developed in the Ancient Egypt where the beer yeast (*Sacharomyces cerevesiae* L.) began to be used to ferment the dough[45].

Along the History, this process has suffered scarce changes. Until the Industrial Revolution, all baking processes were carried out by hand; this permitted the use of wheat varieties with rheological properties very different to those of the current wheat varieties. When the use of machinery in the baking processes started, producers were forced to look for varieties with very specific qualities[46]. The dough made with these flours must have a certain tolerance to mechanical mixing and over-mixing, a process that is very different from the manual process. Consequently, many traditional wheat varieties were neglected, mainly due to their smaller yields and in many cases to their difficult mechanization. Along with this, part of the allelic prolamin variation present in these ancient materials was lost, mainly in those regions where the replacement of landraces with improved varieties was indiscriminate, which has been particularly intense in the last century[47,48]. Fortunately, part of this variation missed in the fields was stored in Germplasm Banks, and now can be used to enlarge the genetic pool of the modern cultivars.

4.1. The Role of the Old Farmers in Wheat Breeding

The artificial selection of the plants is as old as the Agriculture. The old farmers began to select the traits that were better adapted to the use of each crop. This empirical process has made possible the generation of different materials for a same crop, for example, the classically mentioned case of *Brassica oleraceae*, one species that in hands of these farmers gave rise to such different crops as cabbage, cauliflower, kohlrabi, Brussels sprouts, broccoli and kale. If we think of wheat, it is possible to find peoples that throughout their history used hulled wheats as emmer or spelt, while others readily associated the naked wheat (durum or common wheat) to their diet.

Equally, the use or not of yeast originated wheat with different breadmaking characteristics. All these processes entailed the selection of the different allelic prolamin variants implicated in the technological processes, which joined to the physiological role that these proteins have in the wheat plant (source of amino acids during the germination) might have allowed the fixation of mutations in the repetitive domain of these proteins where the reactive peptides in relation to CD are located.

The dispersion of wheat cultivation from the Fertile Crescent has been documented around the 5th millennium B.C. both for the tetra- and hexaploid species[23]. This expansion was linked to the human migrations and the commercial exchanges between the Near-East peoples and other peoples of Asia, Europe and North of Africa. In this context, numerous events of genetic drift, due to serial founder effects and subsequent expansions, might have taken place. Furthermore, the adaptation to the climatic and edaphic conditions, together with the diversification of end uses, should have generated a large diversity within the crop. For this reason, the possibility that old farmers unconsciously selected the most toxic prolamins is scarce and without any scientific base.

4.2. The Scientific Wheat Breeding.

Although along of the 18th and 19th centuries, the effort of wheat breeders was notable, it was in the 20th century when the wheat breeding was significantly improved. At this respect, the introduction of the reduced height gene (*Rht8*), together with daylight-insensitive gene, had great importance in the development of wheat cultivars during the first decades of the past century. These genes, in combination with the increased availability of nitrogen fertilizer due to the Haber-Bosch process, substantially increased wheat yields around the World[49].

A central figure in the introduction of these traits into European germplasm was Nazareno Strampelli, an Italian wheat breeder that in 1913 developed varieties with shorter straw, lodging resistance and earlier maturity by the use of the Japanese variety Akakomugi. The Strampelli wheats were

lately used to develop wheat varieties worldwide. In 1952, other dwarfing genes (*Rht1* and *Rht2*) from other Japanese variety (Norin-10) were incorporated into the modern wheat varieties by US Department of Agriculture (USDA) breeders[49]. Norin-10 and its derivatives were transferred to the International Maize and Wheat Improvement Center (CIMMYT), in Mexico, and used by Norman Borlaug as part of the key varieties in the Green Revolution, which permitted increase yields worldwide and, in particular, some developing nations as India or Pakistan to greatly improve their food security.

As commented above, in some countries the substitution of the old landraces by these new wheat varieties was indiscriminate, and generated the loss of local genetic diversity. However, although some traits of these improved varieties come from a little number of landraces or old varieties, the genotypes used in the breeding programs of the Global Wheat Program of CGIAR (Consultative Group on International Agricultural Research) represent an important part of the worldwide genetic diversity of wheat.

5. Cereal Species and CD

From all the aforementioned cereals, wheat has been the most widely studied and discussed in relation with the development of the CD. Wheat gluten proteins are composed by the monomeric gliadins and the polymeric glutenins. The majority of CD reactive epitopes have been found in the gliadin fraction. Moreover, the immunotoxicity of many gluten peptides has been assessed by activation of gluten-specific T cells isolated from duodenal biopsies of CD sufferers, and α and γ-gliadins have been found to contain the vast majority of the epitopes triggering the CD[50]. These assays also showed that the number of immunotoxic epitopes identified in wheat gluten proteins and other grasses has significantly increased in the last years. Although wheats with different ploidy levels have been tested for differences in the content of immunoreactive peptides, there is very little information on the genetic diversity in cultivated wheat germplasm. Most studies have included

few genotypes from each species (Table 1) with the exception of two works (one of durum wheat and other of bread wheat), which included more than 30 accessions. On the other hand, the methodology used to assess toxicity was not the same for all studies (Table 1) and therefore, comparisons between species and ploidy levels are complicated.

Nevertheless, wheats with different ploidy levels have shown differences in the content of immunoreactive peptides. Some authors have identified diploid and tetraploid wheats, and even some old hexaploid wheat varieties, as a potential source of variability for the introduction of low CD toxic as a new breeding trait[51-53]. Molberg et al.[54] and Spaenij-Dekking et al.[51] found a large variation in the amount of CD4 T cell stimulatory peptides present in α-, γ-gliadins, and glutenins among diploid, tetraploid, and hexaploid wheat accessions. Similarly, variation for immunotoxicity in *Ae. tauschii*, as determined by epitope screening in expressed proteins, was found to be broader than for *T. aestivum* in a study that included 43 genotypes[55]. In that work, some *Ae. tauschii* genotypes expressed relatively less amounts of CD toxic epitopes. However, this variability does not mean lower toxicity as efficient recognition by α and γ-gliadin specific T-cell clones of the gluten digests of all the accessions was reported in another study[56].

Table 1. Revision of studies analyzing immunotoxicity of cultivated wheats of different level of ploidy: number of genotypes included in the study, type of protein and detection method.

Species	Number of accessions	Protein	Detection method	Reference
T. monococcum	15	Alpha and gamma gliadins	T-cell	[54]
T. monococcum	2	Alpha and gamma gliadins HMW and LMW glutenins	mAbs IFN-γ T-cell	[51]
T. monococcum	1	Alpha gliadins	Epitope screening	[69]
T. monococcum	1	NS[(a)]	IFN-γ	[70]

Species	Number of accessions	Protein	Detection method	Reference
T. monococcum	1	All[(b)]	Epitope screening	[71]
T. monococcum	3	Alpha gliadins	Epitope screening mAbs IFN-γ	[72]
T. monococcum	1	Gamma gliadins	Epitope screening T-cell	[73]
T. monococcum	2	NS	IFN-γ T-cell IL-15	[74]
T. monococcum	1	NS	T-cell	[75]
T. durum	10	Alpha and gamma gliadins	T-cell	[54]
T. durum	4	NS	T-cell	[75]
T. durum	6	Alpha gliadins	mAbs	[76]
T. durum	7	Alpha gliadins	Epitope screening	[77]
T. durum	51	Alpha gliadins	mAbs	[52]
T. aestivum	1	Alpha and gamma gliadins	T-cell	[54]
T. aestivum	5	Alpha and gamma gliadins HMW and LMW glutenins	mAbs IFN-γ T-cell	[51]
T. aestivum	1	NS	T-cell	[75]
T. aestivum	8	Alpha gliadins	Epitope screening	[77]
T. aestivum	2	Alpha gliadins	Epitope screening	[78]
T. aestivum	3	Alpha gliadins	mAbs	[52]

Species	Number of accessions	Protein	Detection method	Reference
T. aestivum	86	Alpha and gamma gliadins HMW and LMW glutenins	mAbs	[53]
T. aestivum	2	NS	IFN-γ anti-tTG	[79]

[(a)]Not specified; [(b)]All prolamin fractions.

In barley, all prolamins fractions are immunotoxic, but D- and C-hordeins have been reported as the most active in triggering the CD[57]. Efforts have been made to identify new varieties of barley with a reduced immunotoxicity. In this line, Tanner and colleagues[57] reported that barley lines lacking B- and C-hordeins had 20-fold reduced immunotoxicity compared with wild-type barley.

Rye is also among the "forbidden" cereals for CD patients and T-cell stimulatory epitopes have been detected in it[58]. However, little is known about the variability in the toxicity of different varieties of rye, including those used for wheat breeding in the Green Revolution.

6. Has Domestication and Breeding Increased the Immunotoxicity of Wheat?

Two types of selection operate (and complement each other) under domestication and breeding: (a) conscious or intentional selection applied by breeders for the traits of interest; (b) the unconscious or automatic selection caused by the fact that these plants were taken out of their original habitat and placed in the new (and usually very different) human-managed environments[59]. The genes for storage proteins have no adaptive value, they are neutral genes, and none of the major genes that regulate the main qualitative traits subjected to strong selection pressure in the domestication of wheat (i.e., loss of seed shattering and threshability) are located in chromosomal regions encoding prolamin genes, nor do most of

the quantitative trait loci (QTL) with small effects on the domestication syndrome[60]. Agronomic traits and adaptation to biotic and abiotic stresses have been the targets of this local selection. A meta-QTL analysis carried out to identify the major and consistent QTLs for the yield and its components has shown that among the prolamin loci only *Glu-A1* and *Glu-B1* for HMWGs were located in the vicinity of two of these meta-QTLs[61]. However selection for resistance to diseases and pests, major constraints of yield in crop plants, could have implied the selection of particular alleles of prolamins, as genes for disease resistance are distributed in gene rich regions all over the wheat genome, including those in group 1 and group 6 chromosomes where gliadin loci are encoded[62,63]. So, if the process of domestication increased the number of toxic peptides or favored gliadin genes with greater toxicity this would have been made unconsciously, and due to the small size of the selected population. Among the multiple and groundless criticisms attracted by the wheat varieties of the Green Revolution, is the little number of parents used by the breeders in the first phases, which imply low variation for prolamins, in particular for gliadins.

Proteomic and genomic data available from bread wheat and its diploid and tetraploid ancestors provide valuable information about prolamin proteins, which include the content of proline and glutamine and the abundance and frequency of CD related epitopes. This information is highly relevant as gliadin genes are rich in the amino acids proline and glutamine and the highly antigenic gluten epitopes are mainly located in the proline-rich regions[50]. So, if post-domestication mutation events have affected immunotoxic regions of gliadin genes, that would be reflected in differences in prolamin proteins among wheat species, and in particular between diploid and hexaploid wheats.

Full gliadin-related protein sequences for organisms indicated in Table 2 were analyzed for parameters such as protein length, the content of proline and glutamine, and the number of CD epitopes per sequence. In this analysis,

relevant gluten T-cell epitopes restricted by HLA-DQ molecules were considered[6].

Protein sequences of oats and barley, which separated first during cereal evolution (Figure 1), are shorter than those of rye and wheat and its ancestors (except *T. turgidum* ssp. *turgidum).* Two diploid ancestors of bread wheat; *Ae. speltoides* (BB) and *Ae. tauschii* (DD) show gliadin lengths of 308 and 298 amino acids, respectively, longer than that of bread wheat (Table 2). *T. turgidum* ssp. *Dicoccoides*, the ancestor of cultivated tetraploid wheats has gliadin proteins of 306 amino acids average length, significantly longer than that of *T. turgidum* ssp. *durum* and *T. turgidum* ssp. *turgidum*, with 287 and 259 amino acids, respectively. It seems that the process of domestication and breeding has not increased the length of gliadin-related proteins in cultivated bread or durum wheat. With respect to the content of proline (P) and glutamine (Q), there is a good correlation between protein length and the content of glutamine ($r^2 = 0.8328$), which indicates that the longer the sequence the higher the glutamine content.

Table 2. Proteomic analysis of gliadin-related proteins in cereals. Only complete protein sequences were considered.

Organism	Common name	Genome	Average length (1)	Proline (%)	Glutamine (%)	Epitopes /seq (2)
A. sativa	Oat	AACCDD	207 d	8.9	26.9	0.4 e
H. vulgare	Barley	HH	265 c	16.9	29.2	1.3 de
S. cereale	Rye	RR	296 ab	18.2	32.9	5.2 a
T. urartu	Wild form	A^uA^u	283 abc	15.5	31.6	3.2 bcd
Ae. speltoides	BB genome donor	BB	308 a	14.9	34.3	3.0 bcd
T. monococcum ssp. *aegilopoides*	Wild einkorn	A^mA^m	288 abc	15.4	31.0	3.4 bcd
T. monococcum ssp. *monococcum*	Cultivated einkorn	A^uA^u	281 bc	15.0	31.8	2.7 bcd
Ae. tauschii	DD genome donor	DD	298 a	15.7	33.5	5,1 a
T. turgidum ssp. *dicoccoides*	Wild emmer	A^uA^uBB	306 a	14.7	33.7	4,4 ab
T. turgidum ssp. *durum*	Macaroni wheat	A^uA^uBB	287 abc	14.7	32.8	2.2 cd
T. turgidum ssp. *turgidum*	Cone, rivet wheat	A^uA^uBB	259 c	16.7	31.9	4,1 ab
T. aestivum ssp. *aestivum*	Bread wheat	A^uA^uBBDD	291 ab	15.7	32.2	5,1 a

(1) Number of amino acids in mature peptides.
(2) Relevant gluten T-cell epitopes restricted by HLA-DQ molecules were considered[6].
Means within a column followed by the same letter are not significantly different at $p<0.05$, as determined by the LSD all-pairwise comparisons test.

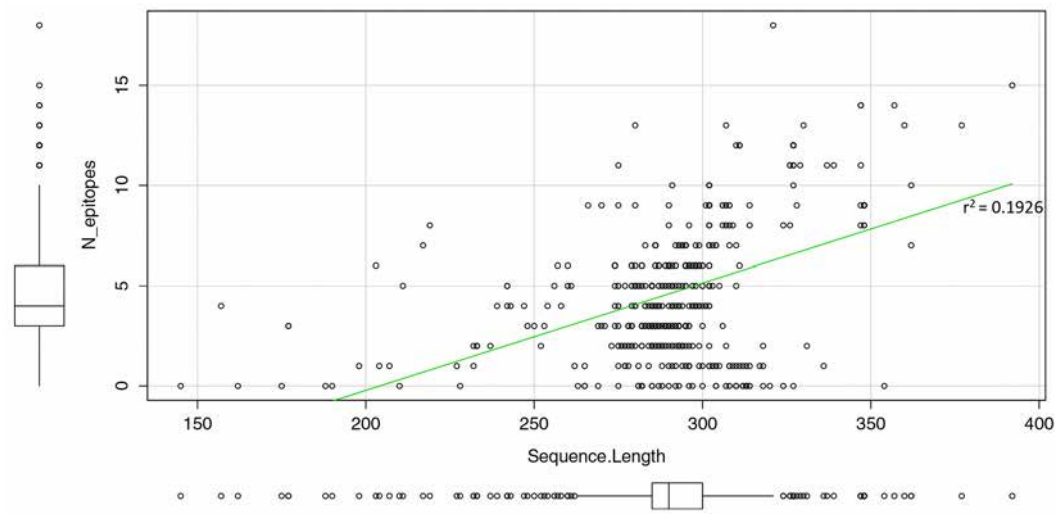


Figure 3. Distribution of number of CD epitopes vs gliadin protein lenght for species indicated in Table 2.

The number of CD related epitopes per sequence is also indicated in Table 2 and plotted in Figure 3. As showed, the lowest number of epitopes per sequence corresponds to oats and barley (Table 2). Rye, *Ae. tauschii*, and bread wheat have the highest number of CD related epitopes per sequence, 5.2, 5.1, and 5.1, respectively. *T. urartu* and *Ae. speltoides*, the donors of the AA and BB genomes, respectively, have a comparable number of epitopes per sequence, and significantly lower than that of *Ae. tauschii,* the donor of the DD genome in bread wheat. The natural hybridization between *T. urartu* and *Ae. speltoides* provided *T. turgidum* ssp. *diccocoides* (Figure 2), wild emmer. According with the gliadin protein data available, this hybridization process seems to have increased the number of CD epitopes per sequence to 4.4 (Table 2). Surprisingly, the development of modern durum wheat varieties (macaroni wheat) led to a significant decrease in the number of epitopes per sequence for this variety (*T. turgidum* ssp. *durum*) but nor for cone or rivet wheat (*T. turgidum* ssp. *turgidum),* which keeps a number of epitopes per sequence comparable to that of its wild ancestor *T. dicoccoides*. *Ae. tauschii* provided the DD genome to bread wheat and hence to modern cultivated hexaploid varieties by the natural hybridization with cultivated emmer

(*T. turgidum* ssp. *dicoccum*) (Figure 2). The number of CD epitopes per sequence was increased in bread wheat (5.1) with respect to wild emmer (4.4). Again, it seems that the hybridization process had increased the number of epitopes per sequence, and this is ascribable to *Ae. tauschii* in the hybridization process. If we look at Figure 3, there are a high number of sequences that contain more than 10 epitopes per sequence. Those sequences are present at a frequency comparable in *Ae. tauschii* and bread wheat (Figure 4A). On the contrary, sequences with low number of CD epitopes per sequence are present at high frequency in *Ae. speltoides*, *T. monococcum* ssp. *monococcum* and *T. turgidum* ssp. *durum* (Figure 4B).

It is clear that the natural hybridization processes described above resulted in genome duplication, and consequently the number of gliadin-related genes should have increased from diploids to tetraploids, and from tetraploids to hexaploids. So, not only the number of epitopes per sequence is relevant but also the number of genes containing those epitopes. There is little information about copy number determination of gliadin genes. Anderson et al.[64] estimated the copy number of α-gliadin genes both in bread and durum wheat. They reported 60 and 150 copies of α-gliadin genes in bread wheat (cv Chinese Spring and Cheyenne, respectively) and 90 for the durum line. However, Lafiandra et al.[65] resolved at least 16 major α-gliadin spots by 2-D PAGE of protein extracts from cv Cheyenne seed. This number is considerably less than the estimated 150 genes for the same cultivar. Among the possible explanations for this discrepancy are that many of the family members are pseudogenes and/or that single protein bands/spots could originate from multiple genes.

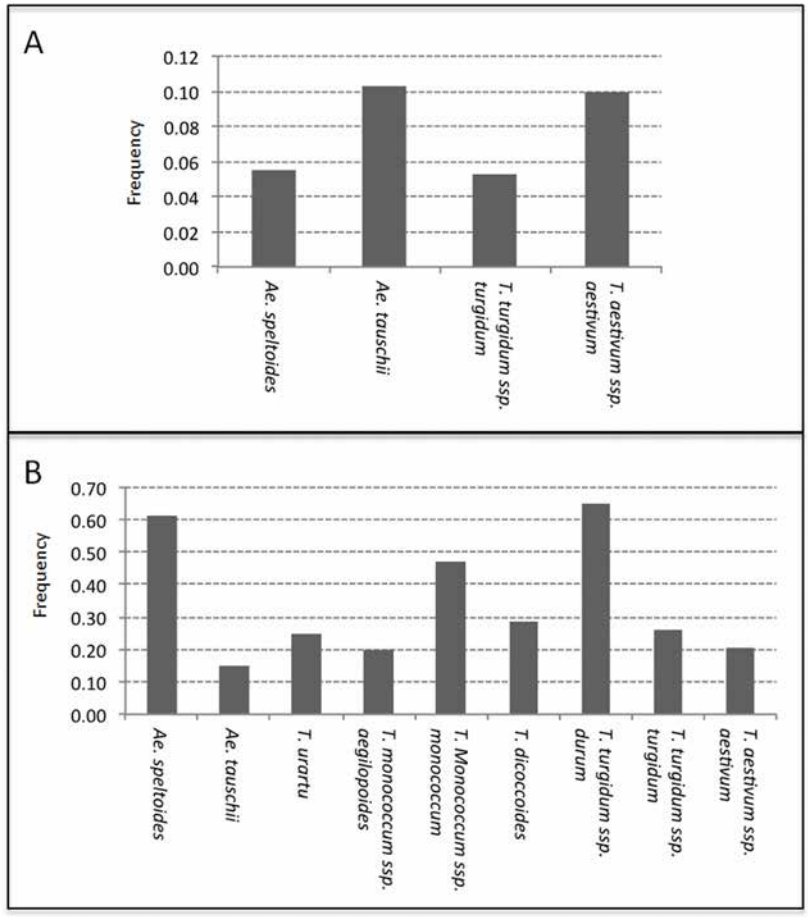


Figure 4. Frequency of sequences containing higher than 10 CD epitopes per sequence (A) or lower than 2 CD epitopes per sequence (B).

Anderson et al.[66] reported the complete set of unique γ-gliadin genes for the wheat cultivar Chinese Spring using a combination of expressed sequence tags (ESTs) and Roche 454 DNA sequences. They reported 11 active genes and two pseudogenes. Four of these genes were assigned to *Ae. tauschii* (the donor of the D genome of bread wheat) while the other γ-gliadins genes were assumed as being encoded in any of the A or B genomes[66].

Regarding the ω-gliadins, the precise number of ω-gliadin proteins and genes in wheat has not been determined. Sabelli and Shewry[67] used Southern blotting to suggest that bread wheat contained about 15-18 ω-gliadin genes. Anderson et al.[68] analyzed all available ω-gliadin DNA sequences and ESTs identified from the large wheat EST collection. They found three groupings of ω-gliadin active gene sequences assigned to each of the three hexaploid wheat genomes, and a fourth group consisting of pseudogenes assigned to the A genome. This is very interesting as active genes reported for each genome were as low as two, and most of ω-gliadin sequences were pseudogenes[68].

7. Conclusions

Wheat is one of the most important crops worldwide, and its extended cultivation is in part due to its high adaptability to different environments and its high yields. Bread wheat accounts for about 95% of cultivated wheat while durum wheat (macaroni wheat) represents only about 5%. The domestication of wheat is the result of a previous natural interspecific hybridization first between diploid, and then between diploid and tetraploid species that resulted in hexaploid wheat. The old farmers began to select the traits that were better adapted to the use. In the 20th century the wheat breeding had its great advance and modern varieties were developed. The gliadin-related genes, responsible for triggering CD, have no adaptive value and therefore, if the toxicity of wheat was increased during the process of domestication and breeding this would have been made unconsciously. During the process of natural hybridization, apparently as a consequence of genome

duplication, the number of gliadin-related genes was increased. Bread wheat, rye, and *Ae. tauschii* have the highest number of CD epitopes per gene, and it seems that in bread wheat, this high number of epitopes is explained by the D genome from *Ae. tauschii*. During the process of domestication and breeding, the number of CD epitopes per gene did not increase and even decreased in some cases. This large variation in the amount of CD4 T cell stimulatory peptides among diploid, tetraploid, and hexaploid wheat accessions is a valuable potential source of variability for the introduction of low CD toxic as a new breeding trait.

Acknowledgments

Financial support from the Spanish Ministry of Economy and Competitiveness (Projects AGL2010-19643, AGL2013-48946-C3-1-R), the European Regional Development Fund (FEDER), and Junta de Andalucía (Project P11-AGR-7920) is acknowledged. Javier Gil-Humanes thanks the 'Fundación Alfonso Martín Escudero' for providing funds through their post-doctoral program.

References

1. Richards MP. *A brief review of the archaeological evidence for Palaeolithic and Neolithic subsistence.* Eur J Clin Nutr. 2002; 56: 1270-78.
   http://dx.doi.org/10.1038/sj.ejcn.1601646
   PMid:12494313
2. Greco L, Timpone L, Abkari A, Abu-Zekry M, Attard T, Bouguerra F et al. *Burden of celiac disease in the Mediterranean area.* World J Gastroenterol. 2011; 17: 4971-8.
   http://dx.doi.org/10.3748/wjg.v17.i45.4971
   PMid:22174546 PMCid:PMC3236588
3. Freeman HJ. *The Neolithic Revolution and Subsequent Emergence of the Celiac Affection.* Int J Celiac Dis. 2013; 1: 19-22.
4. Dicke WK, Weijers HA, Van de Kamer JH. *Coeliac disease. II. The presence in wheat of a factor having a deleterious effect in cases of coeliac disease.* Acta Paediatr. 1953; 42: 34-42.
   http://dx.doi.org/10.1111/j.1651-2227.1953.tb05563.x
   PMid:13050382
5. Troncone R, Auricchio R, Granata V. *Issues related to gluten-free diet in coeliac disease.* Curr Opin Clin Nutr Metab Care. 2008; 11: 329-33.
   http://dx.doi.org/10.1097/MCO.0b013e3282f795f8
   PMid:18403932
6. Sollid LM, Qiao S-W, Anderson RP, Gianfrani C, Koning F. *Nomenclature and listing of celiac disease relevant gluten T-cell epitopes restricted by HLA-DQ molecules.* Immunogenetics. 2012; 64: 455-60.
   http://dx.doi.org/10.1007/s00251-012-0599-z
   PMid:22322673 PMCid:PMC3349865
7. Husby S, Koletzko S, Korponay-Szabo IR, Mearin ML, Phillips A, Shamir R et al. *European Society for Pediatric Gastroenterology, Hepatology, and Nutrition guidelines for the diagnosis of coeliac disease.* J Pediatr Gastroenterol Nutr. 2012; 54: 136-60.
   http://dx.doi.org/10.1097/MPG.0b013e31821a23d0
   PMid:22197856
8. Kang JY, Kang AHY, Green A, Gwee KA, Ho KY. *Systematic review: worldwide variation in the frequency of coeliac disease and changes over time.* Aliment Pharmacol Ther. 2013; 38: 226-45.
   http://dx.doi.org/10.1111/apt.12373
   PMid:23782240

9. Rubio-Tapia A, Kyle RA, Kaplan EL, Johnson DR, Page W, Erdtmann F et al. *Increased prevalence and mortality in undiagnosed celiac disease.* Gastroenterology. 2009; 137: 88-93.

http://dx.doi.org/10.1053/j.gastro.2009.03.059
PMid:19362553 PMCid:PMC2704247

10. Kasarda DD. *Can an increase in celiac disease be attributed to an increase in the gluten content of wheat as a consequence of wheat breeding?* J Agric and Food Chem. 2013; 61: 1155-9.

http://dx.doi.org/10.1021/jf305122s
PMid:23311690 PMCid:PMC3573730

11. Salamini F, Ozkan H, Brandolini A, Schafer-Pregl R, Martin W. *Genetics and geography of wild cereal domestication in the near east.* Nat Rev Genet. 2002; 3: 429-41.

PMid:12042770

12. Huang S, Sirikhachornkit A, Faris J, Su X, Gill B, Haselkorn R et al. *Phylogenetic analysis of the acetyl-CoA carboxylase and 3-phosphoglycerate kinase loci in wheat and other grasses.* Plant Mol Biol. 2002; 48: 805-20.

http://dx.doi.org/10.1023/A:1014868320552
PMid:11999851

13. Marcussen T, Sandve SR, Heier L, Spannagl M, Pfeifer M, Jakobsen KS et al. *Ancient hybridizations among the ancestral genomes of bread wheat.* Science. 2014; 345, 1250092.

http://dx.doi.org/10.1126/science.1250092
PMid:25035499

14. Zohary D, Hopf M, Weiss E. *Domestication of plants in the old world.* Oxford: Oxford Univ. Press. 2012.

15. Weiss E, Kislev ME, Hartmann A. *Anthropology. Autonomous cultivation before domestication.* Science. 2006; 312: 1608-10.

http://dx.doi.org/10.1126/science.1127235
PMid:16778044

16. Lev E, Kislev ME, Bar-Yosef O. *Mousterian vegetal food in Kebara Cave, Mt. Carmel.* J Archaeol Sci. 2005; 32: 475-84.

http://dx.doi.org/10.1016/j.jas.2004.11.006

17. Nevo E. *Evolution of wild barley and barley improvement.* In: Zhang G, Chengdao L, Xu L (Eds.). Advance in barley sciences: Proceedings of 11th International Barley Genetics Symposium. Hangzhou: Zhejiang University Press-Springer. 2013; 1-16.

18. Clegg MT, Brown AHD, Whitfeld PR. *Chloroplast DNA diversity in wild and cultivated barley - Implications for genetic conservation.* Genet Res. 1984; 43: 339-43.

http://dx.doi.org/10.1017/S0016672300026112

19. Neale DB, Saghaimaroof MA, Allard RW, Zhang Q, Jorgensen RA. *Chloroplast DNA diversity in populations of wild and cultivated barley.* Genetics. 1988; 120: 1105-10.

20. Moore AMT, Hillman GC, Legge AJ. *Village on the Euphrates: From Foraging to Farming at Abu Hureyra.* Oxford: Oxford University Press. 2000.

21. Jaaska V. *On the Origin and In Statu Nascendi Domestication of Rye and Barley.* In: Damania AB, Valkoun J, Wilcox G, Qualset CO (Eds.). The Origins of Agriculture and Crop Domestication. Aleppo, Syria: ICARDA, IPGRI, FAO and UC/GRCP. 1998; 210-7.

22. Sencer HA, Hawkes JG. *On the origin of cultivated rye.* Biol J Linn Soc. 1980; 13: 299-313.
http://dx.doi.org/10.1111/j.1095-8312.1980.tb00089.x

23. Zohary D, Hopf M. *Domestication of plants in the Old World.* Oxford, UK: Oxford Science Publications. 1988.

24. Dvořák J, McGuire PE, Cassidy B. *Apparent sources of the A genomes of wheats inferred from polymorphism in abundance and restriction fragment length of repeated nucleotide sequences.* Genome. 1988; 30: 680-9.
http://dx.doi.org/10.1139/g88-115

25. Dvořák J, Zhang HB. *Reconstruction of the phylogeny of the genus Triticum from variation in repeated nucleotide sequences.* Theor Appl Genet. 1992; 84: 419-29.
http://dx.doi.org/10.1007/BF00229502
PMid:24203203

26. McFadden ES, Sears ER. *The origin of Triticum spelta and its free-theshing hexaploid relatives.* J Hered. 1946; 37: 81-7.
PMid:20985728

27. Kerber ER, Rowland GG. *Origin of the free-threshing character in hexaploid wheat.* Can J Genet Cytol. 1974; 16: 145-54.
http://dx.doi.org/10.1139/g74-014

28. Feldman M. *Wheats.* In: Smartt J, Simmonds NW (Eds.). Evolution of crop plants. Harlow, UK: Longman Scientific and Technical. 1995.

29. Shewry PR, Halford NG. *Cereal seed storage proteins: structures, properties and role in grain utilization.* J Exp Bot. 2002; 53: 947-58.
http://dx.doi.org/10.1093/jexbot/53.370.947

30. Miflin BJ, Field JM, Shewry PR. *Cereal storage proteins and their effect on technological properties.* In: Daussant J, Mossé J, Vaughan J (Eds.). Seed Proteins. London, UK: Academic Press. Inc. 1983; 255-319.

31. Shewry PR, Parmar S, Field JM. *Two dimensional electrophoresis of cereal prolamins: applications to biochemical and genetic analyses.* Electrophoresis. 1988; 9: 727-37.

http://dx.doi.org/10.1002/elps.1150091108
PMid:3250875

32. Payne PI, Holt LM, Jackson EA, Law CN. *Wheat storage proteins: their genetics and their potential for manipulation by plant breeding.* Phil Trans R Soc Lond B. 1984; 304: 359-71.

http://dx.doi.org/10.1098/rstb.1984.0031

33. Payne PI. *Genetics of wheat storage proteins and the effects of allelic variation on bread-making quality.* Annu Rev Plant Physiol. 1987; 38: 141-53.

http://dx.doi.org/10.1146/annurev.pp.38.060187.001041

34. Liu CY. *Identification of a new low Mr glutenin subunit locus on chromosome 1B of durum wheat.* J Cereal Sci. 1995; 21: 209-13.

http://dx.doi.org/10.1016/0733-5210(95)90037-3

35. Metakovsky EV, Novoselskaya AY, Kopus MM, Sobko TA, Sozinov AA. *Blocks of gliadin components in winter wheat detected by one-dimensional polyacrylamide gel electrophoresis.* Theo Appl Genet. 1984. 67: 559-68.

http://dx.doi.org/10.1007/BF00264904
PMid:24258847

36. McIntosh RA, Yamazaki Y, Dubcovsky J, Rogers WJ, Morris C, Appels R et al. *Catalogue of gene symbols for wheat.* 2013. http://www.shigen.nig.ac.jp/wheat/komugi/genes/macgene/2013/GeneSymbol.pdf

37. Wieser H. *Comparative investigations of gluten proteins from different wheat species I. Qualitative and quantitative composition of gluten protein types.* Eur Food Res Technol. 2000; 211: 262-8.

38. http://dx.doi.org/10.1007/s002170000165

39. Shewry PR, Tatham AS. *The characteristics, structures and evolutionary relationships of prolamins.* In: Shewry PR, Casey R (Eds.). Seed Proteins. Kluwer Academic Publishers, Dordrecht. 1999; 11e33.

40. Shewry PR, Tatham AS. *The prolamin storage proteins of cereal seeds: structure and evolution.* Biochem J. 1990; 267: 1-12.

PMid:2183790 PMCid:PMC1131235

41. Heidecker G, Messing J. *Structural analysis of plant genes.* Annu Rev Plant Physiol. 1986; 37: 439-66.

http://dx.doi.org/10.1146/annurev.pp.37.060186.002255

42. Pelger S, Säll T, Bengtsson BO. *Evolution of hordein gene organization in three Hordeum species.* Hereditas. 1993; 119: 219-31.

http://dx.doi.org/10.1111/j.1601-5223.1993.00219.x

43. Shewry PR, Napier JA, Tatham AS. *Seed Storage Proteins: Structures and Biosynthesis.* Plant Cell. 1995; 7: 945-56.
http://dx.doi.org/10.1105/tpc.7.7.945
PMid:7640527 PMCid:PMC160892

44. Wieser H, Koehler P. *Is the calculation of the gluten content by multiplying the prolamin content by a factor of 2 valid?* Eur Food Res Technol. 2009; 229: 9-13.
http://dx.doi.org/10.1007/s00217-009-1020-5

45. Harlan JR. *The early history of wheat: earliest traces to the sack of Rome.* In: Evans LT, Peacock WJ (Eds.). Wheat science: today and tomorrow. Cambridge, UK: Cambridge University Press. 1981; 1-29.
PMid:7213279

46. Kemp BJ. *Ancient Egypt: anatomy of a civilization.* 2nd ed. London, UK: Routledge. 2005.
http://dx.doi.org/10.4324/9780203468821

47. Matz SA. *Bakery technology and engineering.* Westport CT, USA: AVI Publishers Company Inc. 1960.

48. Frankel OH. *Genetic conservation in perspective.* In: Frankel OH, Bennett E (Eds.). Genetic resources in plants: their exploration and conservation. Oxford and Edinburgh: UK Blackwell Publishers. 1970; 469-89.

49. Frankel OH, Hawkes JG. *Genetic resources: the past ten years and the next.* In: Frankel OH, Hawkes JG (Eds.). Crop genetic resources for today and tomorrow. Cambridge, UK: Cambridge University Press. 1975; 1-11.

50. Borojevic K, Borojevic K. *The transfer and history of "Reduced Height Genes" (Rht) in wheat from Japan to Europe.* J Hered. 2005; 96: 455-9.
http://dx.doi.org/10.1093/jhered/esi060
PMid:15829727

51. Arentz-Hansen H, Mcadam SN, Molberg Ø, Fleckenstein B, Lundin KE, Jorgensen TJ et al. *Celiac lesion T cells recognize epitopes that cluster in regions of gliadins rich in proline residues.* Gastroenterology. 2002; 123: 803-9.
http://dx.doi.org/10.1053/gast.2002.35381
PMid:12198706

52. Spaenij-Dekking L. *Natural variation in toxicity of wheat: Potential for selection of nontoxic varieties for celiac disease patients.* Gastroenterology. 2005; 129: 797-806.
http://dx.doi.org/10.1053/j.gastro.2005.06.017
PMid:16143119

53. van den Broeck H, Chen HB, Lacaze X, Dusautoir JC, Gilissen L, Smulders M et al. *In search of tetraploid wheat accessions reduced in celiac disease-related gluten epitopes.* Mol Biosyst. 2010; 6: 2206-13.
http://dx.doi.org/10.1039/c0mb00046a
PMid:20714643

54. van den Broeck HC, de Jong HC, Salentijn EMJ, Dekking L, Bosch D, Hamer RJ et al. *Presence of celiac disease epitopes in modern and old hexaploid wheat varieties: wheat breeding may have contributed to increased prevalence of celiac disease.* Theor Appl Genet. 2010; 121: 1527-39.

http://dx.doi.org/10.1007/s00122-010-1408-4
PMid:20664999 PMCid:PMC2963738

55. Molberg Ø, Uhlen AK, Jensen T, Flæte NS, Fleckenstein B, Arentz-Hansen H et al. *Mapping of gluten T-cell epitopes in the bread wheat ancestors: Implications for celiac disease.* Gastroenterology. 2005; 128: 393-401.

http://dx.doi.org/10.1053/j.gastro.2004.11.003
PMid:15685550

56. Chidzanga C. *Analysing the Celiac Disease toxicity in transcribed α-gliadin genes of Aegilops tauschii genotypes using deep RNA-amplicon sequencing.* MSc Thesis. Wageningen University. 2012.

57. Tanner GJ, Howitt CA, Forrester RI, Campbell PM, Tye-Din JA et al. *Dissecting the T-cell response to hordeins in coeliac disease can develop barley with reduced immunotoxicity.* Aliment Pharmacol Ther. 2010; 32: 1184-91.

http://dx.doi.org/10.1111/j.1365-2036.2010.04452.x
PMid:21039679

58. Spaenij-Dekking EH, Kooy-Winkelaar EM, Nieuwenhuizen WF, Drijfhout JW, Koning F. *A novel and sensitive method for the detection of T cell stimulatory epitopes of α/β- and γ-gliadin.* Gut. 2004; 53: 1267-73.

http://dx.doi.org/10.1136/gut.2003.037952
PMid:15306583 PMCid:PMC1774189

59. Zohary D. *Unconscious selection and the evolution of domesticated Plants.* Econ Bot. 2004; 58: 5-10.

http://dx.doi.org/10.1663/0013-0001(2004)058[0005:USATEO]2.0.CO;2

60. Peng JH, Sun D, Nevo E. *Domestication evolution, genetics and genomics in wheat.* Mol Breed. 2011. 28: 281-301.

http://dx.doi.org/10.1007/s11032-011-9608-4

61. Zhang L-Y, Liu D-C, Guo X-L, Yang W-L, Sun J-Z, Wang D-W et al. *Genomic distribution of quantitative trait loci for yield and yield-related traits in common wheat.* J Integr Plant Biol. 2010; 52: 996-1007.

http://dx.doi.org/10.1111/j.1744-7909.2010.00967.x
PMid:20977657

62. Erayman M, Sandhu D, Sidhu D, Dilbirligi M, Baenziger PS, Gill KS. *Demarcating the gene-rich regions of the wheat genome.* Nucleic Acids Res. 2004; 32: 3546-65.

http://dx.doi.org/10.1093/nar/gkh639
PMid:15240829 PMCid:PMC484162

63. Dilbirligi M, Erayman M, Sandhu D, Sidhu D, Gill KS. *Identification of Wheat Chromosomal Regions Containing Expressed Resistance Genes.* Genetics. 2004; 166: 461-81.

http://dx.doi.org/10.1534/genetics.166.1.461
PMid:15020436 PMCid:PMC1470719

64. Anderson OD, Litts JC, Greene FC. *The α-gliadin gene family. I. Characterization of ten new wheat α-gliadin genomic clones, evidence for limited sequence conservation of flanking DNA, and Southern analysis of the gene family.* Theor Appl Genet. 1997; 95: 50-8.

http://dx.doi.org/10.1007/s001220050531

65. Lafiandra D, Kasarda DD, Morris R. *Chromosomal assignment of genes coding for the wheat gliadin protein components of the cultivars "Cheyenne" and "Chinese Spring" by two-dimensional (two-pH) electrophoresis.* Theor Appl Genet. 1984; 68: 531-9.

http://dx.doi.org/10.1007/BF00285007
PMid:24257826

66. Anderson OD, Huo N, Gu YQ. *The gene space in wheat: the complete γ-gliadin gene family from the wheat cultivar Chinese Spring.* Funct Integr Genomics. 2013. 13: 261-73.

http://dx.doi.org/10.1007/s10142-013-0321-8
PMid:23564033

67. Sabelli PA, Shewry PR. *Characterization and organization of gene families at the Gli-1 loci of bread and durum wheats by restriction fragment analysis.* Theor Appl Genet. 1991; 83: 209-16.

http://dx.doi.org/10.1007/BF00226253
PMid:24202360

68. Anderson OD, Gu YQ, Kong X, Lazo GR, Wu J. *The wheat ω-gliadin genes: structure and EST analysis.* Funct Integr Genomics. 2009; 9: 397-410.

http://dx.doi.org/10.1007/s10142-009-0122-2
PMid:19367421 PMCid:PMC2700870

69. Herpen TW van, Goryunova SV, Schoot J van der, Mitreva M, Salentijn E, Vorst O et al. *Alpha-gliadin genes from the A, B, and D genomes of wheat contain different sets of celiac disease epitopes.* BMC Genomics. 2006; 7(1): 1.

http://dx.doi.org/10.1186/1471-2164-7-1
PMid:16403227 PMCid:PMC1368968

70. Pizzuti D, Buda A, D'Odorico A, D'Incà R, Chiarelli S, Curioni A et al. *Lack of intestinal mucosal toxicity of Triticum monococcum in celiac disease patients.* Scand J Gastroenterol. 2006. 41: 1305-11.

http://dx.doi.org/10.1080/00365520600699983
PMid:17060124

71. Vaccino P, Becker H-A, Brandolini A, Salamini F, Kilian B. *A catalogue of Triticum monococcum genes encoding toxic and immunogenic peptides for celiac disease patients.* Mol Genet Genomics. 2009; 281: 289-300.

http://dx.doi.org/10.1007/s00438-008-0412-8
PMid:19104838 PMCid:PMC2757618

72. Mitea C, Salentijn EMJ, van Veelen P, Goryunova SV, van der Meer IM, van den Broeck HC et al. *A Universal Approach to Eliminate Antigenic Properties of Alpha-Gliadin Peptides in Celiac Disease.* PLoS ONE. 2010; 5(12).

http://dx.doi.org/10.1371/journal.pone.0015637
PMid:21179575 PMCid:PMC3002971

73. Salentijn EM, Mitea DC, Goryunova SV, Meer IM van der, Padioleau I, Gilissen LJ et al. *Celiac disease T-cell epitopes from gamma-gliadins: immunoreactivity depends on the genome of origin, transcript frequency, and flanking protein variation.* BMC Genomics. 2012; 13(1): 277.

http://dx.doi.org/10.1186/1471-2164-13-277
PMid:22726570 PMCid:PMC3469346

74. Gianfrani C, Maglio M, Aufiero VR, Camarca A, Vocca I, Iaquinto G et al. *Immunogenicity of monococcum wheat in celiac patients.* Am J Clin Nutr. 2012; 96: 1339-45.

http://dx.doi.org/10.3945/ajcn.112.040485
PMid:23134879

75. Šuligoj T, Gregorini A, Colomba M, Ellis HJ, Ciclitira PJ. *Evaluation of the safety of ancient strains of wheat in coeliac disease reveals heterogeneous small intestinal T cell responses suggestive of coeliac toxicity.* Clin Nutr. 2013; 32: 1043-9.

http://dx.doi.org/10.1016/j.clnu.2013.02.003
PMid:23465776

76. Gregorini A, Colomba M, Ellis HJ, Ciclitira PJ. *Immunogenicity Characterization of Two Ancient Wheat α-Gliadin Peptides Related to Coeliac Disease.* Nutrients. 2009; 1: 276-90.

http://dx.doi.org/10.3390/nu1020276
PMid:22253984 PMCid:PMC3257593

77. Salentijn EM, Goryunova SV, Bas N, van der Meer IM, van den Broeck HC, Bastien T et al. *Tetraploid and hexaploid wheat varieties reveal large differences in expression of alpha-gliadins from homoeologous Gli-2 loci.* BMC Genomics. 2009; 10(1): 48.

http://dx.doi.org/10.1186/1471-2164-10-48
PMid:19171027 PMCid:PMC2636828

78. Xie Z, Wang C, Wang K, Wang S, Li X, Zhang Z et al. *Molecular characterization of the celiac disease epitope domains in α-gliadin genes in Aegilops tauschii and hexaploid wheats (Triticum aestivum L.).* Theor Appl Genet. 2010; 121: 1239-51.

http://dx.doi.org/10.1007/s00122-010-1384-8
PMid:20556595

79. Carroccio A, Di Prima L, Noto D, Fayer F, Ambrosiano G, Villanacci V et al. *Searching for wheat plants with low toxicity in celiac disease: Between direct toxicity and immunologic activation.* Dig Liver Dis. 2011; 43: 34-9.

http://dx.doi.org/10.1016/j.dld.2010.05.005
Pmid:20554485

Chapter 16

Analytical Tools for Gluten Detection. Policies and Regulation

Mª Carmen Mena[1], Carolina Sousa[2]

[1] Proteomics Facility and Gluten Unit. Spanish National Centre for Biotechnology. Spanish National Research Council (CSIC), Consejo Superior de Investigaciones Científicas, Madrid, Spain.

[2] Departamento de Microbiología y Parasitología, Facultad de Farmacia, Universidad de Sevilla, Sevilla, Spain.

mcmena@cnb.csic.es, csoumar@us.es

Doi: http://dx.doi.org/10.3926/oms.264

How to cite this chapter

Mena MC, Sousa C. *Analytical Tools for Gluten Detection. Policies and Regulation.* In Arranz E, Fernández-Bañares F, Rosell CM, Rodrigo L, Peña AS, editors. *Advances in the Understanding of Gluten Related Pathology and the Evolution of Gluten-Free Foods*. Barcelona, Spain: OmniaScience; 2015. p. 527-564.

Abstract

Gluten proteins are mixture of two groups of proteins named prolamins and glutelins. Many of these proteins are resistant to digestive enzymes and therefore after ingestion of gluten containing foods, there may be immunological potentially toxic peptides in small-bowel mucosal for celiac disease predisposed individuals. Since the only effective treatment of celiac disease is the avoidance of gluten containing foods, and taking into account the high prevalence of this disease, is mandatory to have reliable methods for gluten determination to ensure that consumption of labeled "gluten-free" food is safe for celiacs. Several factors may affect the results in gluten analysis such as the modifications of proteins produced during manufacturing of foods, the interference of the mixture of ingredients, and the use of the appropriate standard for gluten analysis. There are different available techniques for gluten analysis in foods. The most widely used are those based in the classical immunological techniques using different antibodies mainly enzyme-linked-immunosorbent assays, western blot, and lateral flow devices dipsticks. In addition, biosensors technologies can be applied to gluten analysis. Regarding the non-immunological tools, the most useful ones are the proteomics techniques and real time quantitative PCR. In most of the countries, regulations concerning the composition and labeling of foodstuffs suitable for people intolerant to gluten states that limit values for "gluten-free" foods and foods "specially processed to reduce the gluten content" are 20 and 100 mg/Kg of gluten respectively. Therefore any technique used must have at least a sensitivity to reach this lower limit.

Keywords

Gluten-free foods, ELISA, prolamins, glutelins.

1. Introduction

Gluten proteins are an extremely polymorphic mixture of two different groups of proteins named prolamins and glutelins, which are present as either monomers or as oligomers and polymers linked by interchain disulphide bonds. Many of these proteins are resistant to digestive enzymes and therefore after ingestion of gluten containing foods, there may be immunological potentially toxic peptides in small-bowel mucosal for celiac disease (CD) predisposed individuals. Tradicionally only prolamins were considered as immunotoxic in adults, but in children and certain adults, there has been proved an immune response to glutenins. Prolamins are the major class of storage proteins in wheat, rye, barley and oats and their function is to store nitrogen, carbon and sulfur in the grain endosperm. They belong to the prolamin superfamily together with several plant food allergens such as 2S albumins, nonspecific lipid transfer proteins and cereal alpha-amylase/tripsin inhibitors[1,2]. Osborne was the first to suggest the name prolamins for this group of cereal proteins because of their high content of proline and amide nitrogen[3]. Osborne characterized cereal prolamins as freely soluble in relatively strong ethyl alcohol, but insoluble in absolute alcohol, slightly in water, and easily soluble in very dilute acids and bases[3].

Prolamins are distinct from other proteins in their high content in the amino acids proline (Pro or P) and glutamine (Gln or Q) that comprise 15 and 35 % of the gluten proteins, respectively[4]. A special characteristic of proline is its ability to make β-turns. These turns form a tighter helix than an α-helix and thus enable proteins to be packed more efficiently into a small space. This is convenient for a plant to store vital amino acids, but makes it difficult for enzymes to hydrolyze the tight structures of prolamins. As a consequence, these proteins are poorly degraded by gastric and pancreatic digestive proteases in gastrointestinal tract. Some partially hydrolyzed peptides may enter into the intestinal epithelium and have access to the propia lamina by a mechanism than remain unknown, causing damage in celiac disease patients. The glutamine residues of these peptides are deamidated by a tisular transglutaminase (tTG) turning them into glutamic

acid, increasing the immunoestimulatory potential of the peptides as the negative charge enhance the joining of the peptide to the DQ2 or DQ8 receptors of antigen presenting cells causing a response from intestinal CD4+ T cells and damage in mucosal villi. However, this disease not only affects the gut, but also it is a systemic disease that may cause injury to the skin, liver, joints, brain, heart, and other organs.

Gluten proteins can be classified in different ways regarding its characteristics and species. Wheat, barley and rye contain celiac-active prolamins, whereas maize, rice, millet and sorghum do not. Oats contains low amounts of the prolamin type avenin. Wheat, rye, barley and some oat cultivars have been established to trigger celiac disease, whereas maize, rice and buckwheat were found not to be harmful.

Prolamins can be divided into groups based on their sulfur content, size or sequence homologies[5]. Shewry and Tatham divided prolamins based on their sulfur content into S-poor, S-rich and High molecular weight (HMW) prolamins. Whereas Wieser[6] divided prolamins into three groups based on their size: HMW (80000-120000 Da), medium molecular weight (MMW) (52000-80000 Da) and low molecular weight (LMW) (30000-52000 Da) groups. The HMW group consists of HMW glutenin subunits of wheat, HMW secalins, and D-hordeins. The MMW group consists of omega-type gliadins and secalins and C-hordeins. The LMW group consists of alpha/beta gliadins and gamma-gliadins, gamma secalins (monomeric gamma-40 and polymeric gamma-75), gamma-hordeins, LMW glutenins and B-hordeins.. The storage proteins of oats are different from those of wheat, barley and rye. Avenins are monomeric and polymeric proteins, and can be divided into groups based their molecular weights. The molecular weights of alpha-avenins are about 12000-18000 Da and those of gamma-avenins about 22000-35000 Da. The HMW are similar to LMW-GS (Low molecular weight glutenin subunits) from wheat[7,8] (Table 1).

Table 1. Characterization of storage protein types of wheat, barley rye and oats.

Group	Wheat	Barley	Rye	Oats	Type
Prolamins	Alpha/Beta gliadins Gamma gliadins Omega gliadins	Gamma hordeins C hordeins B Hordeins	Gamma 40k-secalins Omega secalins	Alpha avenins Gamma avenins	Monomeric
Glutelins	HMW glutenins LMW glutenins	D hordeins	Gamma 75k-secalins HMW secalins	LMW avenins	Polymeric

The calculation of gluten content is usually performed based on the assumption of a 1:1 ratio between gliadins and glutenins, as traditionally, gluten proteins have been divided into roughly equal fractions according to their solubility in alcohol–water solutions: the soluble gliadins and the insoluble glutenins. Nevertheless, some studies have reported slight differences in the ratio between gliadins and glutenins, suggesting a factor around 65:35 mainly for barley and rye and depending on the variety and specie of the cereal[9].

CD goes in remission when the patients are put on a gluten-exclusion diet, and patients relapse when gluten is reintroduced into the diet[10,11]. Complying with a gluten-free diet (GFD) is difficult and affects the patients' quality of life, but a strict diet is critical to reduce morbidity and mortality[12]. However, this generates numerous social and economic repercussions. It is not easy to maintain a diet with zero gluten content because gluten contamination of food is commonplace. Even products specifically targeted at dietary treatment of CD may contain tiny amounts of gluten proteins, either because of the cross-contamination of originally gluten-free cereals during the milling,

storage, and manipulation, or because of the presence of wheat starch as a major ingredient. Therefore, standardized methods of analysis are needed to quantitatively determine the gluten content of food and provide the basis for enforcing regulations regarding use of the term "gluten-free" in food labelling.

2. Analytical Tools for Gluten Analysis

2.1. Factors Affecting Gluten Analysis

2.1.1. Modifications of Proteins During Manufacturing of Foods

Proteins in foods are modified during manufacturing by different processes to improve their functionally and increase their usage in different applications in the food industry. These modifications include mainly deamidation, transamidation and degradation by different types of hydrolysis. All of these modifications can also happen naturally due to enzymes in cereal seeds that are released when cells are broken down during processing. Hydrolysis may decrease the toxicity of gluten[13] but this fragmentation of peptides can make more difficult the analysis of gluten in these foods[14]. Deamidation of gluten proteins decreases the affinity and recognition of antibodies to gluten proteins and peptides, which may lead to underestimation when immunoassays are used to quantify gluten content of foods[15]. During the processing of some foods, proteins are treated at high temperatures in a dry state at a neutral pH, forming isopeptide bonds between lysine and asparagine and glutamine residues. Furthermore, the heat-treatment of cooked and baked products leads to the formation of protein aggregates in an insoluble matrix that makes analyses even more difficult. Therefore it is necessary to use an extraction system giving complete recovery of both prolamins and glutelins. The so-called cocktail solution combines reducing and disaggregating agents to extract gluten proteins completely as this ensures that protein aggregates are disrupted[16]. However, it is not compatible with all the techniques used for gluten analysis because beta-mercaptoethanol interferes with the specific binding of the antibodies. In order to solve this problem, another extraction

solution called UPEX (universal prolamin and glutelin extractant solution) leads to a complete extraction and it is compatible with all gluten analysis procedures[14]. This solution includes the odourless reducing agent, Tris (2-carboxyethyl)-phosphine(TCEP) that is more specific for breaking disulphide bridges and less toxic than the other reducing agents commonly used[17] and the disaggregating agent N-Lauroylsarcosine, widely used in plant cell lysis, which contributes to opening polypeptide chains and is even more efficient than guanidine hydrochloride (patent WO 2011/07039 A2).

In addition, a novel universal gluten extraction solution (UGES) has recently been described (Biomedal Diagnostics, Seville, Spain). The components of this gluten extraction solution are a reducing agent, a solubilising agent (arginine) and an antiseptic agent in ethanolic solution (patent WO 201231612). The UGES procedure gave high extraction efficiency from both simple and complex matrices even if they had been heat-processed.

2.2.2. Interference of Ingredients

There are certain foods in which ingredients may interfere with the results yielding lower or higher values than real gluten content. For instance, in the case of chocolate and other foods containing tannins, when a spiked sample with a known value of gluten is analyzed, the observed recovery is lower than expected. Tannins are plant polyphenols that bind and precipitate proteins (such as gliadins) and yield large tannic acid-gliadin complexes, therefore interfering in the determination of the gluten content in food. As well as gliadins, other proteins such as gelatin are susceptible to bind polyphenols. In order to solve this problem a modified extraction protocol combining the UPEX solution with fish gelatin and polyvinylpyrrolidone (PVP) must be applied. This modified protocol should be applied routinely or at least when analyzing foods containing unknown ingredients[14].

In addition, other proteins may interfere in the analysis resulting in an overestimation of gluten content. This phenomenon has been observed when gluten is analyzed in soy based foods such as soy drinks after extraction with 60% ethanol. Nevertheless, when UPEX solution is used for extracting gluten

proteins, the interference components do not remain in solution and there is no overestimation. As that interferences were not observed in the main ingredient in soy drinks (soybeans) it was suggested that processing soybeans to produce soy drinks might cause changes in the solubility of these proteins leading them to remain in suspension in 60% ethanol but nor in UPEX/60% ethanol[14].

2.1.3. Standards for Gluten Analysis

Another critical point in gluten analysis is the use of a correct standard representative of gluten proteins to be analyzed in any kind of foods. The Working Group on Prolamin Analysis and Toxicity (PWG) gliadin standard is the most used internationally standard for gluten analysis. The PWG standard preparation was developed as part of a multi-centre project whose aim was to produce an international reference standard which would enable validation of quantitative results obtained using different methods. This standard is obtained from a mixture of 28 wheat cultivars representative of the European wheat-producing countries[18]. A conventional protocol for prolamin extraction was followed, with some modifications made for the purpose of obtaining a large quantity with few contaminants. Characterization was then begun by the most wide-ranging methodology available (RP-HPLC, polyacrylamide gel electrophoresis, capillary electrophoresis, MALDI-TOF MS, immunoassays). Its stability and solubility were also evaluated. In this manner, a highly stable and completely soluble reagent was obtained which has been extensively characterized and can be used as reference material[19].

Nevertheless, cereals contain a greater number of proteins than those present in the PWG standard. Some authors have suggested that it would be more correct to use a hydrolyzed standard to quantify peptides of partially hydrolyzed gluten in fermented wheat, rye, and barley products[19]. Commercial foods usually have only partial hydrolysis of proteins and, when the proteins are exhaustively hydrolyzed, the toxicity for celiac patients of the peptides generated usually disappears. Comparison of the intact PWG gliadin standard

with a partially enzymatically digested gliadin standard demonstrated that the resulting curves were similar in each case when applying a competitive immunoassay and therefore the intact PWG gliadin standard can be used as a more accessible gold standard as it is more difficult to prepare a reproducible hydrolyzed standard[14].

However, other strategies based on the use of immunotoxic peptides of gluten as standard are being developed for the analysis of samples using hydrolyzed gluten. This standard presented a high degree of repeatability, reproducibility and stability and the results obtained were correlated with the potential relative immunotoxicity of gluten[20,21].

2.2. Immunological Techniques

The most used methods for gluten analysis in foods are based on immunological analysis. Immunological methods are based on the antibodies raised against the different prolamin fractions or specific sequences found in gluten proteins. The requirement for the assays is that they should measure the harmful proteins and peptides, regardless of the type of food or manufacturing process[22].

There are many immunoanalytical-based commercial kits available for the quantification of gliadin/gluten/wheat proteins, including rapid test kits (lateral flow device assay format).

2.2.1. Enzyme-Linked Immunosorbent Assays (ELISAs)

Since the mid-1980s, multiple immunochemical gluten analysis methods have been developed[23]. The earlier methods were reviewed by Howdle and Losowsky[24]. Two ELISAs formats, sandwich and competitive, are the recommended methods for gluten analysis in gluten-free foods. The sandwich method is based on two antibodies. The first is called coating antibody and the second detecting antibody. The coating antibody is bound to the bottom of the microplate wells and the detecting antibody is used to recognize the antigens attached to the coating antibody. An enzyme is linked to the

detecting antibody. Commonly used enzymes include horseradish peroxidase (HRP) and alkaline phosphatase (AP). The purpose of the enzyme is to induce a color reaction involving a chromogen, which can be measured by spectrophotometric methods. The coating and detecting antibody can be the same antibody or they can be different. For this type of analysis, the sample protein must have at least two epitopes recognized for the two antibodies. Therefore, the sandwich technique is not suitable for hydrolyzed proteins.

The competitive method is based on the competition between sample proteins and standard proteins. Only one antibody is used in this assay, which makes it suitable for also detecting small, hydrolyzed proteins and peptides. However, the robustness of the method may not be as good as that obtained with the sandwich format, since nonspecific binding is more likely when only one binding site is needed for detection. The enzyme in competitive systems can be conjugated with the antibody or with the standard peptide/protein. In the competitive assay format, the intensity of the color reaction is inversely proportional to the amount of antigen in the sample.

Many ELISA assays, both sandwich and competitive, are commercially available (Table 2). However, the results obtained with such kits are often non comparable, since they target different gluten components and differ in antibody specificity, extraction conditions and matrix effects[25-27].

Other immunological ELISA systems based on different antibodies have also been developed. McKillop et al.[28] and Troncone et al.[29] developed ELISAs based on polyclonal rabbit antisera against gliadin with very low detection limits. The assay of McKillop was not tested with heated samples and that of Troncone reacted with proteins from rice and maize that are not harmful for celiac patients. In 1988 Friis[30] also developed an ELISA using a polyclonal rabbit antibody; however, this antibody additionally recognized proteins from buckwheat. Other antibodies were raised against different epitopes of prolamins as proposed Freedman et al.[31] and Chirdo et al.[32,33].

Ellis et al.[34] developed an ELISA based on the PN3 antibody, for the toxic 19-mer peptides[35]. Subsequently, a competitive ELISA was developed with the

same antibody[36]. The competitive assay based on the PN3 detected equally harmful peptides from wheat, barley, rye and oats. Neither of these above-mentioned methods are commercially available.

Table 2. Enzyme-linked immunosorbent assays for gluten detection.

Name of antibody	Type of antibody*	Antibody raised against	Main recognition epitope	ELISA	LOD**	Reference
-	pAb	α-Gliadin Gliadin	-	Sandwich Competitive	1-20 ng/ml***	(23)
-	pAb	Gliadin	-	Sandwich	22 ng/ml	(28)
-	pAb	Gliadin	-	Sandwich	5 ng/ml	(29)
-	mAb	Gliadin	-	Sandwich	15 ng/ml	(31)
-	pAb	Gliadin	-	Competitive	13 ng/ml	(30)
401/21	mAb	ω-Gliadin	-	Sandwich	100-150 ng/ml	(38)
-	pAb	Gliadin	-	Competitive	1 ng/ml	(33)
13B4 12A1	mAb	Gliadin	-	Sandwich (12A1) Competitive (13B4) Competitive (12A1)	1 ng/ml 20 ng/ml 5 ng/ml	(34)
R5	mAb	Secalin	QQPFP	Sandwich Competitive	1.5 ng/ml 0.36 ng/ml	(46) (14)
PN3	mAb	19-mer	QQQPFP	Sandwich Competitive	4 ng/ml 128 ng/ml	(34) (36)
Gliaα-2/9 Gliaγ-1	mAb	α-Gliadin γ-Gliadin	LQPFPQPQ QQRPFI	Competitive	12 ng/ml	(42)
8D4 7C6	2 x mAb 1 x pAb	Gliadin	QQSFPQQ QQTFPQP QPFRPQ	Sandwich	5 ng/ml	(40) (41)
G12 A1	mAb	33-mer	QPQLPY QLPYPQP	Sandwich Competitive	0.6 ng/ml 0.4 ng/ml	(50) (52)
-	pAb	Gliadin	-	Sandwich	0.3 µg/ml	Morinaga Institute of Biological Science Inc., Crystal Chem Inc.)
-	-	-	-	Sandwich	5 µg/ml	Neogen

*polyclonal antibody (pAb), monoclonal antibody (mAb); **limit of detection (LOD); ***limit of quantification.

2.2.1.1. ω-Gliadin ELISA and Others

Skerrit and Hill[37,38] developed a sandwich format that was approved as an official method by AOAC (Association of Official Agricultural Chemist) and it was used for many years in gluten analysis. This method is based on an antibody that recognizes the heat-stable ω-gliadin fraction. This is an advantage, since that fraction remains unchanged during the processing of food. However, the disadvantage of the method is that the different relative content of the ω-fraction among cereal species causes considerable variation in the quantitative result[2,39]. In addition, fertilization may have strong effect on the protein composition of the grain. These changes in protein composition affect the immunological analysis results, especially when using the ω-specific antibody. Other disadvantage is that this method has only a weak response to barley hordeins. This method is no longer in general use; however it can still be obtained from different companies.

ImmunoTech (Pardubice, Czech Republic), developed a gliadin ELISA kit based on two monoclonal antibodies against two different epitopes of gliadin and one polyclonal antibody[40,41]. It recognizes wheat, rye and spelt with the same efficiency, but barley with an efficiency of only about 20-30%.

An ELISA method for detecting α-gliadins was developed by Koning and co-workers. Initially, the research group developed several antibodies against T-cell stimulatory epitopes. The antibodies were raised against synthetic peptides that represented T-cell stimulatory epitopes in α-gliadin, γ-gliadin, LMW glutenin and HMW glutenin[42,43]. The antibodies were very specific to the epitopes they were raised against and were able to detect homologous epitopes in other cereals (barley, oats, wheat, rye and triticale). However, as the method was further developed, only the α-gliadin antibody was selected for inclusion in the final ELISA. Because of this, the results of the method are expressed as α-gliadin contents.

The Morinaga Wheat Protein ELISA method has been validated in an interlaboratory study supported by The Japanese Ministry of Health, Labor and Welfare and is based in the use of a polyclonal antibody to wheat gliadin that detects multiple epitopes. The antibody also cross-reacts with hordeins

and secalins with a lesser degree than with wheat and therefore this assay underestimates both barley and rye protein content in contaminated foods[44].

2.2.1.2. R5 ELISA

The sandwich R5 ELISA is the most common enzyme immunoassay format used in detection of gluten proteins. It is highly sensitive and specific for gluten proteins and it is especially useful for the quantification of antigens when their concentration is low, when they are contained in samples with a large amount of other non-gluten proteins, or both. This assay is based on the R5 antibody, using two antibodies (R5 antibody and the R5 conjugated antibody) that bind to different sites on the antigen. The R5 antibody recognizes potential toxic-celiac epitopes which occur repeatedly in prolamins, mainly QQPFP, QQQFP, PQPFP, LQPFP, QQPYP, QLPYP, that are contained in toxic-celiac peptides such as Gliadin 33 mer peptide, Gliadin 26 mer peptide and Gliadin 25 mer peptide[45]. This ELISA has a limit of quantification of 1.56 ppm of gliadins and, combined with what is known as the cocktail extraction solution[46], it is internationally accepted by the Codex Alimentarius Commission as the method for determining gluten content in gluten-free foods[47]. In hydrolyzed foods, the quantification of gluten by the sandwich R5 ELISA is not accurate enough as two intact epitopes are required to quantify the gluten content.

The competitive R5 ELISA, based on the R5 monoclonal antibody, leads to a precise quantification of both intact and fragmented gluten because in this technique only one antibody is used and therefore only one epitope is required for complete determination of gluten. In addition, the competitive system is cheaper and faster than the sandwich system ELISA[14]. The Codex Alimentarius Commission states that a modification of the R5 assay (competitive ELISA) has to be applied for the detection of hydrolyzed gluten[47]. The cocktail extraction solution is not compatible with this competitive technique, but the combination of the competitive assay with the UPEX solution described above leads to accurate and complete gluten analysis. The limits of detection and quantification of the competitive R5

ELISA are 0.36 and 1.22 ng/ml of gliadins, respectively, being lower in liquid samples (LOQ of 0.30 ppm of gliadins). Recently, a collaborative study has confirmed that the two R5 antibody-based ELISA test kits are able to detect gliadin at the lower level of the limit of detection with good reproducibility and repeatability[25].

2.2.1.3. G12 and A1 ELISA

An ideal antibody for gluten analysis in foods should be not only a reliable indicator of the presence of prolamins from cereal species known to be toxic to CD patients but also should recognize the specific intramolecular regions responsible for such toxicity. Nevertheless, there are many such regions and even today not all have been identified.

Recent advances in the celiac field strongly recommend updating the concept of "gluten detection" to "potential relative immunotoxicity of gluten" for the safety of celiac consumers of food. Two monoclonal antibodies, A1 and G12, were raised against the immunodominant peptide 33-mer[48]. The 33-mer peptide from α-2 gliadin is a principal contributor to gluten immunotoxicity[49]. The reactivity of these antibodies was correlated with the potential immunotoxicity of the dietary grains from which the proteins were extracted[50,51].

A sandwich ELISA using the monoclonal G12 and A1 antibodies gave very promising results for gluten analysis across a range of samples[52,53]. This method had a detection limit for wheat, barley, and rye prolamins of 0.6 ng/mL. Furthermore, the reactivity of these antibodies were correlated with the potential immunotoxicity of those dietary grains from which the proteins were extracted, thereby providing a rational explanation for why some cereal varieties trigger immunological response, and enabling the presence of such varieties to be avoided in gluten-free diet[20,50,52].

A competitive ELISA method was also developed for the detection of toxic gluten peptides in hydrolyzed foods based on G12 antibody. This assay is highly sensitive and reproducible with a detection limit of 0.44 ppm gliadin. This ELISA system showed high reproducibility and repeatability[50,51].

2.2.2. Western Blot

Single and two-dimensional gel electrophoresis (both SDS-PAGE and A-PAGE) have been used by different authors in order to characterize wheat, barley, rye and oat proteins from cereal grains of different species[7,54,55]. Nevertheless, these techniques have not enough sensibility for detecting gluten in gluten-free foods. The western blot techniques lead to a qualitative or semi-quantitative analysis of these proteins and therefore are very useful for the confirmation of gluten content in foods avoiding false positives or negative results. Proteins separated in one-dimensional SDS-PAGE are electrotransferred onto a polyvinylidene difluoride membrane where proteins are adsorbed. Afterwards, a specific antibody is added, such as the R5 antibody[16], G12 antibody[51,53] or anti cells T gliadin alpha-20 antibody[56].

2.2.3. Lateral Flow Devices (LFDs) and Dipsticks

LFDs are used to qualitatively or semi-quantitatively determine whether gluten is present in a food product. LFDs and dipsticks for rapid and sensitive qualitative detection of gluten are available[57]. LFDs are usually what we think of as “dipstick” tests. Most employ sandwich type methodologies. They utilize a line of fixed antibody on a surface strip and a second antibody conjugated with colored “nano” size particles. When a liquid sample extract is applied to the strip, the conjugate and the sample start to migrate across the surface of the strip together. If the sample extract has the protein or compound present (gluten) and the conjugate can recognize its epitope (binding site), under the right conditions they will bind together. Now that they are “hooked” together as they come in contact with the line of antibodies that are fixed to the strip, these antibodies will also bind to the protein forming a sandwich complex, “sandwiching” the protein (gluten) between the two antibodies. As the conjugate complex starts to accumulate on the surface of the strip the “nano” particles start to become visible.

2.2.4. Biosensors

A number of biosensors for detecting gliadin contamination in gluten-free foods have been developed but are not yet commercially available. Two electrochemical biosensors have been described[58,59]. One uses an antibody raised against the immunodominat epitope of gliadin with a LOD of 5.5 µg/L. The second is based on the adsorption of anti-gliadin Fab fragments on gold surfaces. The LOD for gliadin was evaluated by impedance (LOD=0.42 mg/L) and amperometry (LOD=3.29 µg/L).

A quartz crystal microbalance biosensor incorporating gold nanoparticles was able to detect gliadin with a LOD of 8 µg/Kg[60]. Another biosensor used anti-gliadin antibody-conjugated immunomagnetic beads and fluorescence-dye-loaded immunoliposomal nanovesicles (IMLNs) to form sandwich[61], the LOD for gliadin was 0.6 mg/L.

Recently, Amaya-González et al.[62] have described an electrochemical competitive enzyme –linked assay on magnetic particles, which allows the measurement of as low 0.5 ppb of gliadin standard.

2.3. Non-Immunological Techniques

The quantitative analysis of prolamins is mainly based on immunological methods, but mass spectrometric and chromatographic techniques have also been used[63,64]. In addition, in non-processed foods, the PCR techniques have an interesting role confirming the presence of gluten by a DNA pathway. The use of complementary and alternative non-immunological systems to confirm the results of the immunological methods are essential for validation of methods and avoiding false negative or positive results.

2.3.1. Proteomic Techniques

Applying proteomics to analysis of gluten in foods is of great interest to complement other techniques and to achieve the maximum accuracy in the results. There are several studies that use proteomics techniques for characterization of gluten proteins in grains to confirm and increase flour

quality[65-69]. Nevertheless, in gluten-free foods the wide dynamic range of gluten proteins (low amount compared with the other major proteins) represents a major problem in analyzing them[70].

Mass spectrometry (MS) methods have a high sensibility and they are widely used nowadays for identification, characterization and quantification of proteins and peptides. Depending on the different method of ionization, separation and detection, there are several MS techniques, used in different applications. MALDI-TOF MS was the first technique used to identify toxic prolamins involved in celiac disease and to observe the different patterns of gliadins, hordeins, secalins, and avenins in grains depending on the type of cultivar and variety studied[71]. Afterwards, the technique was optimized for gluten analysis in foods[72].

Even though MALDI-TOF gluten analysis is very useful, identifying gluten based on the analysis of intact proteins is not enough, due to extensive sequence similarities among gluten proteins; the results for hydrolyzed gluten are also insufficiently accurate. For unambiguous gluten protein identification, a proteomic approach involving tandem mass spectrometry (MS/MS) or multistage MS experiments would be beneficial. MS/MS is a process in which an ion formed in an ion source is mass-selected in the first phase, reacted and fragmented, and then the charged products from the reaction are analyzed in the second phase. The classical workflow approach consists of separating protein mixtures by electrophoresis, digest the sample by the enzyme trypsin breaking down proteins into peptides, and, finally, identifying those using MS. Most scientists engaged in proteomics separate proteins by electrophoresis[73]. Nevertheless, more advanced shotgun proteomics approaches overcome the protein separation stage by digesting the entire protein mixture into peptides and separating them with one or two liquid chromatography (LC) steps. In addition to the classical methods of 2-DE and DIGE, MS-based quantification methods have gained increasing popularity. There are two broad groups of quantitative methods in MS-based proteomics: relative and absolute quantitative proteomics. In addition, quantitative proteomics can be classified into two major approaches: differential stable isotope labeling and label-free techniques (Figure 1).

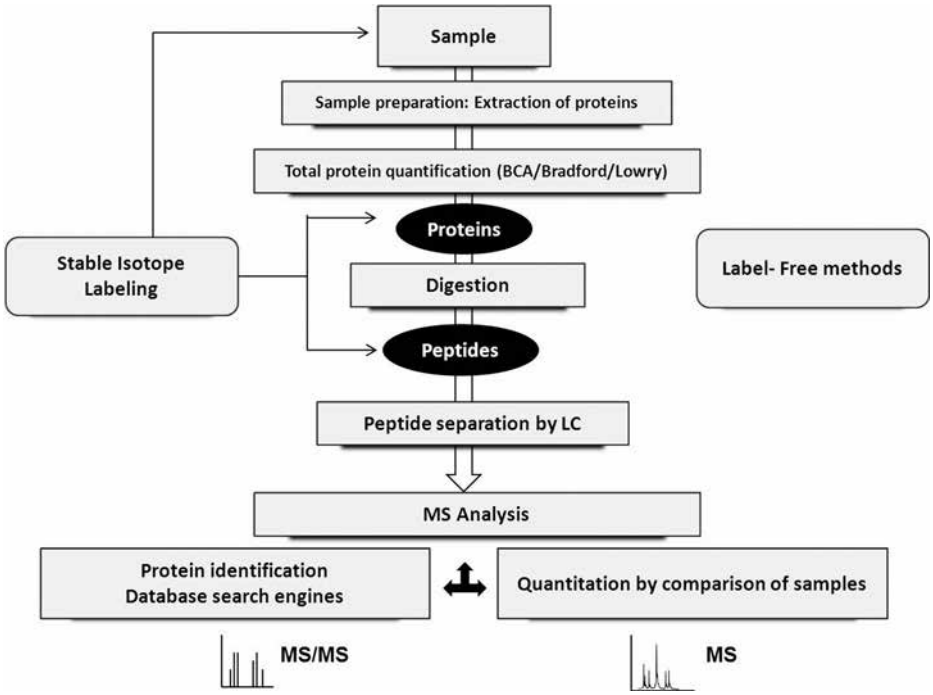


Figure 1. Workflow of protein analysis by proteomics techniques.

There are several difficulties associated with proteomic analysis of prolamins and glutelins as they are a complex mixture of proteins. In addition, there are a limited number of sequences of wheat, barley, and rye that are loaded and registered in public databases, especially for barley and rye, whose sequences are less registered than in the case of wheat, making more difficult the identification of proteins and peptides. Appropriate sample preparation procedures are also essential for correct sample analysis. Enrichment strategies are essential for successful protein identification[74], because the dynamic ranges of the proteins are very different and the proteins of interest are present in a substantially smaller quantity than other major proteins. The fact that gluten is formed by a high number of different proteins is another rate-limiting step in proteomic workflows[75]. Enzymatic digestion of proteins by endoproteases is a key step in protein identification by MS[76]. Trypsin, the most widely-used enzyme for this purpose, cleaves C-terminal to

lysine and arginine, but in the case of gluten proteins these cleavage points are not appropriate for generating peptides easily quantifiable by these techniques. Therefore it is necessary to use different enzymes with different cleavage points to obtain other, more appropriate gluten fragments and obtain a complete characterization, but the digestion by these enzymes is less reproducible[77].

The analysis of hydrolyzed peptides, such as in beers, is also of great interest to test the possible remaining toxic peptides for people with celiac disease, but the comprehensive annotation of the beer proteome is challenged both by the high concentration range of the protein entities and by a severe degree of processing-induced modifications[78]. Other authors have characterized by proteomics techniques the content of prolamins in beer finding different peptides considering different types of beer[79].

The application of analysis of proteotypic peptides in gluten analysis is very promising. The first step in developing a method for gluten detection by identification of proteotypic peptides by MS is the selection of the best proteotypic peptides to be monitored that represent the most important prolamin and glutelin proteins, including those with proven immunogenicity and toxicity[53]. The peptides must be unique to gluten proteins and must ionize efficiently and chromatograph in a stable, reproducible manner. Prior to MS analysis, proteins need to be broken up into peptides by enzymatic digestion. The common peptides for wheat, barley, and rye would be the best potential representative peptides for gluten analysis in all kind of foods when the origin of the contamination is not clear.

2.3.2. Quantitative Real-Time PCR (Q-PCR)

Several polymerase chain reaction (PCR) based methods for the detection and quantification of DNA of gluten-containing cereals have been described and they are very useful to achieve the characterization of different cultivars and selection of genotypes coding for gluten proteins with the best bread-making quality[17,80-83]. Nevertheless, there are few studies regarding the application of this technique to the analysis of gluten in foods. One of the

first studies used a PCR combined with agarose gels to detect wheat contaminations in oats[84]. Afterwards, a quantitative PCR system combined with agarose gels was developed to detect simultaneously contamination of wheat, barley and rye in gluten-free food[85]. Nevertheless, using agarose gels has some disadvantages and the most efforts for the detection and quantification of wheat, barley and/or rye DNA, have been in Q-PCR[86-88].

A Q-PCR system for reliable and rapid quantification of wheat DNA in gluten-free foods and in raw materials has been developed based on the fluorescent dye SYBR Green I and a modified SDS/Guanidine-HCl/Proteinase K DNA extraction protocol. This is a highly specific and sensitive system which presents a quantification limit of 20 pg DNA/mg. Comparing this Q-PCR system with the prolamin levels determined with the most commercially available R5 ELISA it was demonstrated that with the exception of some hydrolyzed and highly processed food samples (such as beers, syrups, malt extracts, breakfast cereals...), the rest of the food with prolamin levels above the R5 ELISA quantification limit (1.5 mg/kg) gave positive signals with the Q-PCR system. Therefore, this Q-PCR system can be used as a non-immunological tool in order to confirm, by the ''DNA pathway'', the presence of wheat in food not only for celiacs but also for individuals with wheat allergy[89].

Other authors have developed a DNA-based allergen-multiplex ligation-dependent probe amplification method that includes the determination of gluten in foods which might constitute a complementary method to the traditional protein-based methods[90].

3. Analytical Tools for the Selection of Oat Varieties with No Toxicity in Celiac Disease

Cultivated oats are hexaploid cereals belonging to the genus *Avena* L., which is found worldwide in almost all agricultural environments[91]. Recently, oats have been receiving increasing interest as human food, mainly because this cereal could be suitable for consumptions by celiac patients. Several varieties of oats are available and all of them present very interesting nutritional and other healthy properties.

The presence of oats in a GFD is still a subject of controversial. Oats differ from other cereals in their prolamin content. The percentage of proline and glutamine (amino acids abundant in toxic regions) in avenin is lower than in other toxic cereals. Some clinical researchers state that patients with celiac disease tolerate oats without signs of intestinal inflammation[92]. In contrast, other studies confirmed the toxicity of oats in certain types of patients with celiac disease. Arentz-Hansen et al.[93] described the intestinal deterioration suffered by some patients with celiac disease following the consumption of oats while on a GFD. Avenin can trigger an immunological response in these patients similar to the response produced by the gluten of wheat, rye or barley. The monitoring of 19 adult patients with celiac disease who consumed 50 g/day of oats over 12 weeks showed that one of the subjects was sensitive to oats. Therefore, it is critical to clarify either qualitatively or quantitatively the potential immunotoxicity of oats to patients with celiac disease[94,95].

Comparison of the different studies are complicated by the different study designs, the different conditions used in the testing, the number of subjects included in each study and the reporting of the purity control of the oat material used in the clinical trials. Another relevant factor in different designs is the absence of information on the oat variety used. Silano et al.[94] investigated the immunogenic effect of avenins from four oat cultivars using peripheral lymphocytes from patients with CD. All the varieties of oats tested (Lampton, Astra, Ava, and Nave) by these investigators were immunogenic with differences in their capacity to induce a response. However, other study

confirmed that *Avena genziana* and *Avena potenza* do not display *in vitro* activities related to CD pathogenesis[95].

The utility of the G12 antibody to identify potentially toxic oat varieties for celiac patients has been reported[96] (Patent No.: WO2013098441 A1). This finding allowed classification of oat varieties into three groups based in their degree of affinity for the G12 antibody: a highly recognized group, one of moderate recognition, and one with no reactivity[96]. These results were confirmed by MALDI-TOF, SDS-PAGE and western blot by showing that the number, relative intensity of the peaks and protein profile obtained for the nine oat varieties differ from one another. The potentially immunotoxicity of the different types of oats was determined by T cell proliferation and interferon γ release. The reactivity that T-cells isolated from celiac patients exhibited with three oat varieties (one from each of the classified groups) correlated directly with the moAb G12 reactivity. The diversity observed in the reactivity to the different oat cultivars suggests variations in the avenin composition, and therefore in the amount of immunotoxic epitopes similar to the 33-mer present in these varieties. This gives a rational explanation for why only some oats trigger an immunological response.

In comparison with wheat gliadins, the avenins have been little studied, and the number of full avenin genes present at the moment in the databases is limited and from few genotypes, so that the variability of avenin genes in oats is not well represented. It has recent been known that, like wheat, oat grains have both monomeric and polymeric avenins[7]. A direct correlation between the immunogenicity of the different varieties of oats and the presence of the specific peptides with a higher/lower potential immunotoxicity has been found, that could explain why certain varieties of oats are toxic for celiac patients and other not[7]. The incorporation of some varieties of oats in food products not only may improve the nutritional quality but also may provide a treatment for various illnesses and would be welcomed by patients with celiac disease (Patent No.: WO2013098441 A1).

4. Policies and Regulation

The presence of high number of gluten components, the variation in the extraction efficiency, and the lack of reference materials representative of all kind of foods, are some issues that hinder the implementation of equivalent laws at national level and the comparison of data across the different methods[97].

In January 2009, the European Commission published a new European Regulation concerning the composition and labeling of foodstuffs suitable for people intolerant to gluten. "Gluten-free" food were defined as dietary foods consisting of or made only from one or more ingredients which do not contain wheat, rye, barley, or oats, and the gluten content does not exceed 20 mg/kg in them as sold to the final consumer[98]. In addition, foods specially processed to reduce the gluten content to a level above 20 up to 100 mg/Kg were defined as food consisting of one or more ingredients from wheat, rye, barley oats or their crossbred varieties which have been specially processed to reduce the gluten content to that levels. Based on this, labeling, advertising and presentation of the products shall bear the terms, "gluten-free" (not exceeding 20 mg/Kg) and "very low gluten" (not exceeding 100 mg/Kg). This Regulation shall apply as from 1 January 2012. Regarding to oats content in food, according to the Codex Alimentarius for food for special dietary use for persons intolerant to gluten, CODEX STAN118-1979 (revised 2008, http://www.codexalimentarius.net/web/more_info.jsp?id_sta=291), oats can be tolerated by most but not all people who are intolerant to gluten. Moreover, the Commission Regulation (EC) No 41/2009 (http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:016:0003:0005:EN:PDF) concerning the composition and labeling of foodstuffs suitable for people intolerant to gluten, also states that the possible adverse effect of oats is an issue of ongoing study and investigation by the scientific community. In addition, a major concern is the contamination of oats with wheat, rye or barley that can occur during grain harvesting, transport, storage and processing, that should be taken into consideration with regard to labeling of those products.

Nowadays, the method for determination of the gluten content in gluten-free foods accepted internationally by the Codex Alimentarius Commission is the sandwich ELISA based on the R5 antibody. As mentioned above, the principal limitation of the sandwich R5 ELISA is that it is essential that at least two epitopes of the sequences recognized by the monoclonal antibody R5 be present simultaneously in a protein or peptide. However, in hydrolyzed foods (such as baby foods, syrups and beers), gluten proteins are fragmented during food processing and converted into peptides in which only one toxic peptide may appear. In this case, the quantification of gluten by sandwich R5 ELISA would be incorrect, yielding less than the real gluten content. The Codex Alimentarius Commission states that "for the detection of hydrolyzed gluten a modification of the R5 assay (competitive ELISA) has to be applied".

In 2013 the Protein & Enzymes Technical Committee of AACC International initiated a collaborative study of a method for gluten quantitation in selected foods using a G12 antibody sandwich ELISA system. Recently, this method has been approved as AACC International Method (AACCI 38-52.01; NewsLetter 2014; 5 (1): 1-5). In March 2014, at the AOACI mid-year meeting, the G12 Sandwich ELISA has been adopted as an AOACI 1st Action Method[99].

In August 2013, gluten-free labeling regulations for gluten-free foods were established for the first time by the Food and Drug Administration (FDA) of the United States. These gluten limits are based on Codex standards and define the term "gluten-free" for voluntary use in the labeling of foods when any presence of gluten is less than 20 ppm. In general, foods may be labeled "gluten-free" if the food either is inherently gluten free; or does not contain an ingredient that is: 1) a gluten-containing grain (any type of wheat, rye, barley), or crossbreeds of these grains; 2) derived from a gluten-containing grain that has not been processed to remove gluten; or 3) derived from a gluten-containing grain that has been processed to remove gluten (e.g., wheat starch), if the use of that ingredient results in the presence of 20 parts per million (ppm) or more gluten in the food. The final rule applies to all FDA-regulated foods, including dietary supplements. The rule excludes those foods

whose labeling is regulated by the U.S. Department of Agriculture (USDA) and the Alcohol and Tobacco Tax and Trade Bureau (TTB). Generally, USDA regulates the labeling of meats, poultry, and certain egg products (FDA regulates the labeling of shell eggs). TTB regulates the labeling of most alcoholic beverages, including all distilled spirits, wines that contain 7 percent or more alcohol by volume, and malted beverages that are made with both malted barley and hops. All foods imported into the United States must meet also these requirements to make a gluten-free claim. Manufacturers who elect to analyze their foods for gluten can select the test methods most appropriate for them, considering the type of foods they manufacture, and FDA recommends the use of scientifically valid methods to obtain reliable and consistent results (http://www.fda.gov/food/guidanceregulation/guidancedocumentsregulatoryinformation/allergens).

In Australia and New Zealand claims in relation to gluten content of food are prohibited unless the expressly permitted cases. A claim to the effect that a food is gluten free must not be made in relation to a food unless the food contains no detectable gluten; and no oats or their products; or cereals containing gluten that have been malted, or their products. In addition, a claim to the effect that a food has low gluten content must not be made in relation to a food unless the food contains no more than 20 mg gluten per 100 g of the food (Standard 1.2.8 Federal Register of Legislative Instruments F2012C00218).

5. Conclusions

CD is a common autoimmune disorder that has genetic, environmental, and immunological components. The ingestion of gluten proteins contained in wheat, barley, rye, and in some cases oats, leads to characteristic inflammation, villous atrophy, and crypt hyperplasia in the CD patient's upper small intestine. Safety of gluten free foods can be only ensured by providing reliable methods of gluten detection and quantitation. The high variety of gluten components and other ingredients contained in foods after manufacturing make extraction efficiency and detection very difficult.

Methods for gluten analysis are available for the control of "gluten-free" products. Different immunological and non-immunological techniques are being applied to increase the sensitivity and provide supplementary information on gluten protein identification, taking into account that methods for gluten analysis must be sensitive enough to quantify low levels of gluten in foods to fit in the food regulations.

The quantitative analysis of gluten is mainly performed by ELISA methods. Proteomics techniques are promising tools for quantification of gluten, whereas DNA-based methods are useful tools to detect eventual contaminations. The limit values of 20 and 100 mg/Kg of gluten in "gluten-free" and "very low gluten" foods, respectively, help managing the diet of most celiac patients efficiently.

References

1. Breiteneder H, Radauer C. *A classification of plant food allergens.* J Allergy Clin Immunol. 2004; 113(5): 821-30

   http://dx.doi.org/10.1016/j.jaci.2004.01.779
   PMid:15131562
2. Shewry PR, Tatham AS. *The characteristics, structures and evolutionary relationships of prolamins.* In: Shewry PR, Casey R (Eds.). Seed Proteins. The Netherlnads: Kluwer Academic Publishers. 1999; 11-34.

   http://dx.doi.org/10.1007/978-94-011-4431-5_2
3. Osborne TB. *The proteins of the wheat kernel.* Publication no. 84 Carnegie Institution of Washington, Press of Judd & Detweiler, Inc, Washington DC, USA. 1907.

   http://dx.doi.org/10.5962/bhl.title.22763
4. Stern M, Ciclitira PJ, van Eckert R, Feighery C, Janssen FW, Mendez E et al. *Analysis and clinical effects of gluten in coeliac disease.* European journal of gastroenterology & hepatology. 2001; 13(6): 741-7.

   http://dx.doi.org/10.1097/00042737-200106000-00023
5. Shewry PR, Tatham AS. *The prolamin storage proteins of cereal seeds: structure and evolution.* Biochem J. 1990; 267(1): 1-12.

   PMid:2183790 PMCid:PMC1131235
6. Wieser H. *Comparative investigations of gluten proteins from different wheat species I. Qualitative and quantitative composition of gluten protein types.* Eur Food Res Technol. 2000; 211(4): 262-8.

   http://dx.doi.org/10.1007/s002170000165
7. Real A, Comino I, de Lorenzo L, Merchan F, Gil-Humanes J, Gimenez MJ et al. *Molecular and immunological characterization of gluten proteins isolated from oat cultivars that differ in toxicity for celiac disease.* PloS one. 2012; 7(12): e48365.

   http://dx.doi.org/10.1371/journal.pone.0048365
   PMid:23284616 PMCid:PMC3524229
8. Chesnut RS, Shotwell MA, Boyer SK, Larkins BA. *Analysis of avenin proteins and the expression of their mRNAs in developing oat seeds.* Plant Cell. 1989; 1(9): 913-24.

   http://dx.doi.org/10.1105/tpc.1.9.913
   PMid:2535531 PMCid:PMC159827
9. Wieser H. *Chemistry of gluten proteins.* Food Microbiol. Review. 2007; 24(2): 115-9.

   http://dx.doi.org/10.1016/j.fm.2006.07.004
   PMid:17008153

10. Sollid LM. *Coeliac disease: dissecting a complex inflammatory disorder.* Nat Rev Immunol. 2002; 2(9): 647-55.
http://dx.doi.org/10.1038/nri885
PMid:12209133

11. Bernardo D, Pena AS. *Developing strategies to improve the quality of life of patients with gluten intolerance in patients with and without coeliac disease.* Eur J Intern Med. Editorial. 2012; 23(1): 6-8.

12. Corrao G, Corazza GR, Bagnardi V, Brusco G, Ciacci C, Cottone M et al. *Mortality in patients with coeliac disease and their relatives: a cohort study.* Lancet. 2001; 358(9279): 356-61.
http://dx.doi.org/10.1016/S0140-6736(01)05554-4

13. Greco L, Gobbetti M, Auricchio R, Di Mase R, Landolfo F, Paparo F et al. *Safety for patients with celiac disease of baked goods made of wheat flour hydrolyzed during food processing.* Clinical gastroenterology and hepatology: the official clinical practice journal of the American Gastroenterological Association. 2011; 9(1): 24-9.
http://dx.doi.org/10.1016/j.cgh.2010.09.025
PMid:20951830

14. Mena MC, Lombardia M, Hernando A, Mendez E, Albar JP. *Comprehensive analysis of gluten in processed foods using a new extraction method and a competitive ELISA based on the R5 antibody.* Talanta. 2012; 91: 33-40.
http://dx.doi.org/10.1016/j.talanta.2011.12.073
PMid:22365676

15. Kanerva P, Brinck O, Salovaara H, Loponen J. *Deamidation of gluten proteins and peptides decreases the antibody affinity in gluten analysis assays.* J Cereal Sci. 2011; 53(3): 335-9.
http://dx.doi.org/10.1016/j.jcs.2011.02.003

16. Garcia E, Llorente M, Hernando A, Kieffer R, Wieser H, Mendez E. *Development of a general procedure for complete extraction of gliadins for heat processed and unheated foods.* European journal of gastroenterology & hepatology. 2005; 17(5): 529-39.
http://dx.doi.org/10.1097/00042737-200505000-00010

17. Han Z, Wu F, Deng G, Qian G, Yu M, Jia Y. *Structural and expressional analysis of the B-hordein genes in Tibetan hull-less barley.* Genetica. 2010; 138(2): 227-39.
http://dx.doi.org/10.1007/s10709-009-9415-6
PMid:19856114

18. van Eckert R, Berghofer E, Ciclitira PJ, Chirdo F, Denery-Papini S, Ellis HJ et al. *Towards a new gliadin reference material–isolation and characterisation.* J Cereal Sci. 2006; 43: 331-41.
http://dx.doi.org/10.1016/j.jcs.2005.12.009

19. Gessendorfer B, Koehler P, Wieser H. *Preparation and characterization of enzymatically hydrolyzed prolamins from wheat, rye, and barley as references for the immunochemical quantitation of partially hydrolyzed gluten.* Analytical and bioanalytical chemistry. 2009; 395(6): 1721-8.

http://dx.doi.org/10.1007/s00216-009-3080-6
PMid:19763549

20. Comino I, Real A, Gil-Humanes J, Piston F, de Lorenzo L, Moreno ML et al. *Significant differences in coeliac immunotoxicity of barley varieties.* Mol Nutr Food Res. 2012; 56(11): 1697-707.

http://dx.doi.org/10.1002/mnfr.201200358
PMid:22968973

21. Comino I, Real A, Moreno ML, Montes R, Cebolla A, Sousa C. *Immunological determination of gliadin 33-mer equivalent peptides in beers as a specific and practical analytical method to assess safety for celiac patients.* Journal of the science of food and agriculture. 2013; 93(4): 933-43.

http://dx.doi.org/10.1002/jsfa.5830
PMid:22886585

22. Denery-Papini S, Nicolas Y, Popineau Y. *Efficiency and Limitations of Immunochemical Assays for the Testing of Gluten-free Foods.* Journal of Cereal Science. 1999; 30(2): 121-31.

http://dx.doi.org/10.1006/jcrs.1999.0268

23. Windemann H, Fritschy F, Baumgartner E. *Enzyme-linked immunosorbent assay for wheat alpha-gliadin and whole gliadin.* Biochimica et biophysica acta. 1982; 709: 110-21.

http://dx.doi.org/10.1016/0167-4838(82)90428-9

24. Howdle PD, Losowsky MS. *Review of methods for measuring gliadins in food.* Gut. Review. 1990; 31(6): 712-3.

25. Immer U, Haas-Lauterbach S. *Gliadin as a measure of gluten in foods containing wheat, rye, and barley-enzyme immunoassay method based on a specific monoclonal antibody to the potentially celiac toxic amino acid prolamin sequences: collaborative study.* Journal of AOAC International. 2012; 95(4): 1118-24.

http://dx.doi.org/10.5740/jaoacint.CS2012_01
PMid:22970580

26. van Eckert R, Bond J, Rawson P, Klein CL, Stern M, Jordan TW. *Reactivity of gluten detecting monoclonal antibodies to a gliadin reference material.* Journal of Cereal Science. 2010; 51(2): 198-204.

http://dx.doi.org/10.1016/j.jcs.2009.11.012

27. Diaz-Amigo C, Popping B. *Accuracy of ELISA detection methods for gluten and reference materials: a realistic assessment. Journal of agricultural and food chemistry.* 2013; 61(24): 5681-8.

http://dx.doi.org/10.1021/jf3046736
PMid:23713744

28. McKillop DF, Gosling JP, Stevens FM, Fottrell PF. *Enzyme immunoassay of gliadin in food.* Biochem Soc Trans. 1985; 13: 486-7.

29. Troncone R, Vitale M, Donatiello A, Farris E, Rossi G, Auricchio S. *A sandwich enzyme immunoassay for wheat gliadin.* 1986: 0022-1759.

30. Friis SU. *Enzyme-linked immunosorbent assay for quantitation of cereal proteins toxic in coeliac disease.* Clin Chim Acta. 1988; 178(3): 261-70.
http://dx.doi.org/10.1016/0009-8981(88)90234-3

31. Freedman AR, Galfre G, Gal E, Ellis HJ, Ciclitira PJ. *Monoclonal antibody ELISA to quantitate wheat gliadin contamination of gluten-free foods.* J Immunol Methods. 1987; 98(1): 123-7.
http://dx.doi.org/10.1016/0022-1759(87)90445-5

32. Chirdo FG, Añón MC, Fossati CA. *Development of high-sensitive enzyme immunoassays for gliadin quantification using the streptavidin-biotin amplification system.* Food and Agricultural Immunology. 1998; 10(2): 143-55.
http://dx.doi.org/10.1080/09540109809354977

33. Chirdo FG, Añón MC, Fossati CA. *Optimization of a competitive ELISA with polyclonal antibodies for quantification of prolamins in foods.* Food and Agricultural Immunology. 1995; 7(4): 333-43.
http://dx.doi.org/10.1080/09540109509354893

34. Ellis HJ, Rosen-Bronson S, O'Reilly N, Ciclitira PJ. *Measurement of gluten using a monoclonal antibody to a coeliac toxic peptide of A gliadin.* Gut. 1998; 43(2): 190-5.
http://dx.doi.org/10.1136/gut.43.2.190
PMid:10189843 PMCid:PMC1727224

35. Sturgess R, Day P, Ellis HJ, Lundin KE, Gjertsen HA, Kontakou M et al. *Wheat peptide challenge in coeliac disease.* Lancet. 1994; 343(8900): 758-61.
http://dx.doi.org/10.1016/S0140-6736(94)91837-6

36. Bermudo Redondo MC, Griffin PB, Garzon Ransanz M, Ellis HJ, Ciclitira PJ, O'Sullivan CK. *Monoclonal antibody-based competitive assay for the sensitive detection of coeliac disease toxic prolamins.* Analytica Chimica Acta. 2005; 551(1-2): 105-14.
http://dx.doi.org/10.1016/j.aca.2005.07.023

37. Skerritt JH, Hill AS. *Enzyme immunoassay for determination of gluten in foods: collaborative study.* J Assoc Off Anal Chem. 1991; 74(2): 257-64.
PMid:2050607

38. Skerritt JH, Hill AS. *Monoclonal antibody sandwich enzyme immunoassays for determination of gluten in foods. Journal of agricultural and food chemistry.* 1990; 38(8): 1771-8.
http://dx.doi.org/10.1021/jf00098a029

39. Thompson T, Mendez E. *Commercial assays to assess gluten content of gluten-free foods: why they are not created equal.* J Am Diet Assoc. Review. 2008; 108(10): 1682-7.

40. Gabrovskà D, Rysov J, Filov V, Plicka J, Cuhra P, Kubk M et al. *Gluten Determination by Gliadin Enzyme-Linked Immunosorbent Assay Kit: Interlaboratory Study.* Journal of AOAC International. 2006; 89(1): 154-60.
PMid:16512241

41. Sánchez D, Tučková L, Burkhard M, Plicka J, Mothes T, Hoffmanová I et al. *Specificity Analysis of Anti-gliadin Mouse Monoclonal Antibodies Used for Detection of Gliadin in Food for Gluten-free Diet.* Journal of agricultural and food chemistry. 2007; 55(7): 2627-32.
http://dx.doi.org/10.1021/jf0630421
PMid:17335223

42. Spaenij-Dekking EH, Kooy-Winkelaar EM, Nieuwenhuizen WF, Drijfhout JW, Koning F. *A novel and sensitive method for the detection of T cell stimulatory epitopes of alpha/beta- and gamma-gliadin.* Gut. 2004; 53(9): 1267-73.
http://dx.doi.org/10.1136/gut.2003.037952
PMid:15306583 PMCid:PMC1774189

43. Mitea C, Havenaar R, Drijfhout JW, Edens L, Dekking L, Koning F. *Efficient degradation of gluten by a prolyl endoprotease in a gastrointestinal model: implications for coeliac disease.* Gut. 2008; 57(1): 25-32.
http://dx.doi.org/10.1136/gut.2006.111609
PMid:17494108

44. Sharma GM. *Immunoreactivity and detection of wheat proteins by commercial ELISA kits.* Journal of AOAC International. 2012; 95(2): 364-71.
http://dx.doi.org/10.5740/jaoacint.SGE_Sharma
PMid:22649920

45. Kahlenberg F, Sanchez D, Lachmann I, Tuckova L, Tlaskalova H, Méndez E et al. *Monoclonal antibody R5 for detection of putatively coeliac-toxic gliadin peptides.* Eur Food Res Technol. 2006; 222(1-2): 78-82.
http://dx.doi.org/10.1007/s00217-005-0100-4

46. Valdes I, Garcia E, Llorente M, Mendez E. *Innovative approach to low-level gluten determination in foods using a novel sandwich enzyme-linked immunosorbent assay protocol.* Eur J Gastroenterol Hepatol. 2003; 15(5): 465-74.
http://dx.doi.org/10.1097/01.meg.0000059119.41030.df
PMid:12702901

47. Codex Alimentarius Commission. *Joint Food and Agriculture Organization of the United Nations/World Health Organization Food Standards Program. Report of the Twenty- Seventh Session of the Codex Committee on Methods of Analysis and Sampling.* 2006; ALINORM 06/29/23.

48. Morón B, Cebolla Á, Manyani H, Álvarez-Maqueda M, Megías M, Thomas MC et al. *Sensitive detection of cereal fractions that are toxic to celiac disease patients by using monoclonal antibodies to a main immunogenic wheat peptide.* The American journal of clinical nutrition. 2008; 87(2): 405-14.
PMid:18258632

49. Shan L, Molberg O, Parrot I, Hausch F, Filiz F, Gray GM et al. *Structural basis for gluten intolerance in celiac sprue.* Science. 2002; 297(5590): 2275-9.

http://dx.doi.org/10.1126/science.1074129
PMid:12351792

50. Moron B, Bethune MT, Comino I, Manyani H, Ferragud M, Lopez MC et al. *Toward the assessment of food toxicity for celiac patients: characterization of monoclonal antibodies to a main immunogenic gluten peptide.* PloS one. 2008; 3(5): e2294.

http://dx.doi.org/10.1371/journal.pone.0002294
PMid:18509534 PMCid:PMC2386552

51. Ehren J, Moron B, Martin E, Bethune MT, Gray GM, Khosla C. *A food-grade enzyme preparation with modest gluten detoxification properties.* PloS one. 2009; 4(7): e6313.

http://dx.doi.org/10.1371/journal.pone.0006313
PMid:19621078 PMCid:PMC2708912

52. Moron B, Cebolla A, Manyani H, Alvarez-Maqueda M, Megias M, Thomas MC et al. *Sensitive detection of cereal fractions that are toxic to celiac disease patients by using monoclonal antibodies to a main immunogenic wheat peptide.* The American journal of clinical nutrition. 2008; 87(2): 405-14.

PMid:18258632

53. Mena MC, Hernando A, Lombardía M, Albar J. *The application of proteomics in gluten analysis: Identification and characterization of prolamins and glutelins through mass spectrometry.* Proceeding of the 23rd Meeting Working Group on Prolamin Analysis and Toxicity. 2009; 23-8.

54. Salmanowicz BP, Nowak J. *Diversity of monomeric prolamins in triticale cultivars determined by capillary zone electrophoresis.* Journal of agricultural and food chemistry. 2009; 57(6): 2119-25.

http://dx.doi.org/10.1021/jf803326z
PMid:19228059

55. Shuaib A, Hussain MS. *The past and future of neuroprotection in cerebral ischaemic stroke.* European neurology. 2008; 59(1-2): 4-14.

http://dx.doi.org/10.1159/000109254
PMid:17917451

56. van den Broeck HC, America AH, Smulders MJ, Bosch D, Hamer RJ, Gilissen LJ et al. *A modified extraction protocol enables detection and quantification of celiac disease-related gluten proteins from wheat.* J Chromatogr B Analyt Technol Biomed Life Sci. 2009; 877(10): 975-82.

http://dx.doi.org/10.1016/j.jchromb.2009.02.035
PMid:19282254

57. Immer U, Haas Lauterbach S. *Gluten detection in molecular biological and immunological techniques and applications for food chemist.* In: Propping B, Diaz-Amigo C, Hoenicke K (Eds.). Molecular Biological and Immunological Techniques and Applications for Food Chemists. John Wiley & Sons, Inc. 2010: 359-75.

http://dx.doi.org/10.1002/9780470637685.ch19

58. Nassef HM, Bermudo Redondo MC, Ciclitira PJ, Ellis HJ, Fragoso A, O'Sullivan CK. *Electrochemical immunosensor for detection of celiac disease toxic gliadin in foodstuff.* Anal Chem. 2008; 80(23): 9265-71.

http://dx.doi.org/10.1021/ac801620j
PMid:19551990

59. Nassef HM, Civit L, Fragoso A, O'Sullivan CK. *Amperometric immunosensor for detection of celiac disease toxic gliadin based on Fab fragments.* Anal Chem. 2009; 81(13): 5299-307.

http://dx.doi.org/10.1021/ac9005342
PMid:19469538

60. Chu PT, Lin CS, Chen WJ, Chen CF, Wen HW. *Detection of gliadin in foods using a quartz crystal microbalance biosensor that incorporates gold nanoparticles.* Journal of agricultural and food chemistry. 2012; 60(26): 6483-92.

http://dx.doi.org/10.1021/jf2047866
PMid:22694361

61. Chu PT, Wen HW. *Sensitive detection and quantification of gliadin contamination in gluten-free food with immunomagnetic beads based liposomal fluorescence immunoassay.* Analytica Chimica Acta. 2013; 787: 246-53.

http://dx.doi.org/10.1016/j.aca.2013.05.014
PMid:23830446

62. Amaya-Gonzalez S, de-Los-Santos-Alvarez N, Miranda-Ordieres AJ, Lobo-Castanon MJ. *Aptamer binding to celiac disease-triggering hydrophobic proteins: a sensitive gluten detection approach.* Anal Chem. 2014; 86(5): 2733-9.

http://dx.doi.org/10.1021/ac404151n
PMid:24502317

63. Sealey-Voyksner JA, Khosla C, Voyksner RD, Jorgenson JW. *Novel aspects of quantitation of immunogenic wheat gluten peptides by liquid chromatography-mass spectrometry/mass spectrometry.* J Chromatogr A. 2010; 1217(25): 4167-83.

http://dx.doi.org/10.1016/j.chroma.2010.01.067
PMid:20181349

64. Wieser H. *Investigations on the extractability of gluten proteins from wheat bread in comparison with flour.* Z Lebensm Unters Forsch A. 1998; 207: 128-32.

http://dx.doi.org/10.1007/s002170050306

65. Hurkman WJ, Tanaka CK, Vensel WH, Thilmony R, Altenbach SB. *Comparative proteomic analysis of the effect of temperature and fertilizer on gliadin and glutenin accumulation in the developing endosperm and flour from Triticum aestivum L. cv. Butte 86.* Proteome science. 2013; 11(1): 8.
http://dx.doi.org/10.1186/1477-5956-11-8
PMid:23432757 PMCid:PMC3599944

66. Pompa M, Giuliani MM, Palermo C, Agriesti F, Centonze D, Flagella Z. *Comparative Analysis of Gluten Proteins in Three Durum Wheat Cultivars by a Proteomic Approach.* Journal of agricultural and food chemistry. 2013. 61(11): 2606-17.
http://dx.doi.org/10.1021/jf304566d
PMid:23414385

67. Guo G, Lv D, Yan X, Subburaj S, Ge P, Li X et al. *Proteome characterization of developing grains in bread wheat cultivars (Triticum aestivum L.).* BMC plant biology. 2012; 12: 147.
http://dx.doi.org/10.1186/1471-2229-12-147
PMid:22900893 PMCid:PMC3480910

68. Altenbach SB, Vensel WH, Dupont FM. *Analysis of expressed sequence tags from a single wheat cultivar facilitates interpretation of tandem mass spectrometry data and discrimination of gamma gliadin proteins that may play different functional roles in flour.* BMC plant biology. 2010; 10: 7.
http://dx.doi.org/10.1186/1471-2229-10-7
PMid:20064259 PMCid:PMC2827424

69. Mamone G, De Caro S, Di Luccia A, Addeo F, Ferranti P. *Proteomic-based analytical approach for the characterization of glutenin subunits in durum wheat.* Journal of mass spectrometry: JMS. 2009; 44(12): 1709-23.
http://dx.doi.org/10.1002/jms.1680

70. Mamone G, Picariello G, Addeo F, Ferranti P. *Proteomic analysis in allergy and intolerance to wheat products.* Expert review of proteomics. 2011; 8(1): 95-115.
http://dx.doi.org/10.1586/epr.10.98
PMid:21329430

71. Camafeita E, Alfonso P, Mothes T, Mendez E. *Matrix-assisted laser desorption/ionization time-of-flight mass spectrometric micro-analysis: the first non-immunological alternative attempt to quantify gluten gliadins in food samples.* Journal of mass spectrometry: JMS. 1997; 32(9): 940-7.
http://dx.doi.rg/10.1002/(SICI)1096-9888(199709)32:9<940::AID-JMS550>3.0.CO;2-2

72. Hernando A, Valdes I, Mendez E. *New strategy for the determination of gliadins in maize- or rice-based foods matrix-assisted laser desorption/ionization time-of-flight mass spectrometry: fractionation of gliadins from maize or rice prolamins by acidic treatment.* Journal of mass spectrometry: JMS. 2003; 38(8): 862-71.
http://dx.doi.org/10.1002/jms.502
PMid:12938107

73. Granvogl B, Ploscher M, Eichacker LA. *Sample preparation by in-gel digestion for mass spectrometry-based proteomics.* Anal Bioanal Chem. Review. 2007; 389(4): 991-1002.

http://dx.doi.org/10.1007/s00216-007-1451-4
PMid:17639354

74. Gao M, Deng C, Zhang X. *Magnetic nanoparticles-based digestion and enrichment methods in proteomics analysis.* Expert Rev Proteomics. 2011; 8(3): 379-90.

http://dx.doi.org/10.1586/epr.11.25
PMid:21679118

75. Vaezzadeh AR, Deshusses JM, Waridel P, Francois P, Zimmermann-Ivol CG, Lescuyer P et al. *Accelerated digestion for high-throughput proteomics analysis of whole bacterial proteomes.* J Microbiol Methods. 2010; 80(1): 56-62.

http://dx.doi.org/10.1016/j.mimet.2009.10.019
PMid:19913576

76. Zhang K, Wu S, Tang X, Kaiser NK, Bruce JE. *A bifunctional monolithic column for combined protein preconcentration and digestion for high throughput proteomics research.* J Chromatogr B Analyt Technol Biomed Life Sci. 2007; 849(1-2): 223-30.

http://dx.doi.org/10.1016/j.jchromb.2006.11.029
PMid:17150420

77. Salplachta J, Marchetti M, Chmelik J, Allmaier G. *A new approach in proteomics of wheat gluten: combining chymotrypsin cleavage and matrix-assisted laser desorption/ionization quadrupole ion trap reflectron tandem mass spectrometry.* Rapid Commun Mass Spectrom. 2005; 19(18): 2725-8.

http://dx.doi.org/10.1002/rcm.2092
PMid:16124027

78. Picariello G, Mamone G, Nitride C, Addeo F, Camarca A, Vocca I et al. *Shotgun proteome analysis of beer and the immunogenic potential of beer polypeptides.* Journal of proteomics. 2012; 75(18): 5872-82.

http://dx.doi.org/10.1016/j.jprot.2012.07.038
PMid:22868252

79. Colgrave ML, Goswami H, Howitt CA, Tanner GJ. *What is in a beer? Proteomic characterization and relative quantification of hordein (gluten) in beer.* Journal of proteome research. 2012; 11(1): 386-96.

http://dx.doi.org/10.1021/pr2008434
PMid:21999962

80. Salmanowicz BP, Dylewicz M. *Identification and characterization of high-molecular-weight glutenin genes in Polish triticale cultivars by PCR-based DNA markers.* Journal of applied genetics. 2007; 48(4): 347-57.

http://dx.doi.org/10.1007/BF03195231
PMid:17998591

81. Huang XQ, Cloutier S. *Molecular characterization and genomic organization of low molecular weight glutenin subunit genes at the Glu-3 loci in hexaploid wheat (Triticum aestivum L.).* TAG Theoretical and applied genetics Theoretische und angewandte Genetik. 2008; 116(7): 953-66.
http://dx.doi.org/10.1007/s00122-008-0727-1
PMid:18305921

82. Liu SW, Gao X, Lu BR, Xia GM. *Characterization of the genes coding for the high molecular weight glutenin subunits in Lophopyrum elongatum.* Hereditas. 2008; 145(1): 48-57.
http://dx.doi.org/10.1111/j.0018-0661.2008.2029.x
PMid:18439233

83. Zhang X, Liu D, Jiang W, Guo X, Yang W, Sun J et al. *PCR-based isolation and identification of full-length low-molecular-weight glutenin subunit genes in bread wheat (Triticum aestivum L.).* TAG Theoretical and applied genetics Theoretische und angewandte Genetik. 2011; 123(8): 1293-305.
http://dx.doi.org/10.1007/s00122-011-1667-8
PMid:21830110

84. Köppel E, Stadler M, Lüthy J, Hübner P. *Detection of wheat contamination in oats by polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA).* Zeitschrift für Lebensmitteluntersuchung und -Forschung A. 1998; 206(6): 399-403.
http://dx.doi.org/10.1007/s002170050281

85. Dahinden I, von Büren M, Lüthy J. *A quantitative competitive PCR system to detect contamination of wheat, barley or rye in gluten-free food for coeliac patients.* Eur Food Res Technol. 2001; 212(2): 228-33.
http://dx.doi.org/10.1007/s002170000252

86. Hernandez M, Esteve T, Pla M. *Real-time polymerase chain reaction based assays for quantitative detection of barley, rice, sunflower, and wheat.* Journal of agricultural and food chemistry. 2005; 53(18): 7003-9.
http://dx.doi.org/10.1021/jf050797j
PMid:16131102

87. Zeltner D, Glomb M, Maede D. *Real-time PCR systems for the detection of the gluten-containing cereals wheat, spelt, kamut, rye, barley and oat.* European Food Research and Technology A. 2009; 228(3): 321-30.
http://dx.doi.org/10.1007/s00217-008-0937-4

88. Rønning SB, Berdal KG, Andersen CB, Holst-Jensen A. *Novel Reference Gene, PKABA1, Used in a Duplex Real-Time Polymerase Chain Reaction for Detection and Quantitation of Wheat- and Barley-Derived DNA.* Journal of agricultural and food chemistry. 2006; 54(3): 682-7.
http://dx.doi.org/10.1021/jf052328n
PMid:16448168

89. Mujico JR, Lombardía M, Mena MC, Méndez E, Albar JP. *A highly sensitive real-time PCR system for quantification of wheat contamination in gluten-free food for celiac patients.* Food Chemistry. 2011; 128(3): 795-801.

http://dx.doi.org/10.1016/j.foodchem.2011.03.061

90. Mustorp SL, Dromtorp SM, Holck AL. *Multiplex, quantitative, ligation- dependent probe amplification for determination of allergens in food. Journal of agricultural and food chemistry.* 2011; 59(10): 5231-9.

http://dx.doi.org/10.1021/jf200545j
PMid:21452891

91. Suttie JM, Reynolds SG. *Fodder oats: A world overview, 2004.* Food and Agriculture Organization of the United Nations (FAO). Accessed on 16 October 2012.

http://www.fao.org/docrep/008/y5765e/y5765e00.htm

92. Pulido OM, Gillespie Z, Zarkadas M, Dubois S, Vavasour E, Rashid M et al. *Introduction of oats in the diet of individuals with celiac disease: a systematic review.* Adv Food Nutr Res. Review. 2009; 57: 235-85.

93. Arentz-Hansen H, Fleckenstein B, Molberg O, Scott H, Koning F, Jung G et al. *The molecular basis for oat intolerance in patients with celiac disease.* PLoS Med. 2004; 1(1): e1.

http://dx.doi.org/10.1371/journal.pmed.0010001
PMid:15526039 PMCid:PMC523824

94. Silano M, Di Benedetto R, Maialetti F, De Vincenzi A, Calcaterra R, Cornell HJ et al. *Avenins from different cultivars of oats elicit response by coeliac peripheral lymphocytes.* Scandinavian journal of gastroenterology. 2007; 42(11): 1302-5.

PMid:17852883

95. Maglio M, Mazzarella G, Barone MV, Gianfrani C, Pogna N, Gazza L et al. *Immunogenicity of two oat varieties, in relation to their safety for celiac patients.* Scandinavian journal of gastroenterology. 2011; 46(10): 1194-205.

http://dx.doi.org/10.3109/00365521.2011.603159
PMid:21843037

96. Comino I, Real A, de Lorenzo L, Cornell H, Lopez-Casado MA, Barro F et al. *Diversity in oat potential immunogenicity: basis for the selection of oat varieties with no toxicity in coeliac disease.* Gut. 2011; 60(7): 915-22.

http://dx.doi.org/10.1136/gut.2010.225268
PMid:21317420 PMCid:PMC3112367

97. Diaz-Amigo C, Popping B. *Gluten and gluten-free: issues and considerations of labeling regulations, detection methods, and assay validation.* Journal of AOAC International. 2012; 95(2): 337-48.

http://dx.doi.org/10.5740/jaoacint.SGE_Diaz-Amigo
PMid:22649917

98. Commision Regulation (EC) No 41/2009 of 20 January 2009 concerning the composition and labelling of foodstuffs suitable for people intolerant to gluten. 2009.
99. Codex Alimentarius International Food Standards. Accessed on 28 August 2013. http://www.codexalimentarius.net/web/more_info.jsp?id_sta=291

Chapter 17

Gluten-Free Bakery Products and Pasta

Manuel Gómez[1], Lorena S. Sciarini[2]

[1] Food Technology Area, College of Agricultural Engineering (ETSIIAA). University of Valladolid, Palencia, Spain.

[2] Institute of Food Science and Technology (ICYTAC), National Scientific and Technical Council (CONICET), National University of Cordoba, Cordoba, Argentina.

pallares@iaf.uva.es, losciarini@agro.unc.edu.ar

Doi: http://dx.doi.org/10.3926/oms.265

How to cite this chapter

Gómez M, Sciarini LS. *Gluten-Free Bakery Products and Pasta.* In Arranz E, Fernández-Bañares F, Rosell CM, Rodrigo L, Peña AS, editors. *Advances in the Understanding of Gluten Related Pathology and the Evolution of Gluten-Free Foods*. Barcelona, Spain: OmniaScience; 2015. p. 565-604.

Abstract

Wheat-based bakery products are basic components in the diet of most countries all over the world. Intuitively, obtaining gluten-free bakery products of similar characteristics to wheat-based products is a difficult task and because of this, over the last decades extensive research has been done to get gluten-free bread with the adequate crumb structure and texture. Based on this research, this chapter will focus on the strategies for the development of gluten-free bakery products of good technological, sensory and nutritional properties. In gluten-free products, wheat flour has to be replaced by a mixture of flour and starch from different sources. Nevertheless, for products like bread, pasta and some cookies, gluten network development is required; in this case, a gluten substitute –usually hydrocolloids– must be added in the formulation. Some other products, such as cakes, wafers and crepes, do not need this continuous network and, thus, their gluten-free counterparts are more easily obtained. Gluten-free products are usually very rich in starch and contain few proteins and fibers. To overcome this problem, proteins and fibers are common ingredients in these products. Additives and enzymes are being increasingly added to gluten-free products, as well; but their functionality has to be explored since some discrepancies with their function in wheat-based and gluten-free products are observed.

Keywords

Gluten-free flours, gluten replacement, gluten-free bread, gluten-free cakes, gluten-free cookies, gluten-free pasta.

1. Introduction

Among cereals, wheat has specific proteins that make it ideal for certain applications. Thus, wheat gliadins and glutenins, in the presence of water and mechanical work, form a continuous phase named *gluten network*. It is responsible of the extensible and cohesive properties of the dough while reducing its stickiness. Wheat dough is characterized for its tenacity (dough resistance to stretching) and elasticity (dough ability of regaining its original shape after being stretched). These characteristics allow the retention of gas produced during proofing, resulting in a volume increment and the development of an alveolar structure responsible for a sponge-like product after baking. It is still unclear why this network develops in wheat based dough, and why it is absent in dough prepared with other cereals, but a number of variables are known to influence its development, such as the type and ratio of aminoacids that influence the tertiary and quaternary structure of proteins. In this network, the occurrence of many types of bonds among proteins has been suggested, like hydrogen bonds, hydrophobic interactions and, particularly, disulfide bonds among sulfur residues. The strength of gluten network will depend on glutenin and gliadin quantity –and ratio–, their molecular weight and, more generally, of overall quality of wheat proteins. The characteristics of these proteins will determine the strength, elasticity and extensibility of dough. The way in which specific products need specific gluten characteristics will also be addressed in the chapter.

It is important to clarify the concept of *gluten*, since this word is used in different areas and within each area it has different meanings. In the bakery industry, *gluten* is usually related to the protein that makes the dough both cohesive and extensible, easily sheeted and shaped, as well as capable of retaining the gases produced during fermentation and proofing. According to bakers, the only cereal that possesses gluten is wheat. Under certain circumstances, *gluten* makes reference to the network formed by wheat proteins under wet conditions and after the application of mechanical work (during mixing). Using this concept, wheat would not contain any gluten, but

dough and bread or pasta would. Finally, in the food industry, *gluten* is usually used to refer to corn proteins obtained after wet milling. This particular corn gluten can be consumed by celiac patients, unless it is contaminated with other toxic cereals. This gluten does not form a continuous network for bakery processes. In this chapter, *gluten* will be used to refer to wheat protein network.

Another important issue that must be considered is the need for establishing a gluten network in certain processes. Products where the gluten network plays a key role should contain a "gluten substitute" in their formulation; hydrocolloids usually play this role. However, if gluten does not have a basic function or its formation is even undesirable, the production of their gluten-free counterparts will be easier. As a general rule, gluten plays a basic role in doughs needing a minimal consistency, for example to be sheeted and/or in doughs which should retain fermentation gases, such as bread, pizza, croissants, puff pastries and Marie-like cookies, among other products. Gluten network is also needed in pasta production, while, in liquid dough –batter– it does not develop. Examples of this type of batters are layer and sponge cakes, wafers, crepes and waffles. During the processing of some cookies, mixing time is much reduced to avoid gluten formation, since gluten network is not desirable in these products. Finally, in certain products, such as scalded doughs and *churros* –not addressed in this chapter–, processing includes the addition of hot water. In these cases, the incorporation of hot water increases dough temperature inducing partial gelatinization of starch and protein denaturation, thus, avoiding the development of gluten network.

In the first part of this chapter, the production of bakery products where the gluten network is required is analyzed. Then, in section 3, it is discussed the development of pastry products considering that in certain processes gluten network is not required. Finally, in section 4, gluten-free pasta production is explored.

2. Development of Gluten-free Bakery Products

2.1. Raw Materials: Flours and Starches

When wheat flour is removed from any bakery formulation, a large amount of starch is eliminated and has to be replaced by some other ingredient. Among gluten-free raw materials that can be used for this purpose, the most important ones are cereal flours without gluten, as well as native starches. Flours present a more complex composition including starch, a variable amount of proteins, a low quantity of lipids and some minor components, such as fiber, vitamins and minerals. Rice flour is one of the most suitable raw materials due to its hypoallergenic properties, low sodium content, bland taste and light color[1], and its easy availability in the market. Although less frequently, corn flours[2], mainly those from white varieties, and sorghum and millets[3] are also used. Cereal flours have a variable particle size, usually between 0-200 µm, whereas starches have, on average, smaller particles than flours and a simpler composition (almost 100% of dry matter are carbohydrates). Among the starches most traditionally used for the elaboration of gluten-free products, corn and potato starches are found, due to their functional characteristics, price and availability, but also cassava and cereal starches, such as rice and sorghum. Some years ago, gluten-free breads were based on wheat starch, as it had the advantage of presenting a similar taste to wheat flour; but since it contained trace amounts of allergic protein, it was discarded as a possible raw material for gluten free products. However, in recent years, starch isolation procedures have been significantly improved and, since 2008, a gluten-free wheat starch with gluten content lower than 20 mg kg^{-1} –the limit set by the *Codex alimentarius*– is available on the market; this product does not have harmful effects on most celiac patients[4]. Nevertheless, celiac people are still reluctant to consume products with wheat-based ingredients.

Traditionally, oats have been considered harmful for celiac patients. Recent research, though, considers it safe for celiac population, as long as cross contamination with some toxic cereals is avoided[5,6]. A small percentage of

celiac people, however, do not tolerate avenins (proteins present in oat). A variation in oat addition in gluten-free formulations can be observed from one country to another: while its consumption is allowed and extended in Finland, in other countries celiac people are encouraged to seek medical advice before eating it, while in some others there is still a great reluctance towards its consumption by celiac population. Dough prepared with oat flour does not develop a continuous protein network and some "gluten substitute" has to be added. Breads obtained using oat flour have higher protein and beta-glucan contents, and better sensorial score than those obtained with flours from other gluten-free cereals[7], therefore its incorporation is an alternative to be considered in gluten-free bread production.

For wheat-based products, it is known that every particular case requires a particular type of flour, and thus optimum flours for cookie production will not be appropriate for pan bread and vice versa. However, for gluten-free product development, information about flour requirements is scarce. These flours differ mainly in protein quantity, starch characteristic (e.g. amylose and amylopectin ratio) and particle size distribution. It has been established that corn variety or milling process affect bread physicochemical and sensory properties[2]. In the case of rice, low amylose flours lead to breads with better texture, but waxy varieties (ca. 0% amylose) are not appropriate on their own for gluten-free bread production[8]. Ylimaki et al.[9] have also found that medium-length grain varieties are preferred to long grain varieties for obtaining breads with better sensorial properties. The particle size of the rice flour is also known to have an effect on gluten-free bread-making[10,11]. Consistently, studies carried out with rice and corn flours have shown that particle size is the most important variable affecting bread quality[12,13]. Bigger particle size is preferred, while fractions below 80-100 µm should be discarded for the production of gluten-free bread with high volume and soft crumb. However, using flours with extremely large particles may lead to breads with sandy texture, therefore 200 µm should not be exceeded. Flours with larger particles have been shown to reduce the gas retention capacity of the dough and the batter, as well as final bread volume, and this effect was attributed to differences in internal dough structure.

When starches are added in gluten-free formulations, breads with higher volume and more closed crumb structure are obtained, although crust is lighter because Maillard reactions are largely reduced due to the lack of proteins. The addition of starch lead to softer, more cohesive and resilient crumbs. These conclusions apply to breads based on rice flour[14,15] and other cereal flours[16]. The type of starch also influences bread quality but the conclusions drawn in different studies are sometimes contradictory. Thus, while Sanchez et al.[15] obtained an optimum formulation using higher contents of corn than cassava starch, Onyango et al.[16] found that breads based on cassava and rice starches have better crumb texture compared to corn or potato starch. Consequently, different kinds of flour and starch mixtures should be optimized according to the specific basic formulation.

During the last years, research has focused on the study of novel raw materials, such as pseudocereals (Andean crops –such as quinoa and amaranth– and buckwheat) and minor cereals, like teff. Usually, these flours are nutritionally more balanced than corn or rice flours or starches, especially if they are whole-grain flours. Moreover, they have higher protein, fiber, vitamin and mineral contents[17-19], but their availability on the market is rather limited and their price, higher than most current flours and starches. Because of this, their commercial use is restricted to supplement recipes based on rice flour and starches. Among the flours used for this purpose are buckwheat, as a main ingredient or combined with starch[20], rice flour[21,22] or as a supplement[23]. Amaranth and quinoa –both from South America– have also attracted attention, and so has teff, a minor cereal grown in Ethiopia. Generally, the incorporation of these flours affects baking[24] and sensorial properties[7], color and taste.

Soy flour has also been considered for gluten-free breads, either as a supplement[25] or as a main ingredient[26], as well as other pulse flours, such as chickpea flour[27]. Soy flour contains higher amounts of isoflavonoids and proteins than cereal flours. Usually, these flours modify the internal structure and rheological properties of the dough, affecting the texture and volume of loaves, and bread sensory properties. However, the effect will depend on the

formulation that is used. When used as a main component, soy flour with a thermal pretreatment and, as a consequence lower lipoxygenase activity, is preferred since the typical beany flavor of pulse flours is much reduced; however, bread quality, is reduced and appearance is negatively affected, when compared to non-treated soy flour. Nonetheless, the specific volume of bread made of soy flour as a main ingredient is usually lower than bread made with rice flour and/or starches. Other raw material that may be interesting is chestnut flour[28], but it is also used as a supplement for starches or other gluten-free flours; besides, it should not exceed 30%, since higher levels may affect bread quality negatively. In addition, chestnut flour has a characteristic flavor and thus its addition may dramatically change sensory properties and consumer acceptance of the final product.

2.2. Gluten Substitutes

In wheat dough, the gluten network formed during mixing is placed among starch granules and gives cohesion to the system, making gas retention during proofing possible. During the development of gluten-free bread, proteins present in non-wheat flours are not able to form this network, therefore other products must be added so that the dough/batter can retain gases and expand. Thus, Jongh[29], in one of the first studies on gluten-free dough, already suggested that any agent joining together starch granules may favor these processes and with this purpose glycerol monoestereate was used. At present, this function is performed by hydrocolloids. Hydrocolloids, nutritionally classified as soluble fibers, show high water absorption capacity; during mixing, they combine with water and form a continuous phase surrounding flour particles; this phase results in an increased cohesiveness. However, not all hydrocolloids behave in the same way, as they have various effects on bread characteristics. The use of hydroxypropylmethylcellulose (HPMC) has been proposed to obtain gluten-free breads with appropriate physical characteristics[9,30]. This hydrocolloid, which can form thermoreversible gels when heated, is preferred to other hydrocolloids due to the higher specific volumes of resulting breads, and their improved sensory scores[31-34]. The use of

xanthan gum (XG) is also very widespread among gluten-free technologists. Acs et al.[35,36] studied the effect of different hydrocolloids on corn-starch bread properties and found higher volumes when using XG, compared to guar gum, locust bean gum and tragacanth. At present, most commercial gluten-free breads, as well as formulations used in different research works, contain one of these two hydrocolloids. However, the results of their incorporation are usually contradictory in the literature. While XG increases specific volume in some works[35-37], in others, it does not modify this parameter[31]. These differences may arise from the addition of different amounts of hydrocolloids[34,38] or from the type of flour used[33], as well as from differences in the formulation and baking procedures employed in each particular research. Another important effect of hydrocolloids is that they modify the alveolar structure of breads, XG, HPMC and carboxymethylcellulose (CMC) being the ones producing a finer structure and a higher cell number, when compared to agar or MC (methylcellulose)[39]; these changes in crumb structure also modify crumb texture.

Since hydrocolloids usually increase dough/batter consistency, high quantities of water should be added during bread-making. Actually, most researchers do this correction, but the way it is done is very variable and it is not always well explained; this may be one reason for the differences of hydrocolloids effect observed in the literature. Among the most common hydrocolloids, XG induces the greatest dough/batter consistency[31,37,38], and thus higher water amounts should be added. In order to optimize gluten-free bread formulations, and in particular hydrocolloid level and water amount, the use of response surface designs could be useful[9,40,41]. Sometimes, other hydrocolloids such as CMC, guar gum, locust bean gum or psyllium are added, along with XG or HPMC, to improve bread texture or shelf life (not to increase bread specific volume), since they retain high amounts of water, affect starch retrogradation and delay staling. In this regard, psyllium is an interesting alternative because it is a natural product obtained from the milling of *Plantago ovata* seed hulls; it improves bread sensory properties and shows anti-constipation properties[42] as well as a complementary effect with HPMC[43].

2.3. Proteins and Fibers

The use of starch or flours with low protein and fiber contents for obtaining gluten-free bakery goods leads to a poor nutritional quality of these products compared to their wheat counterparts. As a consequence, research on proteins and fiber incorporation in gluten-free breads has significantly increased in the last years. Both fibers and proteins, not only enhance nutritional properties of bread, but also have an important functional role.

Among proteins proposed for gluten-free bread-making, particularly noteworthy are dairy proteins –both whey proteins and caseins–[44-48], egg proteins[45,46,49-51] and soy proteins[46-51]. Proteins from other sources, such as collagen or lupine[49], pea[48,49,51], yeast[52], or even structured corn proteins[53] or whey[54-56] have also been studied. These studies present contradictory results either because they use different formulations or different levels of protein incorporation. In general, it can be said that the addition of proteins reduces crust lightness[44] because Maillard reactions produced during baking, reduce cell density[49] and modify dough rheology. It has also been observed that using animal proteins, especially egg proteins, leads to breads with higher specific volume compared to vegetable proteins, such as soy proteins[46,49,50]. Moreover, most studies dealing with the incorporation of animal proteins report an increase in loaf volume, while vegetable proteins do not affect this parameter, or even reduce it. This result could be related to the effect of these proteins on dough/batter rheology, since animal proteins –egg proteins– reduce batter consistency, but vegetable proteins –soy proteins– increase it[49-51]; this effect on batter consistency may, in turn, be explained in terms of changes in dough structure. The relationship between dough consistency and bread volume has been reported in other studies[57].

The effect of fiber, as well as proteins, addition will depend on the type of fiber used. Studies on fiber addition into gluten-free breads have mainly focused on the effect of a single type of fiber and fructooligosaccharides[49,58,59], resistant starch[60] or cereal fibers[61,62]. Results are, once again, contradictory and while in some cases fiber enrichment reduces bread volume, in others the contrary effect is observed. Nevertheless, no direct comparison can be made

due to the different formulations, the bread-making procedure, the correction of water amount and the amount of fiber addition used in each case. Hager et al.[63] did compare the effect of inulin and oat β-glucans, but both fibers were added in different amounts. In general, the addition of soluble fibers, such as inulin, polydextrose or nutriose, improves bread quality by enhancing volume and produces darker crust, whereas insoluble fibers, such as celluloses, usually reduce bread volume. However, works carried out by Gómez (unpublished) show that, among celluloses, those with lower particle size and elongated shape lead to the production of loaves with higher volume and lower firmness. The overall effect of insoluble fibers can be related to the fact that they remain intact during mixing and interrupt dough structure; this interruption is less important when small, elongated fibers are used. Soluble fibers, on the other hand, interact with water, hydrocolloid and soluble ingredients in the continuous phase, enhancing its cohesiveness and, as a consequence, gas retention capacity. These studies show that the effect of fibers on dough/batter structure affects its rheology, while those fibers that reduce consistency increase specific volume. Additionally, in the case of fibers like polydextrose and inulin, a partial hydrolysis is produced during bread-making, and the resulting simple sugars, through Maillard reactions, increase crust darkness.

2.4. Additives and Enzymes

Additives and enzymes used in wheat-bread production usually act either on starch fraction or on proteins and gluten network. Those acting on starch fraction will also be appropriate for gluten-free bread production, since their functionality will be similar. Thus, alpha-amylases producing fermentable sugars from starch, enzymes slowing starch retrogradation (anti-staling effect) such as certain amylases, or emulsifiers interacting with starch to reduce retrogradation –such as monoglycerides–, are also suitable for gluten-free bread-making.

On the other hand, additives acting on gluten network will not necessarily have a positive effect on gluten-free breads. Therefore, it has been shown that

DATEM or SSL (emulsifiers used to strengthen gluten doughs) do not have the same effect on gluten-free systems, do not modify loaf volume, or even decrease bread overall quality with firmer crumbs, coarser crumb structure[64], or very slight volume increments[65]. However, other emulsifiers with better air-stabilizing properties, such as monoglycerides or lecithins lead to higher volumes[65].

Unlike wheat breads, the use of proteases with good results in gluten-free systems has been reported[66]. But these results may vary according to the type and the reactivity of protease employed[67], as well as the type of flour used[68]. In this last study, the effect of protease is even negative. It seems that the level of enzyme application also affects bread quality.

The use of transglutaminase has also been explored in gluten-free dough[69,70], sometimes combined with exogenous proteins[45-48,51,71]. Although results are variable and depend on the substrate, the transglutaminase level and the formulation used, it has been proved that transglutaminase acts on proteins increasing their molecular weight[45] and modifying rheological properties of doughs[48,51,69]. In spite of these findings, quality enhancement of gluten-free breads is negligible, and overall quality can even be reduced. This shows that it is not only the presence of a protein continuous network responsible for the volume increment produced during proofing of gluten-free doughs, but its properties are highly relevant as well. The use of glucose oxidase –enzyme capable of enhancing disulphide bonds among protein molecules– has been proposed to increase rice bread volume[72], but once again, the effect of this enzyme depends on the flour used[68] and in some cases it does not significantly influence loaf volume[64].

Starches or flours with a hydrothermal pretreatment –process during which starch is gelatinized– show high water absorption and high thickening properties even at room temperature. Their effect is thus comparable to that of hydrocolloids, and their use has also been suggested to improve the quality of gluten-free breads, increasing their volume and extending their shelf life[73,74], although results are still quite variable and it is important to adjust dough moisture to achieve the optimal rheological properties.

Some enzymes that are not employed in the wheat bread-making process have been proposed as gluten-free breads improvers. Such is the case of cyclodextrin glycosyl transferase (CGTase)[75]; its positive effect on specific volume could be related to the consistency decrease produced after starch hydrolysis. This enzyme, as well as amylases, has been employed to reduce staling in gluten-free breads[76], probably one of the most important problems of these products. Both enzymes hydrolyze starch and reduce its retrogradation. Similarly, the use of lipases –which produce emulsifiers *in situ*– reduces this phenomenon[73], since emulsifiers also present anti-staling properties.

2.5. Processing

As already mentioned, the incorporation of hydrocolloids increases dough/batter consistency, and the water amount needed. Thus, the moisture level in gluten-free bread formulations is higher than 80%, and in most cases, even higher than 100%, whereas the usual water level in wheat dough is lower than 60%. It is generally assumed that higher water amounts lead to breads with higher specific volumes[11-13,30], but large holes are found between crust and crumb when water incorporation is too high or proofing is extended. In addition, breads with excessive hydration present a weak final structure which becomes difficult to cut without breaking. The higher water amount also requires a modification in the baking process. Thus, for gluten-free bread production, baking is longer and usually at lower temperatures than conventional wheat breads.

The existence of an inverse relationship between dough/batter consistency and gluten-free bread volume is widely accepted[57]. A lower consistency can be obtained by adding higher water amounts, but also by using oil in the formulation. The incorporation of oil produces breads with higher volume, but also more cohesive and moister crumbs. This effect is worth highlighting, since a typical defect of gluten-free breads is lack of cohesiveness and very dry texture.

During wheat bread-making, mixing is essential for gluten network development because it is at this point when the energy necessary for this process is generated. So, mixing time is defined as a function of gluten development. In gluten-free bread-making, the mixing process is different for two main reasons. Firstly, gluten-free formulations present lower consistency than wheat formulations and are considered batter rather than dough, and thus mixing accessories are also different. Secondly, mixing ingredients together takes shorter than in conventional systems. However, a very short mixing may lead to lower volumes[77], either because yeast does not adapt to the medium or because the incorporation and distribution of air into the dough is insufficient. After mixing, dough is placed into molds and fermented. Fermentation is critical in gluten-free bread production, since dough structure is usually weaker than in wheat dough, and a long fermentation may produce a collapse and reduction of dough volume, especially in doughs with high water contents[12,13]. This results in flat breads or breads presenting a depression in the central region, with low volume and coarse crumb structure. Therefore, fermentation time should be defined for each particular formulation and bread-making procedure, considering amount of yeast and fermentation temperature. Usually, fermentation time for gluten-free systems is shorter than for conventional wheat formulations. The behavior of dough during proofing can be studied with a rheofermentometer, an instrument used for the study of gluten systems, avoiding the use of overweight on doughs, considering the weakness of gluten free doughs.

2.6. Sourdough

This process consists in fermenting a mixture of flour and water for a relatively long period of time at a moderate temperature. This technique, employed as such almost since the beginning of bread history, allows naturally occurring microorganisms (lactic acid bacteria and yeasts) in the flour to leaven. To facilitate continuous production, bakers started to save a portion of ripe sourdough to seed subsequent doughs; this procedure continued until the nineteenth century. During fermentation, cereal-associated lactic acid bacteria

(LAB) produce lactic and acetic acids, typically lowering pH below 5, and yeast produces CO_2 and ethanol. These conditions favor the activation of enzymes that are beneficial for bread-making. In wheat and barley breads, it is known that this technique improves loaf volume, texture, taste, nutritional value and shelf-life since it delays staling and protects bread from mold spoilage[78,79].

Although sourdough application was replaced in the nineteenth century for other technologies that reduced production time –and thus, overall costs–, its use has become popular again in the last decade, due to the nutritional and technological benefits obtained with this procedure.

In gluten-free breads, incorporating sourdough into the formulation has also resulted in breads with better technological and nutritional properties. The lower pH activates amylolytic and proteolytic enzymes. Moore et al.[80] have shown a decrease in the size of both protein particles and starch granules after 24 h of fermentation of the sourdough, as well as softer breads with a lower firming rate. For oat-based breads, a higher specific volume has been observed when using sourdough[81]. This effect was attributed to a decrease in dough consistency produced by a change in the viscosity profile of the starch, probably caused by acid and enzymatic hydrolysis. As a result of the change in starch behavior, a stronger gel is produced during heating, increasing dough stability and allowing to obtain breads with a better texture[82].

Another effect of the change in pH is the activation of phytases that degrade the phytic acid normally present in most cereal flours, as reported for sorghum-based breads[83]. Phytic acid forms complexes with the minerals present in the dough, reducing their bioavailability. Sourdough also carries nutritional benefits, since it favors mineral availability. Another beneficial effect of sourdough in gluten-free breads that is being a subject of intense research is the *in situ* production of exopolysaccharides[84,85] which will act as hydrocolloids. The importance of hydrocolloids in gluten-free bread production has been discussed earlier in this chapter. Also, by applying sourdough technology, a decrease in mold spoilage rate has been observed, thus enhancing breads' shelf-life[86].

Most studies performed so far have focused on working with autochthonous flora present in the system, with the obvious advantage of being already adapted to the medium. Thus, they show a clear competitive advantage over any other strain. However, in the industry, sourdough is preferentially obtained by using commercial starters which favor the production of breads with constant quality. The selection of strains of LABs and yeast to be used as starters is a condition to ensure the constant quality of end-products; in particular, such selection must be oriented to find those microorganisms that are adapted to the substrate and are able to dominate the fermentation process inhibiting the development of contaminants or autochthonous strains[87].

3. Gluten-Free Pastry

3.1. Cake-Making

Cake production consists in mixing the ingredients together to make a batter that will be finally baked. During the baking process, an increase in batter volume is observed, partly due to the expansion of gas bubbles contained in the batter as a consequence of temperature rise, and partly to the effect of leavening agents, in case they are present in the formulation. This volume increase is produced during the first stages of baking until starch gelatinization, which renders the structure more rigid and makes further expansion more difficult, if not impossible.

The batter is an emulsion composed of air (discontinuous phase) in a mixture of ingredients (continuous phase). In this system, not only is air incorporation of fundamental importance, but its distribution and the viscosity of the continuous phase surrounding gas bubbles are so too. Consequently, the smaller the gas bubbles, the higher their stability in the batter, and the higher the final volume of the cake. In addition, the viscosity of the continuous phase should be high enough to avoid bubbles coalescence and at the same time allow volume increase during baking. There is an enormous variety of cake formulations leading to quite different products.

But, in general, they can be divided in two groups. The first one includes those formulations with no added fat and usually with no leavening agent, in which the cake is obtained by incorporating a huge amount of tiny gas bubbles. In this group we can find *sponge cakes* or *chiffon cakes*, where the performance of egg-albumin as foaming agent is of key importance. The second group is composed of *layer* or *pound cakes*, where the presence of oil or fat is fundamental. In these cases, the use of a leavening agent to enhance the volume increment during baking is very frequent.

It is worth highlighting that gluten does not have a key role in cake production, and in most cases a gluten network is not developed at all. In liquid batters, mixing accessories do not apply enough mechanical work as to develop the network. On the other hand, the starch present in the batter does have a basic function, since it confers viscosity; this, in turn, stabilizes the emulsion and, after gelatinization, confers structure, preventing the dough from flattening. Thus, gluten-free cake-making is, in most cases, rather simple; it is only a matter of changing wheat flour for other gluten-free flour, such as rice or corn, or for a starch. Nevertheless, some factors should be taken into account when making this substitution. First, the proteins present in wheat flour, although they have no functional properties, act on Maillard reactions producing the brown color of the cake surface. Hence, if wheat flour is substituted for a starch, the color of the product will probably not be satisfactory, and the viscosity of the batter will be modified, and thus its capacity to obtain a good air-emulsion will change as well. When starch content in the batter is increased –and as a consequence, protein content is decreased–, the viscosity of the system changes as well as the capacity of producing an appropriate emulsions[88]. Another aspect to consider is starch gelatinization temperature, since this parameter varies according to the botanical source of starch. The end of dough/batter expansion in the oven depends on gelatinization temperature; the latter will also depend on other factors, such as type or quantity of sugar in the formulation.

Usually, the formulation of different cakes is optimized for the use of wheat flour, therefore its substitution for a starch should be accompanied with the

addition of some kind of protein (vegetal or animal) or the complete reformulation of the recipe. The final result will depend on the type and quantity of the starch and protein employed[88]. Proteins usually absorb more water than starch, and this should be taken into account when replacing wheat starch: the water present in the system will be in a more free state and because of this the final batter will be wetter. Adding proteins is a useful strategy when using flours such as rice flour where the protein content is lower than wheat[89]. These authors have shown that the amount of the protein added is important, and so is the type of protein. In this regard, they obtained better results when incorporating animal proteins (caseins and egg proteins) than vegetal proteins (soy and pea).

When wheat flour is substituted for a gluten-free flour, an important aspect to take into consideration is the sensorial effect that this substitution may have, since different flours have quite different color and flavor. Rice flour usually has a neutral flavor and a pale color, which makes it similar to wheat flour; corn flour has a stronger flavor and its color is rather yellowish, which will influence the characteristics of the final product. In Western countries, the use of rice flour to substitute wheat flour is usually preferred. Nonetheless, consumers' preferences may be different from one country to another based on cultural and traditional aspects, and the use of different flours (corn or sorghum) is not only possible but sometimes also advisable. The selection of the leavening agent is also critical, since it should produce gases when the dough has the necessary structure to retain them and be able to expand; this critical point depends on starch gelatinization temperature. As a general rule, the differences in gelatinization temperature for wheat starch and gluten-free cereal flours is not very important, but any problem arise during the development of these products should be solved by changing the leavening agent or the amount of sugar in the formulation.

One of the most important issues when choosing gluten-free flour for cake-making is probably particle size. *Flour* particle size is usually considered to be smaller than 200 µm, but in the case of corn and rice –which are harder grains requiring higher energy input for milling–, particle size is larger. The

japonica variety of rice grains is usually softer than *indica* and produces finer flours. When wheat flour is used for cake-making, finer flours are preferred because a more stable emulsion with larger amounts of smaller bubbles is obtained. When coarse flours, such as rice and corn, are used the emulsions are unstable, and better results are obtained if bigger particles (bigger than 100-140 µm) are removed. Optimum particle size will depend on the type of cake that is produced. Thus, finer flours are preferred for sponge-cakes and coarser flours for layer-cakes[90]. It is possible to re-mill this flour fraction, but at the expense of a higher content of damaged starch that may affect the quality of the final product.

Once wheat flour is substituted for an adequate gluten-free flour, the same additives used for wheat-based cakes can be used with satisfactory results in stabilizing the emulsion[91], reducing mold spoilage or improve batter viscosity (hydrocolloids and modified starches). The use of extruded flours, with a high thickening ability has also been proposed[92]. By using a small amount of hydrocolloids, such as XG, cake quality may improve[91-94], however, they are not indispensable and their addition does not exceed 0.5-1%.

The recommendations made in this section are also applicable to muffin and Madeleine production because these products are very similar to cakes, even though they are baked in smaller cups (cupcakes). Nevertheless, in products where batter viscosity is higher, the mechanical force applied during mixing may develop gluten network (in wheat based products), and for their gluten-free counterparts it may be useful to follow the recommendations made for gluten-free breads, including the addition of hydrocolloid in the formulation.

3.2. Other Products Obtained from Batters

There is a huge amount of products made from a batter that is poured into molds and then baked, such as tea pasta, or put between two hot metal sheeting, such as wafers and waffles, or even heated in a metallic surface, such as crepes. In these products, as well as in cakes, gluten network is not developed and gluten-free counterparts are easy to obtain. Wheat flour is

replaced by gluten-free flour, taking into account the aspects mentioned for cake-making. For this type of products the differences in gelatinization temperature are not important. Nonetheless, it is important to select the gluten-free flour according to its particle size, since this parameter has a great influence on product quality and sensory properties such as taste, flavor and color. It is not possible to make general recommendations due to differences among products and regional preferences and different flours may be used in each case. It is essential, however, to use flour with regular characteristics, like particle size, color and starch properties, to obtain homogeneous products with a regular quality.

3.3. Cookie-Making

Considering that most cookies are made of dough in which the gluten network is not developed, gluten-free cookie-making should not represent a difficult task. In this group of cookies where gluten network is not important, we find batter cookies, such as tea-cookies (extruded) or wafers (discussed in section 3.2 above), and those in which mixing is reduced to avoid gluten network formation, such as wire-cut or molded short-dough cookies. Once again, it is important to carefully select the gluten-free flour or starch to be used. As in the case of bread- and cake-making, several authors have suggested the use of different gluten-free flours, like rice flour[1,95], buckwheat[1,96], amaranth[97], teff[98] or chick peas[99], in addition to corn and potato flours and starches[100]. Sensory characteristics and particle size are aspects to be considered. Finer flours facilitate particles hydration, but also affect the final texture of cookies since batter/dough emulsion properties are also modified.

In addition, there are cookies where a continuous gluten network is formed, such as crackers or Marie-type cookies. In these cases, dough must be sheeted and then cut, and crackers proofed. Due to the gluten network, these cookies present a less brittle texture.

Usually, gluten-free Marie-type cookies do not include any gluten substitute, like hydrocolloids, in their formulation and are made in the same

way as conventional cookies, without gluten development, shaped or sheeted into a circle or rectangle, similar to their wheat counterparts, but with a slightly different texture. If hydrocolloids are added, water amount should be corrected; since cookies are products with low water contents –typically less than 5% and particularly less than 2% for the Marie type–, adding high amounts of water may have a negative effect during the drying process (baking). And thus, adding hydrocolloids makes it more difficult to reduce moisture and achieve the typical crispy texture in this product.

3.4. Puff-Pastry Making

Puff-pastry making is based on the formation of multiple interspersed layers of dough and fat, in such a way that when water is evaporated during baking, a laminar structure is obtained. The first step in puff-pastry making is to prepare the dough –similar to bread dough–, place a piece of fat with it and bring together. The product is then sheeted and folded several times to increase the number of layers. On the one hand, fat should present a high melting point to prevent it from melting during sheeting, as it would mix with dough, which is undesirable. On the other hand, dough should be cohesive, extensible (easy to sheet) and have low stickiness. This type of dough usually requires a gluten network able to confer cohesion and allow the dough to be stretched without breaking. In this way, for the formulation of gluten-free puff-pastry, a gluten substitute should be incorporated. The same hydrocolloids used for gluten-free bread-making, such as xanthan gum, guar gum, locust bean gum or cellulose derivatives, may be used for puff-pastry. However, after hydrocolloid addition, this type of dough tends to be too sticky. To reduce stickiness, it is important to limit water incorporation. Sometimes it is also important for the dough not to be too consistent so that it can be easily sheeted. A possible alternative is to use oil in the formulation. Also, some extra flour added onto the dough surface or letting air flow can be used to dry out dough surface and thus reduce its stickiness. However, in spite of these alternative techniques, it is not possible to sheet gluten-free puff

pastry to the same extent as wheat puff-pastry; final products present a different texture, are less crunchy and have thicker and coarser layers.

Some puff-pastries include yeast in their formulation, and are fermented after sheeting. During fermentation, an increment in the volume of the pieces is registered. Examples of these products are croissants, where the recommendations for gluten-free bread-making apply to flour/starch selection, gluten substitutes and additives. Stickiness should be reduced to a minimum and extensibility should be enhanced, making the sheeting process easier.

4. Pasta-Making

Pasta is probably the simplest cereal-based product. From the formulation point of view, it consists of a mix of flour or semolina with water and it may also contain egg. Regarding processing, pasta is prepared by following a hydration step, mixing, shaping/cutting and drying. Pasta can be classified according to some of the following parameters: water content, processing type and/or shaping. According to water content of the final product, it can be fresh or dried. Looking at the technology employed to shape it, pasta can be extruded or sheeted. It can also be short, long or filled. This section will be focused on dry extruded pasta, widely consumed all over the world.

High protein content, as well as a strong gluten network, is required for obtaining pasta with a proper cooking performance. During pasta cooking, two main phenomena take place: on the one hand, gluten network –developed during mixing– hydrates and, as temperature rises, coagulates and becomes insoluble, thus creating a strong network that entraps starch granules. On the other hand, water diffusion inside pasta and temperature rise lead to starch gelatinization. During gelatinization, part of the amylose leaches out of the granule and diffuses to pasta surface and –if pasta structure is inadequate– to cooking water as well. Once on the pasta surface, amylose is responsible for the increase in stickiness, with a detrimental effect on its sensory quality.

Starch gelatinization and protein coagulation are competitive phenomena, since they occur at the same temperature range and are both influenced by

water availability[101]. The faster the protein coagulation, the more limited starch swelling, and the lower the amylose quantity that leaches out from the granules, ensuring a firmer texture and a lower stickiness of the final product. Pasta with good technological properties shows high resistance to overcooking, a firm texture, low stickiness and reduced organic matter loss into cooking water. These parameters are of vital importance when pasta is chosen by consumers.

In gluten-free pasta –which lacks the protein network– the structural role may be assumed by starch. The retrogradation of amylose solubilized during gelatinization implies a double-helix formation, stabilized through hydrogen bonding and thus forming a continuous phase surrounding swelled and deformed starch granules. This retrograded amylose is thermally stable and can only be dissociated at temperatures higher than 100°C. The empiric knowledge of this phenomenon has been used in Asia for many years, where the traditional process to obtain rice-based noodles include several –and complex– heating and cooling steps of rice flour that lead to starch pregelatinization –and later retrogradation–, and this flour is then mixed with the rest of flour and water to complete noodle-making. In this way, a tridimensional network with viscoelastic properties is obtained[102]. So, ideal starch for gluten-free pasta production should present high tendency to retrograde, such as high amylose starches or pulse starches. In this last part of the chapter, the main raw materials employed for gluten-free pasta will be covered.

Traditionally, gluten-free pasta is made of rice flour. Pasta of good technological properties has been obtained by using this flour[103,104]. Usually, flour obtained from long grains is preferred since it presents high amylose content. Moreover, parboiled grains have good performance for pasta making, since during parboiling starch gelatinizes and the amylose-lipid complex is formed. These changes in starch structure limit starch swelling and amylose loss during pasta cooking.

Considering corn flour, Dexter and Matsuo[105] have shown that the lower the amylose content, the lower the noodles quality. However, there is a

compromise between amylose content and flour performance, since corn with more than 40% amylose does not completely gelatinize during heating and this limits later retrogradation[106].

In addition to amylose content, it has been found that flour particle size, as well as the pretreatment of flour/water mixture (microwave heating), have a considerable influence on noodle quality[107]. The presence of big particles delays protein and starch dispersion during heating in water (pretreatment). Higher temperatures and moisture during pretreatment favor, on the one hand, gelatinization and retrogradation and, on the other, protein interaction, once glass transition temperature has been reached. However, corn proteins provide a weak and transitory structure unable to stabilize the final product. Therefore, the structural role lies, again, with starch[107].

As already mentioned, pseudocereals have received important attention essentially because of their high nutritional value. A rapid literature search suggests that buckwheat is the favorite pseudocereal for gluten-free pasta-making. It has been shown that these grains have a lower detrimental effect on pasta quality compared to quinoa and amaranth[108], in terms of firmness, cooking time and cooking loss. When using quinoa, and particularly amaranth, pasta firmness is substantially reduced, as well as its tolerance to overcooking. Nevertheless, it has also been reported that buckwheat is susceptible to undergoing Maillard reactions during the drying process because of its high lysine and reducing sugars content, which yields a product of unpleasant brown color[109]. The performance of these flours will also depend on the pretreatment they are subjected to before pasta-making. Thus, extrusion-cooking of a mix of rice and amaranth flours produces pasta with good quality parameters, whereas the same mix of flours without pretreatment shows a low performance for pasta production[110].

Sorghum presents some interesting characteristics, such as being a source of antioxidant and cholesterol lowering compounds[3]. It has been proposed that the main factor determining the high performance of this cereal is grain hardness[111], which determines flour particle size and quantity of damaged starch. In general, pasta with a higher firmness and tensile strength is

obtained when using flours from grains with hard endosperm, subjected to a more extensive milling process; thus flours present lower particle size and higher quantity of damaged starch[112,113]. Liu et al.[112] have corroborated that pasta quality is not related to protein quantity. Moreover, it has been shown that flour pretreatment, such as microwave heating, has a positive effect on the final quality of pasta. Waxy sorghum flour has also been studied, but cooking loss is too high and the resulting pasta presents high stickiness due to a limited starch retrogradation after pretreatment[113].

The most widely used additives for pasta production are undoubtedly hydrocolloids and emulsifiers. With hydrocolloids addition higher consistency is obtained, as well as higher firmness and more pleasant mouth-feel[114,115]. Therefore, the negative effect of adding functional ingredients to the gluten-free flours (corn and rice) traditionally used can be offset by adding hydrocolloids, which confer cohesion to the system. For example, the nutritional quality of corn-based pasta can be enhanced by adding oat flour, and its negative effect counteracted with hydrocolloids, best results being obtained by adding CMC and chitosan[115].

Emulsifiers lubricate the system during the extrusion process, increasing consistency and decreasing stickiness[116]. Furthermore, when the emulsifier is added, starch swelling and amylose leaching are reduced when heated[117] and thus, cooking loss is reduced[118].

Nevertheless, despite the positive effect of emulsifiers and hydrocolloids addition, some researchers[119] suggest that consumers usually associate their presence in gluten-free pasta with an artificial food. In this context, looking for alternatives when selecting raw materials and/or processing conditions seems a viable option for good quality pasta.

As already mentioned, the pretreatment of raw materials has an important effect on pasta quality. Treatments inducing starch gelatinization –and subsequent retrogradation–, such as parboiling[120], pregelatinization[121], annealing and heat moisture treatment[122] among the most important ones, favor the structural development of pasta, increasing the firmness of the final product and decreasing cooking loss.

Regarding pasta-making process itself, extrusion-cooking probably represents the most suitable alternative for gluten-free pasta, since it unifies two different processes: pregelatinization and shaping. Wang et al. produced pea flour-[123] and starch-[124] based pasta employing two extrusion methods: the classic method, consisting in shaping pasta dough at room temperature and atmospheric pressure, and extrusion-cooking (twin-screw), in which dough is subjected to high temperatures for a short period of time; using this method, starch is partially gelatinized and proteins are partially denatured, and thus a restructuration takes place in the extruded dough. These authors found a decrease in cooking time, a lower weight of cooked pasta, a notable decrease in cooking loss and a more pleasant texture for pasta produced with extrusion-cooking, when compared to room temperature extrusion. Extrusion-cooking has been successfully employed in mixtures of corn and broad bean flours[125], rice flour[103], and in mixtures of rice and amaranth flours[110], among other raw materials.

5. Concluding Remarks

Some wheat-based products, such as bread, puff-pastry and pasta, are obtained from a dough where a continuous gluten network has been developed; whereas other products, like cakes, pastries and cookies are obtained from a dough without a developed gluten network –with gluten development being even negative. Thus, in the first case, obtaining gluten-free counterparts is more difficult than in the second. However, there are some alternatives to overcome this problem. Regarding formulation, for products where a gluten network is required, a gluten 'substitute' is usually added in the gluten-free formulation, and this substitute is usually a hydrocolloid. In pasta making, the role of gluten is usually played by pregelatinized starch. It is also important to analyze processing parameters (e.g. mixing, proofing, baking) adapting them to the new needs. Various gluten free flours and starches from different sources have been studied for their use in gluten-free formulations. The huge amount of raw materials and their combinations that

can be used for the elaboration of gluten-free products make it impossible to generalize about their behavior in a gluten-free dough/batter. Moreover, the availability in the market of flours obtained from sources different from wheat is non-continuous; and, moreover, flours from a single botanical origin but commercialized by different suppliers may also present different properties, such as particle size, pasting properties, fiber and protein contents, leading to products of varying quality. It is therefore important to work on understanding, first, the functional properties of the most appropriate flour/starch mixtures for each gluten-free product and, second, the most suitable conditions for continuous processing.

Many additives and enzymes are also analyzed in the literature for improving the technological quality of end products. It is important to note that the functionality of these additives may be different in a gluten-free system compared to a traditional –wheat containing– one, particularly when additives interact with gluten network; in this case, additives effect should be studied for each particular product.

Regarding processing, some alternatives to improve end product quality are also possible, like sourdough for bread-making or extrusion-cooking for pasta-making.

Acknowledgment

Authors would like to thank to University of Valladolid (Spain) and to CONICET and University of Cordoba (Argentina) for financial support.

References

1. Torbica A, Hadnađev M, Dapčević Hadnađev T. *Rice and buckwheat flour characterisation and its relation to cookie quality.* Food Res Int. 2012; 48: 277-83.
   http://dx.doi.org/10.1016/j.foodres.2012.05.001
2. Brites C, Trigo MJ, Santos C, Collar C, Rosell CM. *Maize-Based Gluten-Free Bread: Influence of Processing Parameters on Sensory and Instrumental Quality.* Food Bioprocess Tech. 2010; 707-15.
3. Taylor JRN, Schober TJ, Bean SR. *Novel food and non-food uses for sorghum and millets.* J Cereal Sci. 2006; 44: 252-71.
   http://dx.doi.org/10.1016/j.jcs.2006.06.009
4. Houben A, Höchstötter A, Becker T. *Possibilities to increase the quality in gluten-free bread production: an overview.* Eur Food Res Technol. 2012; 235: 195-208.
   http://dx.doi.org/10.1007/s00217-012-1720-0
5. Hüttner EK, Arendt EK. 2010. *Recent advances in gluten-free baking and the current status of oats.* Trends Food Sci Tech. 2010, 21: 303-12.
   http://dx.doi.org/10.1016/j.tifs.2010.03.005
6. Pawlowska P, Diowksz A, Kordialik-Bogacka E. *State-of-the-Art Incorporation of Oats into a Gluten-Free Diet.* Food Rev Int. 2012; 28: 330-42.
   http://dx.doi.org/10.1080/87559129.2012.660715
7. Hager A-S, Wolter A, Czerny M, Bez J, Zannini E, Arendt EK et al. *Investigation of product quality, sensory profile and ultrastructure of breads made from a range of commercial gluten-free flours compared to their wheat counterparts.* Eur Food Res Technol. 2012B; 235: 333-44.
   http://dx.doi.org/10.1007/s00217-012-1763-2
8. Nishita KD, Bean MM. *Physicochemical properties of rice in relation to rice bread.* Cereal Chem. 1979; 56: 185-9.
9. Ylimaki G, Hawrysh ZJ, Hardin RT, Thomson ABR. *Response-surface methodology in the development of rice flour yeast breads - sensory evaluation.* J Food Sci. 1991; 56: 751-5.
   http://dx.doi.org/10.1111/j.1365-2621.1991.tb05374.x
10. Nishita KD, Bean MM. *Grinding methods: Their impact on rice flour properties.* Cereal Chem. 1982; 59: 46-9.
11. Ylimaki G, Hawrysh ZJ, Hardin RT, Thomson ABR. *Application of response-surface methodology to the development of rice flour yeast breads - objective measurements.* J Food Sci. 1988; 53: 1800-5.
    http://dx.doi.org/10.1111/j.1365-2621.1988.tb07846.x

12. de la Hera E, Martinez M, Gómez M. *Influence of flour particle size on quality of gluten-free rice bread.* LWT-Food Sci Technol. 2013; 54: 199-206.
http://dx.doi.org/10.1016/j.lwt.2013.04.019

13. de la Hera E, Talegón M, Caballero P, Gómez M. *Influence of maize flour particle size on gluten-free breadmaking.* J Sci Food Agr. 2013; 93: 924-32.
http://dx.doi.org/10.1002/jsfa.5826
PMid:22886488

14. Sciarini LS, Ribotta PD, León AE, Pérez GT. *Influence of gluten-free flours and their mixtures on batter properties and bread quality.* Food Bioprocess Tech. 2010; 3: 577-85.
http://dx.doi.org/10.1007/s11947-008-0098-2

15. Sánchez HD, Osella CA, De La Torre MA. *Optimization of gluten-free bread prepared from cornstarch, rice flour, and cassava starch.* J Food Sci. 2002; 67: 416-9.
http://dx.doi.org/10.1111/j.1365-2621.2002.tb11420.x

16. Onyango C, Mutungi C, Unbehend G, Lindhauer MG. *Modification of gluten-free sorghum batter and bread using maize, potato, cassava or rice starch.* LWT-Food Sci Technol. 2011; 44: 681-6.
http://dx.doi.org/10.1016/j.lwt.2010.09.006

17. Alvarez-Jubete L, Arendt EK, Gallagher E. *Nutritive value and chemical composition of pseudocereals as gluten-free ingredients.* Int J Food Sci Nutr. 2009; 60: 240-57.
http://dx.doi.org/10.1080/09637480902950597
PMid:19462323

18. Alvarez-Jubete L, Arendt EK, Gallagher E. *Nutritive value of pseudocereals and their increasing use as functional gluten-free ingredients.* Trends Food Sci Tech. 2010A; 21: 106-13.
http://dx.doi.org/10.1016/j.tifs.2009.10.014

19. Hager A-S, Wolter A, Jacob F, Zannini E, Arendt EK. *Nutritional properties and ultra-structure of commercial gluten free flours from different botanical sources compared to wheat flours.* J Cereal Sci. 2012; 56: 239-47.
http://dx.doi.org/10.1016/j.jcs.2012.06.005

20. Wronkowska M, Haros M, Soral-Smietana M. *Effect of starch substitution by buckwheat flour on gluten-free bread quality.* Food Bioprocess Tech. 2013; 6: 1820-7.
http://dx.doi.org/10.1007/s11947-012-0839-0

21. Peressini D, Pin M, Sensidoni A. *Rheology and breadmaking performance of rice-buckwheat batters supplemented with hydrocolloids.* Food Hydrocolloid. 2011; 25: 340-9.
http://dx.doi.org/10.1016/j.foodhyd.2010.06.012

22. Torbica A, Hadnadev M, Dapčević Hadnađev T. *Rheological, textural and sensory properties of gluten-free bread formulations based on rice and buckwheat flour.* Food Hydrocolloid. 2010; 24: 626-32.
http://dx.doi.org/10.1016/j.foodhyd.2010.03.004

23. Mariotti M, Pagani MA, Lucisano M. *The role of buckwheat and HPMC on the breadmaking properties of some commercial gluten-free bread mixtures.* Food Hydrocolloid. 2013; 30: 393-400.
http://dx.doi.org/10.1016/j.foodhyd.2012.07.005

24. Alvarez-Jubete L, Auty M, Arendt EK, Gallagher E. *Baking properties and microstructure of pseudocereal flours in gluten-free bread formulations.* Eur Food Res Technol. 2010; 230: 437-45.
http://dx.doi.org/10.1007/s00217-009-1184-z

25. Sánchez HD, Osella CA, de la Torre MA. *Use of response surface methodology to optimize gluten-free bread fortified with soy flour and dry milk.* Food Sci Technol Int. 2004; 10: 5-9.
http://dx.doi.org/10.1177/1082013204042067

26. Shin D-J, Kim W, Kim Y. *Physicochemical and sensory properties of soy bread made with germinated, steamed, and roasted soy flour.* Food Chem. 2013; 141: 517-23.
http://dx.doi.org/10.1016/j.foodchem.2013.03.005
PMid:23768388

27. Miñarro B, Albanell E, Aguilar N, Guamis B, Capellas M. *Effect of legume flours on baking characteristics of gluten-free bread.* J Cereal Sci. 2014; 56: 476-81.

28. Demirkesen I, Mert B, Sumnu G, Sahin S. Utilization of chestnut flour in gluten-free bread formulations. J Food Eng. 2010; 101: 329-36.
http://dx.doi.org/10.1016/j.jfoodeng.2010.07.017

29. Jongh G. *The formation of dough and bread structure. I. The ability of starch to form structures and the improving effect of glyceryl-monosterate.* Cereal Chem. 1961; 38: 140-52.

30. McCarthy DF, Gallagher E, Gormley TR, Schober TJ, Arendt EK. *Application of response surface methodology in the development of gluten-free bread.* Cereal Chem. 2005; 82: 609-15.
http://dx.doi.org/10.1094/CC-82-0609

31. Sabanis D, Tzia C. *Effect of hydrocolloids on selected properties of gluten-free dough and bread.* Food Sci Technol Int. 2011; 17: 279-91.
http://dx.doi.org/10.1177/1082013210382350
PMid:21917639

32. Andersson H, Ohgren C, Johansson D, Kniola M, Stading M. *Extensional flow, viscoelasticity and baking performance of gluten-free zein-starch doughs supplemented with hydrocolloids.* Food Hydrocolloid. 2011; 25: 1587-95.
http://dx.doi.org/10.1016/j.foodhyd.2010.11.028

33. Hager A-S, Arendt EK. *Influence of hydroxypropylmethylcellulose (HPMC), xanthan gum and their combination on loaf specific volume, crumb hardness and crumb grain characteristics of gluten-free breads based on rice, maize, teff and buckwheat.* Food Hydrocolloid. 2013; 32: 195-203.
http://dx.doi.org/10.1016/j.foodhyd.2012.12.021

34. Crockett R, Ie P, Vodovotz Y. *How do xanthan and hydroxypropyl methylcellulose individually affect the physicochemical properties in a model gluten-free dough?* J Food Sci. 2011; 76: E274-E282.
http://dx.doi.org/10.1111/j.1750-3841.2011.02088.x
PMid:21535827

35. Acs E, Kovacs Z, Matuz J. *Bread from corn starch for dietetic purposes. I. Structure formation.* Cereal Res Commun. 1996; 24: 441-9.

36. Acs E, Kovacs Z, Matuz J. *Bread from corn starch for dietetic purposes. II. Formation of the visual and technological properties.* Cereal Res Commun. 1996; 24: 451-9.

37. Sciarini LS, Ribotta PD, León AE, Pérez GT. *Effect of hydrocolloids on gluten-free batter properties and bread quality.* Int J Food Sci Tech. 2010; 45: 2306-12.
http://dx.doi.org/10.1111/j.1365-2621.2010.02407.x

38. Lazaridou A, Duta D, Papageorgiou M, Belc N, Biliaderis CG. *Effects of hydrocolloids on dough rheology and bread quality parameters in gluten-free formulations.* J Food Eng. 2007; 79: 1033-47.
http://dx.doi.org/10.1016/j.jfoodeng.2006.03.032

39. Demirkesen I, Kelkar S, Campanella OH, Sumnu G, Sahin S, Okos M. *Characterization of structure of gluten-free breads by using X-ray microtomography.* Food Hydrocolloid. 2014; 36: 37-44.
http://dx.doi.org/10.1016/j.foodhyd.2013.09.002

40. Toufeili I, Dagher S, Sadarevian S, Noureddine A, Sarakbi M, Farran MT. *Formulation of gluten-free pocket-type flat breads: Optimization of methylcellulose, gum arabic and egg albumen levels by response surface methodology.* Cereal Chem. 1994; 71: 594-601.

41. Sabanis D, Tzia C. *Selected structural characteristics of HPMC-containing gluten free bread: A response surface methodology study for optimizing quality.* Int J Food Prop. 2011; 14: 417-31.
http://dx.doi.org/10.1080/10942910903221604

42. Zandonadi RP, Assuncao Botelho RB, Coelho Araujo WM. *Psyllium as a substitute for gluten in bread.* J Am Diet Assoc. 2009; 109: 1781-4.
http://dx.doi.org/10.1016/j.jada.2009.07.032
PMid:19782179

43. Haque A, Morris ER. *Combined use of ispaghula and HPMC to replace or augment gluten in breadmaking.* Food Res Int. 1994; 27: 379-93.
http://dx.doi.org/10.1016/0963-9969(94)90194-5

44. Krupa-Kozak U, Baczek N, Rosell MC. *Application of dairy proteins as technological and nutritional improvers of calcium-supplemented gluten-free bread.* Nutrient. 2013; 5: 4503-20.
http://dx.doi.org/10.3390/nu5114503
PMid:24241213 PMCid:PMC3847745

45. Storck CR, Zavareze E, Gularte MA, Cardoso Eliasa M, Rosell CM, Guerra Dias AR. *Protein enrichment and its effects on gluten-free bread characteristics.* LWT-Food Sci Technol. 2013; 53: 346-54.
http://dx.doi.org/10.1016/j.lwt.2013.02.005

46. Smerdel B, Pollak L, Novotni D, Čukelj N, Benković M, Lušić D et al. *Improvement of gluten-free bread quality using transglutaminase, various extruded flours and protein isolates.* J Food Nutr Res. 2012; 51: 242-53.

47. Shin M, Gang D-O, Song J-Y. *Effects of protein and transglutaminase on the preparation of gluten-free rice bread.* Food Sci Biotechnol. 2010; 19: 951-6.
http://dx.doi.org/10.1007/s10068-010-0133-8

48. Marco C, Rosell MC. *Breadmaking performance of protein enriched, gluten-free breads.* Eur Food Res Technol. 2008; 227: 1205-13.
http://dx.doi.org/10.1007/s00217-008-0838-6

49. Ziobro R, Witczak T, Juszczak Leslaw, Korus J. *Supplementation of gluten-free bread with non-gluten proteins. Effect on dough rheological properties and bread characteristic.* Food Hydrocolloid. 2013; 32: 213-20.
http://dx.doi.org/10.1016/j.foodhyd.2013.01.006

50. Crockett R, Ie P, Vodovotz Y. *Effects of soy protein isolate and egg white solids on the physicochemical properties of gluten-free bread.* Food Chem. 2011; 129: 84-91.
http://dx.doi.org/10.1016/j.foodchem.2011.04.030

51. Marco C, Rosell MC. *Effect of different protein isolates and transglutaminase on rice flour properties.* J Food Eng. 2008; 84: 132-9.
http://dx.doi.org/10.1016/j.jfoodeng.2007.05.003

52. Miñarro B, Normahomed I, Guamis B, Capellas M. *Influence of unicellular protein on gluten-free bread characteristics.* Eur Food Res Technol. 2010; 231: 171-9.

http://dx.doi.org/10.1007/s00217-010-1269-8

53. Erickson DP, Campanella OH, Hamaker BR. *Functionalizing maize zein in viscoelastic dough systems through fibrous, beta-sheet-rich protein networks: An alternative, physicochemical approach to gluten-free breadmaking.* Trends Food Sci Tech. 2012; 24: 74-81.

http://dx.doi.org/10.1016/j.tifs.2011.10.008

54. van Riemsdijk LE, van der Goot AJ, Hamer RJ. *The use of whey protein particles in gluten-free bread production, the effect of particle stability.* Food Hydrocolloid. 2011; 25: 1744-50.

http://dx.doi.org/10.1016/j.foodhyd.2011.03.017

55. van Riemsdijk LE, van der Goot AJ, Hamer R J, Boom RM. *Preparation of gluten-free bread using a meso-structured whey protein particle system.* J Cereal Sci. 2011; 53: 355-61.

http://dx.doi.org/10.1016/j.jcs.2011.02.006

56. van Riemsdijk LE, Peigrom PJM; van der Goot AJ, Boom RM, Hamer RJ. *A novel method to prepare gluten-free dough using a meso-structured whey protein particle system.* J Cereal Sci. 2011; 53: 133-8.

http://dx.doi.org/10.1016/j.jcs.2010.11.003

57. Matos ME, Rosell CM. *Quality indicators of rice-based gluten-free bread-like products: Relationships between dough rheology and quality characteristics.* Food Bioprocess Technol. 2013; 6: 2331-41.

http://dx.doi.org/10.1007/s11947-012-0903-9

58. Korus J, Grzelak K, Achremowicz K, Sabat R. *Influence of prebiotic additions on the quality of gluten-free bread and on the content of inulin and fructooligosaccharides.* Food Sci Tech Int. 2006; 12: 489-95.

http://dx.doi.org/10.1177/1082013206073072

59. Capriles VD, Areas JAG. *Effects of prebiotic inulin-type fructans on structure, quality, sensory acceptance and glycemic response of gluten-free breads.* Food & Function. 2013; 4: 104-10.

http://dx.doi.org/10.1039/C2FO10283H
PMid:23032642

60. Korus J, Witczak M, Ziobro R, Juszczak L. *The impact of resistant starch on characteristics of gluten-free dough and bread.* Food Hydrocolloid. 2009; 23: 988-95.

http://dx.doi.org/10.1016/j.foodhyd.2008.07.010

61. Sabanis D, Lebesi D, Tzia C. *Development of fiber-enriched gluten-free bread: a response surface methodology study.* Int J Food Sci Nutr. 2009; 60: 174-90.
http://dx.doi.org/10.1080/09637480902721196
PMid:19330631

62. Sabanis D, Lebesi D, Tzia C. *Effect of dietary fiber enrichment on selected properties of gluten-free bread.* LWT-Food Sci Technol. 2009; 42: 1380-9.
http://dx.doi.org/10.1016/j.lwt.2009.03.010

63. Hager A-S, Ryan LAM, Schwab C, Gaenzle MG, O'Doherty JV, Arendt EK. *Influence of the soluble fibers inulin and oat beta-glucan on quality of dough and bread.* Eur Food Res Technol. 2011; 232: 405-13.
http://dx.doi.org/10.1007/s00217-010-1409-1

64. Sciarini LS, Ribotta PD, Leon AE, Pérez GT. *Incorporation of several additives into gluten free breads: Effect on dough properties and bread quality.* J Food Eng. 2012; 111: 590-7.
http://dx.doi.org/10.1016/j.jfoodeng.2012.03.011

65. Nunes MHB, Moore MM, Ryan LAM, Arendt EK. *Impact of emulsifiers on the quality and rheological properties of gluten-free breads and batters.* Eur Food Res Technol. 2009; 228: 633-42.
http://dx.doi.org/10.1007/s00217-008-0972-1

66. Hamada S, Suzuki K, Aoki N, Suzuki Y. *Improvements in the qualities of gluten-free bread after using a protease obtained from Aspergillus oryzae.* J Cereal Sci. 2013; 57: 91-7.
http://dx.doi.org/10.1016/j.jcs.2012.10.008

67. Kawamura-Konishi Y, Shoda K, Koga H, Honda Y. *Improvement in gluten-free rice bread quality by protease treatment.* J Cereal Sci. 2013; 58: 45-50.
http://dx.doi.org/10.1016/j.jcs.2013.02.010

68. Renzetti S, Arendt EK. *Effects of oxidase and protease treatments on the breadmaking functionality of a range of gluten-free flours.* Eur Food Res Technol. 2009; 229: 307-17.
http://dx.doi.org/10.1007/s00217-009-1048-6

69. Renzetti S, Dal Bello F, Arendt EK. *Microstructure, fundamental rheology and baking characteristics of batters and breads from different gluten-free flours treated with a microbial transglutaminasa.* J Cereal Sci. 2008; 48: 33-45.
http://dx.doi.org/10.1016/j.jcs.2007.07.011

70. Pongjaruvat W, Methacanon P, Seetapan N, Fuongfuchat A, Gamonpilas C. *Influence of pregelatinised tapioca starch and transglutaminase on dough rheology and quality of gluten-free jasmine rice breads.* Food Hydrocolloid. 2014; 36: 143-50.
http://dx.doi.org/10.1016/j.foodhyd.2013.09.004

71. Moore MM, Heinbockel M, Dockery P, Ulmer HM, Arendt EK. *Network formation in gluten-free bread with application of transglutaminase.* Cereal Chem. 2006; 83: 28-36.

http://dx.doi.org/10.1094/CC-83-0028

72. Gujral HS, Rosell CM. *Improvement of the breadmaking quality of rice flour by glucose oxidase.* Food Res Int. 2004; 37: 75-81.

http://dx.doi.org/10.1016/j.foodres.2003.08.001

73. Martinez MM, Marcos P, Gomez M. *Texture development in gluten-free breads: Effect of different enzymes and extruded flour.* J Texture Stud. 2013; 44: 480-9.

http://dx.doi.org/10.1111/jtxs.12037

74. Purhagen JK, Sjoo ME, Eliasson A-C. *The anti-staling effect of pre-gelatinized flour and emulsifier in gluten-free bread.* Eur Food Res Technol. 2012; 235: 265-76.

http://dx.doi.org/10.1007/s00217-012-1753-4

75. Gujral HS, Guardiola I, Carbonell JV, Rosell MC. *Effect of cyclodextrinase on dough rheology and bread quality from rice flour.* J Agr Food Chem. 2003; 51: 3814-8.

http://dx.doi.org/10.1021/jf034112w
PMid:12797748

76. Gujral HS, Haros M, Rosell CM. *Starch hydrolyzing enzymes for retarding the staling of rice bread.* Cereal Chem. 2003; 80: 750-4.

http://dx.doi.org/10.1094/CCHEM.2003.80.6.750

77. Gómez M, Talegón M, de la Hera E. *Influence of mixing on quality of gluten-free bread.* J Food Quality. 2013; 36: 139-45.

http://dx.doi.org/10.1111/jfq.12014

78. De Vuyst L, Vancanneyt M. *Biodiversity and identification of sourdough lactic acid bacteria.* Food Microbiol. 2007; 24: 120-7.

http://dx.doi.org/10.1016/j.fm.2006.07.005
PMid:17008154

79. Gobbetti M, De Angelis M, Di Cagno R, Rizzello CG. *Sourdough lactic/acid bacteria.* In: Arendt EK, Dal Bello F (Eds.). Gluten-free Cereals Products and Beverages. Elsevier. 2008; 267-88.

http://dx.doi.org/10.1016/B978-012373739-7.50014-9

80. Moore MM, Juga B, Schober TJ, Arendt EK. *Effect of lactic acid bacteria on properties of gluten-free sourdoughs, batters, and quality and ultrastructure of gluten-free bread.* Cereal Chem. 2007; 84: 357-64.

http://dx.doi.org/10.1094/CCHEM-84-4-0357

81. Hüttner EK, Dal Bello F, Arendt EK. *Identification of lactic acid bacteria isolated from oat sourdoughs and investigation into their potential for the improvement of oat bread quality.* Eur Food Res Technol. 2010; 230: 849-57.
http://dx.doi.org/10.1007/s00217-010-1236-4

82. Schober TJ, Bean SR, Boyle DL. *Gluten-free sorghum bread improved by sourdough fermentation: biochemical, rheological, and microstructural background.* J Agric Food Chem. 2007; 55: 5137-46.
http://dx.doi.org/10.1021/jf0704155
PMid:17536829

83. Osman MA. *Changes in sorghum enzyme inhibitors, phytic acid, tannins and in vitro protein digestibility occurring during Khamir (local bread) fermentation.* Food Chem. 2004; 88: 129-34.
http://dx.doi.org/10.1016/j.foodchem.2003.12.038

84. Rühmkorf C, Jungkunz S, Wagner M, Vogel RF. *Optimization of homoexopolysaccharide formation by lactobacilli in gluten-free sourdoughs.* Food Microbiol. 2012; 32: 286-94.
http://dx.doi.org/10.1016/j.fm.2012.07.002
PMid:22986191

85. Galle S, Schwab C, Dal Bello F, Coffey A, Gänzle MG, Arendt EK. *Influence of in-situ synthesized exopolysaccharides on the quality of gluten-free sorghum sourdough bread.* Int J Food Microbiol. 2012; 155: 105-12.
http://dx.doi.org/10.1016/j.ijfoodmicro.2012.01.009
PMid:22342455

86. Moore MM, Dal Bello F, Arendt EK. *Sourdough fermented by Lactobacillus plantarum FST 1.7 improves the quality and shelf life of gluten-free bread.* Eur Food Res Technol. 2008; 226: 1309-16.
http://dx.doi.org/10.1007/s00217-007-0659-z

87. De Vuyst L, Vrancken G, Ravyts F, Rimaux T, Weckx S. *Biodiversity, ecological determinants, and metabolic exploitation of sourdough microbiota.* Food Microbiol. 2009; 26: 666-75.
http://dx.doi.org/10.1016/j.fm.2009.07.012
PMid:19747599

88. Ronda F, Oliete B, Gómez M, Caballero PA, Pando V. *Rheological study of layer cake batters made with soybean protein isolate and different starch sources.* J Food Eng. 2011; 102: 272-7.
http://dx.doi.org/10.1016/j.jfoodeng.2010.09.001

89. Matos ME.; Sanz T, Rosell CM. *Establishing the function of proteins on the rheological and quality properties of rice based gluten free muffins.* Food Hydrocolloid. 2014; 35: 150-8.
http://dx.doi.org/10.1016/j.foodhyd.2013.05.007

90. de la Hera E, Martinez M, Oliete B, Gómez M. *Influence of flour particle size on quality of gluten-free rice cakes.* Food Bioprocess Technol. 2013; 6: 2280-8.
http://dx.doi.org/10.1007/s11947-012-0922-6

91. Ronda F, Gómez M, Caballero PA, Oliete B, Blanco CA. *Improvement of quality of gluten-free layer cakes.* Food Sci Technol Int. 2009; 15: 193-202.
http://dx.doi.org/10.1177/1082013208105170

92. Jeong S, Kang W-S, Shin M. *Improvement of the quality of gluten-free rice pound cake using extruded rice flour.* Food Sci Biotechnol. 2013; 22: 173-80.
http://dx.doi.org/10.1007/s10068-013-0024-x

93. Sumnu G, Koksel F, Sahin S, Basman A, Meda V. *The effects of xanthan and guar gums on staling of gluten-free rice cakes baked in different ovens.* Int J Food Sci Technol. 2010; 45: 87-93.
http://dx.doi.org/10.1111/j.1365-2621.2009.02107.x

94. Preichardt LD, Vendruscolo CT, Gularte MA, Moreira A da S. *The role of xanthan gum in the quality of gluten free cakes: improved bakery products for coeliac patients.* Int J Food Sci Technol. 2011; 46: 2591-7.
http://dx.doi.org/10.1111/j.1365-2621.2011.02788.x

95. Oliveira V, Mariani, M, Vogt E, Venzke J. *Physicochemical and sensory evaluation of gluten-free cookies made with rice flour, rice bran and soy flour.* Ann Nutr Metab. 2013; 63: 1790.

96. Dapčević Hadnađev TR, Torbica AM, Hadnađev MS. *Influence of buckwheat flour and carboxymethyl cellulose on rheological behaviour and baking performance of gluten-free cookie dough.* Food Bioprocess Technol. 2013; 6: 1770-81.
http://dx.doi.org/10.1007/s11947-012-0841-6

97. Calderón de la Barca AM, Rojas-Martinez ME, Islas-Rubio AR, Cabrera-Chávez F. *Gluten-free breads and cookies of raw and popped amaranth flours with attractive technological and nutritional qualities.* Plant Food Hum Nutr. 2010; 65: 241-6.
http://dx.doi.org/10.1007/s11130-010-0187-z
PMid:20734143

98. Coleman J, Abaye AO, Barbeau W, Thomason W. *The suitability of teff flour in bread, layer cakes, cookies and biscuits.* Int J Food Sci Nutr. 2013; 64: 877-81.
http://dx.doi.org/10.3109/09637486.2013.800845
PMid:23730794

99. Yamsaengsung R, Berghofer E, Schoenlechner R. *Physical properties and sensory acceptability of cookies made from chickpea addition to white wheat or whole wheat flour compared to gluten-free amaranth or buckwheat flour.* Int J Food Sci Technol. 2012; 47: 2221-7.
http://dx.doi.org/10.1111/j.1365-2621.2012.03092.x

100. Ergin A, Herken EN. *Use of various flours in gluten-free biscuits.* J Food Agric Environ. 2012; 10: 128-31.

101. Pagani MA. *Pasta products from non-conventional raw materials.* In: Mercier C, Cantarelli C (Eds.). Pasta and extruded products. London: Elsevier Applied Science. 1986; 52-68.

102. Tan H-Z, Li Z-G, Tan B. *Starch noodles: History, classification, materials, processing, structure, nutrition, quality evaluating and improving International.* Food Res Int. 2009; 42: 551-76.
http://dx.doi.org/10.1016/j.foodres.2009.02.015

103. Marti A, Seetharaman K, Pagani MB. *Rice-based pasta: A comparison between conventional pasta-making and extrusion-cooking.* J Cereal Sci. 2010; 52: 404-9.
http://dx.doi.org/10.1016/j.jcs.2010.07.002

104. Marti A, Caramanico R, Bottega G, Pagani MA. *Cooking behavior of rice pasta: Effect of thermal treatments and extrusion conditions.* LWT-Food Sci Technol. 2013; 54: 229-35.
http://dx.doi.org/10.1016/j.lwt.2013.05.008

105. Dexter JE, Matsuo RR. *Effect of starch on pasta dough rheology and spaghetti cooking quality.* Cereal Chem. 1979; 56: 190-5.

106. Tam LM, Corke H, Tan WT, Li J, Collado LS. *Production of Bihon-type noodles from maize starch differing in amylose content.* Cereal Chem. 2004; 81: 475-80.
http://dx.doi.org/10.1094/CCHEM.2004.81.4.475

107. Waniska RD, Yi T, Lu J, Xue-Ping L, Xu W, Lin H. *Effects of preheating temperature, moisture, and sodium metabisulfite content on quality of noodles prepared from maize flour or meal.* Food Sci Technol Int. 1999; 5: 339-46.
http://dx.doi.org/10.1177/108201329900500406

108. Schoenlechner R, Drausinger J, Ottenschlaeger V, Jurackova K, Berghofer E. *Functional Properties of Gluten-Free Pasta Produced from Amaranth, Quinoa and Buckwheat.* Plant Foods Hum Nutr. 2010; 65: 339-49.
http://dx.doi.org/10.1007/s11130-010-0194-0
PMid:20972627

109. Alamprese C, Casiraghi E, Pagani MA. *Development of gluten-free fresh egg pasta analogues containing buckwheat.* Eur Food Res Technol. 2007; 225: 205-13.
http://dx.doi.org/10.1007/s00217-006-0405-y

110. Cabrera-Chávez F, Calderón de la Barca AM, Islas-Rubio AR, Marti A, Marengo M, Pagani MA et al. *Molecular rearrangements in extrusion processes for the production of amaranth-enriched, gluten-free rice pasta.* LWT-Food Sci Technol. 2012; 47: 421-6.
http://dx.doi.org/10.1016/j.lwt.2012.01.040

111. Schober TJ, Messerschmidt M, Bean SR, Park SH, Arendt EK. *Gluten free bread from sorghum: Quality differences among hybrids.* Cereal Chem. 2005; 82: 394-404.
http://dx.doi.org/10.1094/CC-82-0394

112. Liu L, Herald TJ, Wang D, Wilson JD, Bean SR, Aramouni FM. *Characterization of sorghum grain and evaluation of sorghum flour in a Chinese egg noodle system.* J Cereal Sci. 2012; 55: 31-6.
http://dx.doi.org/10.1016/j.jcs.2011.09.007

113. Suhendro EL, Kunetz CF, McDonough CM, Rooney LW, Waniska RD. *Cooking characteristics and quality of noodles from food sorghum.* Cereal Chem. 2000; 77: 96-100.
http://dx.doi.org/10.1094/CCHEM.2000.77.2.96

114. Susanna S, Prabhasankar P. *A study on development of Gluten free pasta and its biochemical and immunological validation.* LWT-Food Sci Technol. 2013; 50: 613-21
http://dx.doi.org/10.1016/j.lwt.2012.07.040

115. Padalino L, Mastromatteo M, De Vita P, Ficco DBM, Del Nobile MA. *Effects of hydrocolloids on chemical properties and cooking quality of gluten-free spaghetti.* Int J Food Sci Technol. 2013; 48: 972-83.
http://dx.doi.org/10.1111/ijfs.12049

116. Charutigon C, Jitpupakdree J, Namsreem P, Rungsardthong V. *Effects of processing conditions and the use of modified starch and monoglyceride on some properties of extruded rice vermicelli.* Food Sci Technol. 2008; 41: 642-51.
http://dx.doi.org/10.1016/j.lwt.2007.04.009

117. Eliasson A-C, Krog N. *Physical properties of amylose-monoglyceride complexes.* J Cereal Sci. 1985; 3: 239-48.
http://dx.doi.org/10.1016/S0733-5210(85)80017-5

118. Kaur L, Singh J, Singh N. *Effect of glycerol monostearate on the physico-chemical, thermal, rheological and noodle making properties of corn and potato starches.* Food Hydrocolloid. 2005; 19: 839-49.
http://dx.doi.org/10.1016/j.foodhyd.2004.10.036

119. Marti A, Pagani MA. *What can play the role of gluten in gluten free pasta?* Trends Food Sci Tech. 2013; 31: 63-71.
http://dx.doi.org/10.1016/j.tifs.2013.03.001

120. Barbiroli A, Bonomi F, Casiraghi MC, Iametti S, Pagani MA, Marti A. *Process conditions affect starch structure and its interactions with proteins in rice pasta.* Carbohyd Polym. 2013; 92: 1865-72.
http://dx.doi.org/10.1016/j.carbpol.2012.11.047
PMid:23399230

121. Yousif EI, Gadallah MGE, Sorour AM. *Physico-chemical and rheological properties of modified corn starches and its effect on noodle quality.* Ann Agric Sci. 2012; 57: 19-27.
http://dx.doi.org/10.1016/j.aoas.2012.03.008

122. Hormdok R, Noomhorm A. *Hydrothermal treatments of rice starch for improvement of rice noodle quality.* Food Sci Technol. 2007; 40: 1723-31.
http://dx.doi.org/10.1016/j.lwt.2006.12.017

123. Wang N, Bherud PR, Sosulski FW, Tyler RT. *Pasta like product from pea flour by twin-screw extrusion.* J Food Sci. 1999; 64: 671-8.
http://dx.doi.org/10.1111/j.1365-2621.1999.tb15108.x

124. Wang N, Maximiuk L, Toews R. *Pea starch noodles: Effect of processing variables on characteristics and optimisation of twin-screw extrusion process.* Food Chem. 2012; 133: 742-53.
http://dx.doi.org/10.1016/j.foodchem.2012.01.087

125. Giménez MA, González RJ, Wagner J, Torres R, Lobo MO, Samman NC. *Effect of extrusion conditions on physicochemical and sensorial properties of corn-broad beans (Vicia faba) spaghetti type pasta.* Food Chem. 2013; 136: 538-45.
http://dx.doi.org/10.1016/j.foodchem.2012.08.068
PMid:23122095

Chapter 18

Gluten-Free Autochthonous Foodstuff (South America and Other Countries)

María Alejandra García[1], Sonia Zulma Viña[1,2]

[1] Universidad Nacional de La Plata (UNLP) - Consejo Nacional de Investigaciones Científicas y Técnicas CONICET-La Plata, Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA), Argentina.

[2] Universidad Nacional de La Plata, Facultad de Ciencias Agrarias y Forestales, Laboratorio de Investigación en Productos Agroindustriales (LIPA) - Curso Bioquímica y Fitoquímica, Argentina.

magarcia@quimica.unlp.edu.ar, soniavia@quimica.unlp.edu.ar

Doi: http://dx.doi.org/10.3926/oms.266

How to cite this chapter

García MA, Viña SZ. *Gluten-Free Autochthonous Foodstuff (South America and Other Countries)*. In Arranz E, Fernández-Bañares F, Rosell CM, Rodrigo L, Peña AS, editors. *Advances in the Understanding of Gluten Related Pathology and the Evolution of Gluten-Free Foods*. Barcelona, Spain: OmniaScience; 2015. p. 605-644.

Abstract

The conservation and sustainable use of biodiversity for agriculture and nutrition have been extensively pointed out as crucial elements for food security and nutrition. Likewise, the relevance of learning from traditional foods and applying indigenous knowledge for the development and production of innovative gluten-free foods has been referred.

South and Central America have supplied a great quantity of plant foods for the sustenance of the humankind. Latin-America is by this time one of the World largest net food exporting area. However, its complete potential to expand agricultural production for regional consumption and global export has not yet been achieved. The region has a large number of skilled farmers that have preserved and transmitted their knowledge through generations.

Feeding a rapidly growing global population without expanding farming into environmentally susceptible areas and reducing the productive ability of the land already cultivated is a challenge that presents an elevated complexity level.

In a framework of a strong need for diet diversification, populations with special nutritional requirements, such as celiac patients, should be benefited with the offer of more balanced, rich and safe diet components. The possibility of learning to a great extent from traditional foods and spread on local and territorial knowledge for the development and production of innovative gluten-free foods appears as a promising alternative.

This chapter collects information about several plant species from the American continent that are more extensively used for the

production of gluten-free foods (e. g. maize, potato, cassava, sweet potato, quinoa, amaranth, some legume grains) as well as other species that could potentially be developed with the same purpose, such as the Andean root and tuber crops: achira, ahipa, arracacha, maca, mashua, mauka, oca, ulluco, and yacon.

Keywords

Plant biodiversity and food, food sources from South and Central America, maize, potato, and cassava, andean root, tuber, and grain crops, innovative gluten-free products, family farming and food production.

1. Biodiversity and Food

The Food and Agriculture Organization of the United Nations (FAO) extensively works on the conservation and sustainable use of biodiversity for agriculture and nutrition[1], which is considered crucial for sustainable diets and for food security and nutrition.

Biodiversity is understood as the variability among living organisms from all sources, comprising terrestrial and aquatic ecosystems and the ecological complexes of which they are part. It includes diversity within species, between species and the diversity of the ecosystems[2].

According to Savard et al.[3], in the last decades biodiversity concerns have been in the head of conservation efforts worldwide[2]. The term 'biodiversity' transcends all levels of life, from genes to communities, and all spatial and temporal scales. Biodiversity concepts can give a helpful support for conservation efforts.

During history, human beings have adapted to the tasks of their local environments to produce food systems with appropriate cultural features that provide healthy diets. Nevertheless, nowadays nearly 900 million people in the world suffer chronic hunger, diverse kinds of malnutrition and they have deficient access to healthy food[4,5]. It has been pointed out that human healthiness relies on the health of the ecosystems that supports people live, which must be carefully protected. The biodiversity inherent to the ecosystems must be safeguarded since it contributes to varied, healthy and sustainable diets.

Unfortunately, there is a worldwide tendency towards dietary simplification and a loss of food biodiversity is verified due to a progressive reliance on a reduced number of varieties of staple food crops[1].

There are over 50,000 edible plants in the world. However, only three crops (rice, wheat and maize) provide 60% of the food energy supply from plant origin. A few hundred of plant species contribute significantly to food supplies. In particular, the *Poaceae* botanical family (the fifth-largest plant

family) comprises more than 10,000 species but few of them have been broadly introduced into cultivation over the past 2,000 years.

It is a very well-known fact that crop genetic diversity is under growing pressure from urban development, disease, and climate change, while mono-cropping (agricultural practices relying on a few high-yielding species and varieties) expose food supply to considerable risks. Little genetic diversity makes crops susceptible to widespread diseases, as happened during the Irish Potato Famine, when the late blight pathogen wiped out entire crops of the dominant potato variety, and one million people starved to death[6].

According to Dini et al.[7], the food supply relying on relatively few crops has negatively affected the competitiveness of minor or heritage crops. They have been restricted to subsistence uses or subjected to a disappearance risk. The renewed interest in neglected and under-utilized species arises from their involvement in agricultural diversification and the enhancement in the use of land, their economic potential and the prospect for diet diversification. These plant species have been used by local populations for many centuries. Their innovation is thus related to the manner in which old and new uses are being readdressed[7].

There are several zones of the Earth that concentrate the major biological richness and they are frequently referred to as 'mega-diverse regions'. Up-to 70% of the biological diversity of our planet is found in 17 countries, representing 10% of the total planet extension.

The American continent joins the highest number of mega-diverse countries (seven in total): Brazil, Colombia, Ecuador, USA, Mexico, Peru, and Venezuela. Five countries with these features are situated in Asia (China, Philippines, Indonesia, India, and Malaysia); three in Africa (Madagascar, Democratic Republic of the Congo, and South Africa); and two in Oceania (Australia and Papua New Guinea).

The unique characteristics that allow to an enormous number of species being originated in and inhabiting these countries are: 1) many of them are in the tropics, where environmental conditions (climate and soils) favor biodiversity; 2) the coexistence of islands and the continental portions allows

to the development of endemic, distinctive flora and fauna; 3) these countries comprise extensive areas that can shelter a lot of biological species, from different origins. Likewise, the domestication of plants and animal by the indigenous communities gave rise to a huge natural richness.

Some of the main messages and conclusions of the Third International Symposium on Gluten-Free Foods and Beverages (GF13 Conference), held in Vienna (Austria) in June 2013 referred to the possibility of learning to a great extent from traditional foods and applying indigenous knowledge for the development and production of innovative gluten-free foods[8]. However, there is an urgent need to be caring of the local livelihood from which the whole mankind could achieve a very valuable knowledge.

Likewise, with the development of new food products and the emerging of genetically modified and other new grain varieties, it becomes necessary to stay alert and constantly communicate potential new risks for gluten intolerant individuals who make up on average 1% and in some areas a lot more than 6% of the general population[8].

Current trends show that the gluten-free (GF) foodstuff market is one of the most growing markets in the sector of food and beverages, considering its evolution in recent years as well as the prospective for the immediate future.

2. Gluten-Free Autochthonous Foodstuff from South and Central America

2.1. Maize

Maize (*Zea mays*) (also known as corn) is native to the western hemisphere, although its exact place of origin is not completely certain. Archeological data found from drill cores at Mexico City were identified as maize pollen grains considered to be 80,000 years old[9]. Likewise, corn cobs that were dated 5,600 years old by radiocarbon determination were found at the bat caves in New Mexico. Most historians accept that corn was

domesticated in the Tehuacan Valley of Mexico and that the original wild forms have long been extinct[9].

Proofs suggest that cultivated maize developed through natural crossings, firstly with gamagrass to yield teosinte and then probably with back-crossing of teosinte to primitive maize to produce modern races.

Maize was an honored food in the Americas. Domesticated by farmers about 8,000 years ago, America's cultures ground maize into dough or boiled, broiled, or popped it over hot coals. For drinking preparation, maize was also combined with water and other ingredients such as honey, chocolate, and pepper[10].

Maize is cultivated throughout the world and the worldwide production in 2012 was 872,066,770 tonnes[11]. According to Singh et al.[12], the main producing countries are USA, China, Brazil, Argentina, India, France, and Indonesia. Different varieties of maize are grown such as *Z. mays* var. *amylacea* (floury corn; soft corn); *Z. mays* var. *indurata* (flint corn; Indian corn); *Z. mays* var. *indentata* (dent corn); *Z. mays* var. *saccharata* and *Z. mays* var. *rugosa* (sweet corn); *Z. mays* var. *everta* (mainly used for popcorn); *Z. mays* var. *ceratina* (waxy corn)[12]. Likewise, maize kernels with different colors are also available, ranging from white to yellow, red and purple. Blue-, purple- and red-pigmented maize show bioactive and antioxidant properties due to high anthocyanin and phenolic content.

The constituents of maize kernel are: the endosperm (82-83%), the germ or embryo (10-11%), the pericarp (5-6%) and the tip cap (0.8-1%). The tip cap is the remaining fibrous material that connects the maize grain to the corncob.

According to Singh et al.[12], the endosperm is composed of numerous cells, each one packed with starch granules embedded in a continuous matrix of proteins. Corn endosperm includes two distinct parts: floury and horny endosperm. Horny endosperm has tightly packed, smaller starch granules placed toward the periphery, meanwhile floury endosperm contains laxly packed starch granules surrounding the central fissure.

The major constituent of maize grain is starch, which reaches up to 88% of the endosperm. Simple sugars are also present, such as glucose, sucrose and fructose varying from 1-3% of the kernel[13]. In common maize, with either the dent or flint type of endosperm, amylose makes up 25-30% of the starch and amylopectin represents the remaining 70-75%. On the other hand, amylopectin constitutes practically 100% of the starch in waxy maize. An endosperm mutant called amylose-extender (ae) shows an increase in the amylose percentage of the starch to 50% and more[13].

Protein content in maize (mostly found in the endosperm, inside sub-cellular bodies or protein bodies which contain the storage proteins of the endosperm) ranges from 8 to 11% of the grain weight in common maize varieties.

At least four different protein fractions in corn kernels are mentioned[13]: albumins and globulins (about 12% percent of total nitrogen); prolamines (52% of the nitrogen in the kernel), being zeins the ones found in the largest concentration; glutelins; and a small amount of residual nitrogen (about 5%). A minimum of four main fractions of the zein storage proteins have been identified: α-, β-, γ- and δ-zein.

While most maize protein (75%) comes from the endosperm, the embryo concentrates the proteins with the best amino acid profile. Those proteins present about three times more albumin, twice as much globulin, and ten times less zein than the whole grain[14].

Concerning the amino acid content of maize proteins, the zein fraction was shown to be very low in lysine (usually less than 30 mg g^{-1} protein) and practically lacking in tryptophan content. Conversely, the albumin, globulin and glutelin fractions contain relatively high levels of lysine and tryptophan, but they are the minor fraction of maize proteins. Another important characteristic of the zein fractions is that they show high content of leucine, an amino acid concerned in isoleucine deficiency[13].

Veloso Naves et al.[14], evaluated the nutritional and protein quality of maize germ with pericarp in relation to the whole corn kernel. The authors pointed out that the germ fraction presented a good profile of essential amino acids

with a lysine level of 57.2 mg g^{-1} protein, approximately 50% higher than that of whole corn. The level of lysine found in whole corn (37.9 mg g^{-1} protein) was also higher than the values reported in the literature for common corn, which varied from 26 to 30 mg g^{-1} protein. The non-essential or conditionally essential amino acid contents of the germ were higher than those of whole corn, mainly due to aspartic acid, arginine and glycine levels[14].

2.2. Potato and Other Andean Root and Tuber (R&T) Crops

Potato (*Solanum tuberosum* L.) is the fourth most important world food crop, after wheat, rice, and maize. Since the sixteenth century, the diversity and adaptability of this tuber crop has made it spreading from South America, in the high Andes, to diverse altitudes in temperate regions of the world. Lately, potato production has been increasing most rapidly in the warm, humid, tropical Asian lowlands during the dry season[15]. In accordance with the International Potato Center, organism that was founded in 1971 in Lima (Peru), more than a billion people worldwide consume potato, and the global total crop production exceeds 300 million metric tons[6].

According to Kiple and Ornelas[16], potato was a subsistence crop on the highlands of all continents. In Europe, it was originally an anti-famine product but then became a basic dietary component. Potato has also developed as a vegetable or co-staple crop in Asia and Africa.

Potato is a critical produce in terms of food security in the face of population growth and increased hunger rates. For example, China, the largest consumer of potatoes in the world, expects to increase potato production to meet about 50% of the food demand for the next 20 years[6].

Potato was probably domesticated between 10,000 and 7,000 years ago in Peru and Bolivia, in the region of the Titicaca Lake. Cultivated potatoes include thousands of varieties that differ in size, shape, color, and other sensory characteristics. Potato originated in the South American Andes, but its core area of wild genetic diversity extends from Venezuela, Colombia, Ecuador, Peru, Bolivia, Argentina, and Chile across the Pampa and Chaco

regions of Argentina, Uruguay, Paraguay, and southern Brazil[16]. Towards the North, it reaches Central America, Mexico, and the southwest of the United States. More than 200 wild potato species can be found in this broad habitat, covering high mountains and uplands, valleys, subtropical forests, drier semiarid basins between elevations, and littoral valleys[16].

According to Rodríguez-Sandoval et al.[17], potato flour contains good quality edible grade protein, dietary fiber, several macronutrients and trace elements, vitamins and negligible fat[18]. It has been positioned as a value-added thickener and color and/or flavor improver. Potato flour can be incorporated in sauces, gravy, bakery and extruded products, manufactured snacks, and in soup mixes[17].

Dini et al.[7], have pointed out that potato flour is probably the oldest commercial processed potato product, widely used in bakery. Small proportions of added potato flour allow retaining the freshness of bread, giving a characteristic flavor and improving toasting qualities. In bread making, potato products can be blended with wheat flour as starch and native or precooked flour.

Potato flour, when used for bread baking, is known to reduce staling and to improve toasting properties. Because of its adequate mineral content (potassium, magnesium and phosphorus) potato behaves like a very good yeast food. Potato flour is also used in the preparation of flat bread[19], such as 'lefse' and 'potetlefse' (Scandinavian potato flat breads).

Potato flour is produced in large quantities in USA and several European countries. The Netherlands, Germany, the United States, and Belgium are the main exporting countries and exported together 0.27 million tons of potato flour in 2007[19].

Rodríguez-Sandoval et al.[17], have studied the effect of quinoa and potato flours on the thermo-mechanical and bread making properties of wheat flour. From a techno-functional point of view, the authors have measured the moisture content (MC), water absorption index (WAI), water solubility index (WSI) and swelling power (SP) of the potato flour used in their assays. Results showed that this product presented slightly lower levels of moisture

content (12.03±0.19%) together with the highest values of the other parameters in comparison with wheat and quinoa flour. Potato flour WAI was 4.48±0.11 g g^{-1}, meanwhile WSI and SP reached values of 7.45±0.72% and 4.84±0.12 g. g^{-1}, respectively. Authors have pointed out that higher WAI, WSI, and SP values of potato flour are possibly due to a higher content of phosphate groups on amylopectin, which resulted in repulsion between phosphate groups on adjacent chains, increasing hydration by weakening the extent of bonding within the crystalline domains.

Although chemical composition of potato flour depends on the variety and the region of provenance, carbohydrate content can vary between 79.0 and 87.3 g per 100 g. Crude protein ranges from 3.9 to 8.1 g/100 g, meanwhile crude fiber is within the range 1.3-2.9 g/100 g[19]. Total dietary fiber reaches 5.9 g/100 g of edible portion, according to USDA National Nutrient Database Reference[20]. Such as in other root and tuber derivate products, fat content is rather low: it can range between 0.3 and 1.3 g/100 g. Potato flour is a very good source of potassium (1000-1380 mg/100 g). Ascorbic acid content can range between 4-19 mg/100 g.

It has been mentioned that potato flour protein content is similar to that present in common cereals. Gahlawat and Sehgal[21] reported that the *in vitro* digestibility of potato flour protein was 73.3% and this value was significantly higher than that of raw potatoes. Potato tubers are a rich source of free asparagine (2010-4250 mg kg^{-1}).

Rodríguez Galdón et al.[22], determined the amino acid profile, amino acid score and total protein content in ten traditional potato cultivars from Tenerife (Bonita, Bonita negra, Azucena negra, Mora, Borralla, Terrenta, Colorada de baga, Negra, Peluca blanca and Palmera lagarteada). The authors have found significant differences among the potato cultivars in total protein content, and in the amino acids that were studied, except methionine. Apparently, the concentration of amino acids was not influenced by the production region. The chemical score of the potato protein varied considerably among the potato cultivars, ranging from 26.2 to 66.5%. Sulphur amino acids were the limiting amino acids for almost all the potato cultivars

studied. Lysine was the limiting amino acid for the Borralla cultivar, and the second limiting amino acid in the rest of the potato cultivars analyzed.

It is worth noting that *Solanum tuberosum* is a cultivated tetraploid species of the series *Tuberosa*. This series includes two subspecies: the world-wide distributed *tuberosum* and the *andigena* (Juz. et Buk.) Hawkes subspecies. However, the last one (*andigena)* has received different taxonomic treatments. The subspecies *andigena* is cultivated at elevations of 2,500-4,300 m in the Andean highlands[23,24].

The *Andigenum* group comprises numerous potato landraces differing in growth habit, flower color as well as in tuber characteristics (distribution and depth of the eyes, shape, and skin and flesh color). In Argentina, these local varieties are grown in the northwest area (mostly in Jujuy, Salta and Catamarca provinces), in phytogeographical areas that correspond to the high mountain valleys and ''quebradas'' of the Puna and Prepuna[24]. Some potato varieties cultivated in Northern Argentina ('Tuni', 'Negra Ojosa', 'Colorada', 'Oca', 'Collareja', 'Runa', 'Moradita', 'Sani', 'Sallama', 'Santa María', 'Azul', 'Blanca', 'Malgacha') were historically selected by the Andean farmers mainly for their resistance to pests and diseases as well as for their nutritional value.

In order to rescue potato varieties with distinctive characteristics, several research projects are being conducted. In this sense, potato landraces are preserved *ex situ* at the Argentinean Potato Genebank of the Instituto Nacional de Tecnología Agropecuaria (INTA). In the 70's the Germplasm Bank was created in the Agricultural Experimental Station of Balcarce (INTA) in order to conserve, characterize and evaluate wild and cultivated potatoes. As a result of numerous germplasm collection trips and the conservation work in the medium and long term, the Bank has at present a collection of all wild species of the country as well as of those cultivated Andean varieties. According to Ispizúa et al.[24], the research group that works in Andean potatoes from northwestern Argentina have reported the intraspecific morphological variation and biochemical variability of potato storage proteins, among other achievements. The researchers Adriana Andreu (Biological Research Institute IIB, CONICET-UNMdP) and Andrea Clausen

(Genebank of the INTA) lead the Project "Treasures of the Andean Biodiversity: native potatoes and their value for the humanity", which promulgate the knowledge of these ancestral varieties and the creation of consciousness about the importance of its conservation, revaluing the phytogenetic patrimony.

Since these potato landraces possess valued agronomic characteristics (i.e. resistance to biotic and abiotic factors), they are frequently used by breeding programs from around the world. However, the agriculture in the Andean valleys of Argentina is nowadays threatened by various factors such as the loss of genetic diversity to reduce cultivation in the Andean potatoes due to the increasing number of different varieties that are planted. However, low cost technologies that can improve the situation are available, such as the choice of varieties with better agronomic and nutritional characteristics.

Referring to other Andean R&T crops, the CIP points out that nine native Andean R&T crops hold economic and nutritional relevance for subsistence farmers in the Andes. They are known by their Quechua aboriginal names: achira, ahipa, arracacha, maca, mashua, mauka, oca, ulluco, and yacon[6]. These crops are highly adapted to adverse environmental conditions: they grow at high altitudes, can surpass conditions of drought, tolerate freezing temperatures, and resist the exposure to ultraviolet radiation. Thus, from a productive point of view, they achieve good yields even with minimal or no inputs. Likewise, these crops show high vitamin, micronutrient, and starch content. Some of them have been mentioned as bearing various medicinal properties.

Achira, edible canna or Queensland arrowroot (*Canna edulis*) is a perennial plant that was a staple food for ancient Peruvians. There are 30-60 species in America and Asia, most of which produce fleshy, starchy rhizomes traditionally baked in earthen ovens, and also used to produce starchy flour for cooking breads and biscuits, and as a thickener in drinks and soups[6].

Achira cultivation has expanded to Asia, especially China, Vietnam, Taiwan, and Thailand, where its starch is used in the food industry for the production of ''noodles'' and employed as a thickening agent for sauces,

condiments, dressings, and soups. Some authors have indicated that this plant has great potential for application in food because its roots are an interesting raw material for the extraction of starch and the development of edible films[25].

Achira rhizomes produce a high-value starch with large starch granules that enables it to be extracted simply and cost-effectively using homemade equipment. The particular composition of this starch makes it an important source of income for Andean communities, where in some villages it is the main cash crop[6]. For example, achira is achieving extensive recognition in Colombia, where there is an increasing demand for biscuits made from the root.

Achira flour consists mainly of starch, proteins, lipids, and fibers. Andrade-Mahecha et al.[25], have pointed out that the fiber content is directly related to the granule size. Fractions with larger mean diameter possessed higher dietary fiber content (ranging from 229.5-322.1 g/kg on a dry basis). These fiber values were higher than the ones of cassava and sweet potato flour. The protein content of achira flour varied between 40.6 and 45.4g/kg on a dry basis[25]. The lipid content of the flour samples ranged from 9.0 to 11.1 g/kg. Achira flour with the largest particle size (59.7 µm) showed the highest ash content (78.5 g/kg), while the highest starch content was found in the flour fraction with the smallest particle size. The authors have mentioned that the achira flour produced in the study can be considered as a functional ingredient for use in the food industry. The amylose content of the achira starch was 390.0 and 407.6 g/kg on a dry basis, for Brazilian and Colombian *Canna indica* starches, respectively[25].

Belonging to the *Fabaceae* (*Leguminosae*) botanical family, the genus *Pachyrhizus* (yam beans) is native to southern and central America. One of its distinctive characteristics is the production of storing tuberous roots. Thus, *Pachyrhizus* species could be developed as a new source of non-traditional flour and starch.

The main cultivated species are: *Pachyrhizus tuberosus*, the ‘‘Amazonian yam bean’’, mainly grown in Bolivia, Peru, Ecuador and Brazil; *Pachyrhizus erosus*, the ‘‘jacatupe’’ or ‘‘Mexican yam bean’’, found in Central America

and the Caribbean; and *Pachyrhizus ahipa*, the ‘‘ahipa’’ or ‘‘Andean yam bean’’, from the Andes of Bolivia and northern Argentina[7].

Yam bean plants were cultivated by the ancient Mayans and Aztecs several centuries ago. The Mexican jıcama (*Pachyrhizus erosus*) has been rediscovered as a root crop of great economic significance. Its tuberous roots show, on a dry weight basis, 3-5 times the protein content of other root crops, such as potato. They are used as human food and for feeding livestock, because of their high energy content and digestibility. This species is currently cultivated in Mexico, Guatemala, El Salvador, and Honduras and it has also been introduced to different pan-tropical zones, with remarkable success in Southeast Asia[7].

Chemical analysis showed that *P. erosus* roots can provide potassium, sodium, phosphorus, calcium, and magnesium, as well as significant amounts of ascorbic acid. Other vitamins, such as thiamine, riboflavin, pyridoxine, niacin and folic acid, were also reported[26].

Concerning *P. ahipa*, this species was cultivated in the past by the Incan civilization although its production and use diminished significantly since the Conquest of America. Ahipa flour can be considered an alternative gluten-free product, appropriate for people with specific nutritional requirements. Compared to other R&T, ahipa flour has a more balanced composition from a nutritional point of view, supplying protein, fiber and minerals, such as potassium, calcium and iron.

Arracacha or Peruvian carrot (*Arracacia xanthorrhiza* Bancroft) belongs to the *Apiaceae* (*Umbelliferae*) botanical family. Three main varieties, with their distinctively yellow-, white-, and purple-colored roots are available[6].

According to Ribeiro et al.[27], the plant is originated in the tropical highlands of the Andes. It is usually grown at heights ranging from 1,500 to 2,500 m above sea level, at temperatures between 15 and 20°C, with an annual precipitation of about 1,400 mm. The edible parts of the plant are their storage roots (which may weigh up to 450 g and contain approximately 73% water), grouped around the central swollen rootstock and secondary cormels, from which shoots and leaves emerge.

The roots has to be roasted, boiled, baked or fried to be consumed and their characteristic taste resembles a blend of celery, carrot and parsnip. With a compact flesh that is richer in texture and taste than potato, the root can be used to garnish and flavor a range of dishes from soups to desserts. Young stems are used in salads or as a cooked vegetable, and the leaves are often fed to livestock[6].

Arracacha starch is easily digestible since the small size of its granules. Thus, it is good pureed or in soups for babies, the elderly, or people with disabilities. The processed roots are used as a thickener for baby food formula and instant soups[6].

Roots postharvest life is markedly short. They decay fast if stored at room temperature, being completely damaged within 12 days. The main factors causing postharvest losses are severe weight loss and *Rhizopus* and *Erwinia carotovora* attack. Since its tropical and subtropical origin, arracacha is sensitive to chilling injury when stored at low temperature, developing internal and external browning at 5°C[27].

Besides being an important food in the Andes, arracacha was introduced to Brazil early in the 20th century and it expanded in the Southern highlands, mainly in Minas Gerais state. Towards 2005, the two major arracacha clones grown in Brazil were Amarela de Carandaí and Roxa de Viçosa[27]. Brazilian crop improvement programs have succeeded in developing varieties that grow in seven months, which could benefit other farmers in the high Andes[6].

Maca (*Lepidium meyenii* Walp.) belongs to the *Brassicaceae* botanical family. It is an annual or biennial herbaceous plant. According to Wang et al.[28], it has been domesticated in the central Andes of Peru at elevations of 3,500-4,500 m above sea level, where it has been grown for at least 2,000 years ago. However, little is known about its origin.

The edible parts of maca are their subterranean hypocotyls, which are eaten fresh, or can be dried and stored for deferred consumption. Maca is also used as a folk medicine, especially to enhance sexual drive and female fertility in human beings and domesticated animals, to relieve rheumatism, ameliorate

respiratory ailments, and as a laxative and antidepressant, among other properties[28].

Because of the initial scientific evidence for the substantiation of maca almost mythical properties, the crop has experienced a commercial boom. According to CIP[6] the root is processed to make flour for bread and biscuits, dried powder, and gelatinized capsules, most of them certified as organic products. Export volume reached over 700,000 kilograms in 2010.

According to Puoci et al.[29], the oral administration of a lipid extract of maca increased the sexual function of mice and rats[30]. Likewise, Sandoval et al.[31], reported on the capacity of this plant to scavenge free radicals and guard cells from oxidative stress.

Maca meal supplementation increased food intake, growth and feed utilization along with improving survival in rainbow trout juveniles[32]. This effect was attributed to the stimulation of growth hormone production. Maca has also been utilized to treat menopausal women since it was found to increase calcium content in the rats' femur[33] and, therefore, to alleviate the reduction of bone mineral density.

Maca chemical composition shows some interesting characteristics, mainly the high protein, unsaturated fatty acid and mineral contents. Water content of fresh maca roots is higher than 80%. On a dry basis, maca roots contain 8.87-11.6% protein, 1.09-2.2% total lipids, 54.6-60.0% carbohydrates, 8.23-9.08% fiber, 4.9-5.0% ash, and an energy content of 663 kJ/100 g. Carbohydrates are represented by sucrose (23.4%), glucose (1.55%), oligosaccharides (4.56%) and polysaccharides (30.4%)[28]. Maca roots contain seven essential amino acids representing 342.6-388.6 mg/g protein. These values are higher than those reported in potatoes and carrots.

The content of linoleic and oleic acids (unsaturated fatty acids) is 52.7-60.3% of total fatty acids. Besides, maca root powders are also abundant in minerals, being the contents of iron 16.6, manganese 0.8, copper 5.9, zinc 3.8, sodium 18.7, potassium 2050 and calcium 150 mg/100 g dry[28].

Recently, Puoci et al.[29], investigated the applicability of maca flour for the preparation of functional breads with improved biological properties. Different

bread compositions (wheat-maca flour blends with 0, 5, 10, 15, 20% of substitution) were tested. They were characterized by specific *in vitro* tests to determine the antioxidant, anti-inflammatory activities, and the ability to reduce the sugar intake by performing enzymatic assays using α-amylase and α-glucosidase. Results revealed that the biological properties of maca flour were retained after the bread making process and that the analyzed breads were suitable as functional foods.

The International Potato Center points out that in terms of food security, oca (*Oxalis tuberosa* Molina), ulluco (*Ullucus tuberosus* Caldas) and añu or mashua (*Tropeolum tuberosum* Ruiz & Pavón) are the three most important Andean R&T crops. They adapt to altitudes between 2,000 and 3,800 meters above sea level and are associated with potato in the Andes of Peru and Bolivia. Cropping potato in combination with oca, ulluco, and mashua is a millennial tradition and this practice offers valuable supplementary nutrients to a diet based on potatoes. For example, oca has been mentioned as a food product high in protein, with a good balance of amino acids, supplying also high quantities of fiber and antioxidants.

Described in the records of the Spanish conquest, ceramic representations show that oca was a highly valued staple dating back to the pre-Colombian era. Because of its high yield and pleasant taste, oca is very popular in rural Andean cuisine. However, most oca production is still for home consumption. The tubers are traditionally boiled in soups or stews or also baked or roasted and often sun dried to sweeten before cooking[6].

Among the known Andean R&T crops, ulluco has been recognized as the most commercially viable. Since ulluco tubers present high water content, they are most suitable for boiling. Plant leaves are also edible and they have been mentioned as containing significant quantities of protein, calcium, and carotene[6].

Mashua tubers vary in color (usually white, yellow, red or purple) and shape. They contain high levels of isothiocyanates (glucosinolates), compounds known for their insecticidal and medicinal properties. This may explain the virtual absence of pests and diseases in the crop. This strong

resistance is one reason why mashua is traditionally intercropped with other plants; farmers use it as a natural way to repel insects and pathogens.

Despite its high nutritional value, mashua is not widely commercialized. Because it is used in traditional medicine to regulate libido (the Incas reported its use to dampen sexual desire in campaigning armies), men are reluctant to eat it.

Campos et al.[34], have studied native potato (*Solanum sp.*), mashua, oca, and ulluco roots and tubers for their antioxidant capacity and associated secondary metabolites. Results showed that the antioxidant capacity in the crops studied ranged from 483 to 9800 µg trolox equivalents g^{-1}; phenolics ranged from 0.41 to 3.37 mg chlorogenic acid equivalents g^{-1}; anthocyanins varied from 0.08 to 2.05 mg cyanidin 3-glucoside g^{-1}; and carotenoid content was between 1-25 µg β-carotene g^{-1}. The content of bioactive compounds was high and variable between crops and within the genotypes studied. Generally, mashua tubers showed the highest antioxidant capacity and phenolic, anthocyanin and carotenoid content related to the other crops. Ulluco was the only crop that contained betalains in the acid form of betaxanthins (22-96 µg g^{-1}) and betacyanins (64 µg g^{-1}) with no presence of carotenoids or anthocyanins. It is worth mentioning that betalains are water-soluble nitrogen-containing pigments, comprising two structural groups: the red-violet betacyanins and the yellow-orange betaxanthins. Several works have demonstrated the potent antioxidant activity of betalains, which has been associated with protection against degenerative diseases[35].

Referring to yacon (*Smallanthus sonchifolius* (Poepp. et Endl.) H. Robinson), this ancient Andean crop has recently attracted worldwide interest due to its particular nutritional properties. Coll Aráoz et al.[36], have pointed out that yacon is a polyploid species (probably a hybrid), belonging to the *Asteraceae* botanical family, which has been classified as a semi-domesticated crop possibly based on a long time of cultivation in the Andean region[37].

Yacon underground system consists of two different types of reserve organs: the tuberous roots, i.e. the commercialized product; and the rhizophores, the organs of vegetative reproduction. The complete system accumulates fructans

and other soluble carbohydrates, such as fructose, glucose and sucrose. The common name 'yacon' has its origin in the Quechua term 'yakku' (equivalent to 'watery' or 'tasteless'). Yacon is cultivated in the Andes from Colombia to northwestern Argentina at altitudes between 1,000 and 3,500 m above sea level. In the last two centuries the area of cultivation has shown a reduction, being cultivated for home consumption.

However, the unique carbohydrate composition of the roots has attracted the international interest since 40 to 70 % of the root dry matter corresponds to fructooligosaccharides (FOS, short polymers of fructose with a polymerization degree of 3-10 fructans which show low caloric value). Roots do not contain starch. Yacon roots also exhibit pharmacological properties such as antioxidant activity and beneficial effects on obesity and insulin resistance[36].

The tuberous root, which is eaten either raw or cooked, is sweet and crispy. Alternatively, yacon roots can be dehydrated and processed into a range of convenience products. They have been used in the production of beverages and bakery products according to their physicochemical properties[7].

2.3. Cassava

Cassava (*Manihot esculenta* Crantz) is a dicotyledonous perennial woody shrub that produces edible starchy roots. Cassava belongs to the *Euphorbiaceae* botanical family. Its roots fit into a class of food that basically provides energy in the human diet in the form of carbohydrates[38].

Cassava is believed to have its centre of origin in the Amazon region of South America[39], in central Brazil. However, there is no total consensus about the exact botanical origins of the progenitors of modern cultivated cassava[40,41].

According to Malandula Chipeta and Bokosi[41] the existence of cassava in Africa dates back to the 16th century, mainly in the West coast of Africa and later to East Africa all the way through Madagascar and Zanzibar carried by Portuguese navigators from Brazil. Further dissemination of cassava in Africa took place during the 20th century probably under the influence of colonial

masters in which it was grown as reserve famine crop and due to its ability to counteract locust's attack[39]. At present, cassava is grown in all African countries.

Cassava was introduced to the Pacific sometime around 1,800 during the early years of European contact. It has become an important dietary staple and in some entities is produced in larger quantity than the traditional root crops of the area (taro, sweet potato, and yam). In the Pacific region, cassava is generally not produced on a large scale. It is grown in subsistence and home gardens, and is available in local markets.

Cassava flour is principally used in baking and confectionery products to substitute wheat flour at different proportions. Other food applications include the manufacture of weaning foods and pasta, and the production of starch used by the food, pharmaceutical, and chemical industries[7]. Cassava flour is widely used in the formulation of products destined to celiac patients. However, the very low protein content (1.0 % dry basis) and absence of gluten are considered disadvantageous for its exclusive use in food formulations, especially if the elasticity of the dough is essential for product quality[7].

According to the USDA National Nutrient Database for Standard Reference[20], 100 g of the edible portion of raw cassava (almost 60% water) provide 160 kcal of energy, 1.36 g of protein, 0.28 g of total fat, 38 g of total carbohydrates (calculated by difference), 1.8 g of total dietary fiber and 1.70 g of total sugars. The mineral content of 100 g of raw cassava corresponds to 16 mg of calcium, 0.27 mg of iron, 21 mg of magnesium, 27 mg of phosphorus, 271 mg of potassium, 14 mg of sodium and 0.34 mg of zinc[20]. Concerning ascorbic acid level, it has been reported to be 20.6 mg/100 g of edible portion; folate (dietary folate equivalent, DFE), 27 µg/100 g; and niacin, 854 µg/100 g.

2.4. Sweet Potato

Although wild forms of sweet potato (*Ipomoea batatas*) are not known to exist today, Central America and Peru are generally accepted as possible centers of origin for this crop, which belongs to the *Convolvulaceae* botanical family.

Sweet potato, native to tropical America, was brought to Spain by Christopher Columbus in 1492 and then introduced to African lands by the Portuguese. Nowadays, it is the third most important crop in seven eastern and central African countries, and fourth in six southern African countries. The highest consumer of sweet potato per capita is one of the African, Caribbean and Pacific Group of States (ACP) countries, the Solomon Islands in the South Pacific[42].

According to the Traditional Pacific Island Crops web site, sweet potato cultivation in the eastern and central Pacific predates European contact by several hundred years, possibly occurring as early as 1,000 CE. This movement of sweet potato from the Americas to the Pacific islands has been the focus of much debate.

Latest archaeological data indicates possible contacts between Polynesians and indigenous people in several locations along the western coast of America. Sweet potatoes may have been introduced into the Pacific as a result of this approximation and subsequently spread throughout Polynesia. Regardless of the means of dispersal, sweet potato remains as an important food crop all over the Pacific and in many other developing countries.

Sweet potato roots of different color (white to red, through yellow and violet, depending on the variety) are rich in starch and sugar. They can be used as human food, animal feed and for the production of alcohol and starch. Sweet potato roots can be consumed boiled, fried or roasted in an oven. The leaves of the plant are also edible (unlike those of the potato which are toxic) and are rich in proteins, vitamins and various minerals.

The International Potato Centre (CIP) keeps the largest bank of sweet potato genes in the world, represented by thousands of wild, traditional and improved varieties. Research works carried out at the beginning of the 20th century has shown that more than one hundred industrial products could be obtained from sweet potato, although their implementation is still to be developed. According to UNCTAD[42], studies have also shown that sweet potato can provide more than twice the carbohydrates than maize.

A program for improving sweet potato has been implemented consisting in crossing varieties obtained from the CIP and selected local varieties[42]. In France, CIRAD (Centre de Coopération Internationale en Recherche Agronomique pour le Développement) has identified a hybrid, since called "Africa" by producers for whom it has been a great success; it is sold on urban African markets. This material outstands for its shorter production cycle (12-16 weeks), better yield, high resistance to disease (leaf and stem scab), long post-harvest shelf life (4 weeks), very good taste and consumers' acceptance and high provitamin A content[42].

According to USDA National Nutrient Database for Standard Reference[20], 100 g of the edible portion of sweet potato provide 20 g of carbohydrates (obtained by difference), 4.2 g of sugars, 3 g of total dietary fiber, 1.6 g of protein and 0.05 g of total lipids. The mineral content of 100 g of sweet potato roots corresponds to 337 mg of potassium, 55 mg of sodium, 47 mg of phosphorus, 30 mg of calcium, and 25 mg of magnesium. The main microelements supplied are iron (0.61 mg/100 g) and zinc (0.30 mg/100 g)[20]. Concerning vitamin contents, ascorbic acid level has been reported to be 2.4 mg/100 g of edible portion; folate (dietary folate equivalent, DFE), 11 µg/100 g; and vitamin A (retinol activity equivalents, RAE), 709 µg/100 g.

Substitution levels above 10% of wheat flour with sweet-potato flour generally bring unacceptable characteristics of bread regarding the loaf volume, flavor, and texture[43].

Early works pointed out that the crude protein content of sweet potato (estimated as Kjeldahl nitrogen x 6.25) had been reported to range from 1.3 to 10% (on a dry basis)[44]. Likewise, significant genetic variability had been noted, thus the prospective for increasing protein content by breeding has been explored. Those early works indicated that sulfur-containing amino acids were the first limiting and lysine was the second limiting amino acid in sweet potato protein[44].

More recently, Sun et al.[45], have reported that the major storage protein in sweet potato root, which accounts approximately 80% of the total root protein called 'sporamin', has a molecular mass 25 kDa under reducing

SDS-PAGE conditions. However, under non-reducing SDS-PAGE conditions, molecular masses of 31 kDa and 22 kDa were reported (sporamin A and B, respectively)[45,46]. Although sweet potato protein amino acid profile and limiting amino acid vary with cultivar, the sweet potato essential amino acid distribution has been mentioned as nutritionally viable.

It is worth noting that sweet potato protein has a strong trypsin inhibitor activity, which could limit effective utilization for human or animal nutrition. Thus, to improve the nutritional value of these proteins, heat processing has been widely applied. In addition, thermal treatment also increases the *in vitro* protein digestibility of some plant products, such as soybean protein[47], probably due to deactivation of trypsin inhibitors.

2.5. Arrowroot

Arrowroot (*Marantha arundinacea*, belonging to the family *Maranthaceae*), also known as 'sagú' and 'uraro', is a perennial herbaceous plant with thick, fleshy roots. Considered as an introduced crop in the Philippines coming from tropical Latin America, the crop is grown specifically for its rhizomes for flour and starch production[48].

Arrowroot is an excellent source of starch (>85%) that has been lately used in the food industry for making biscuits and as a thickener and/or stabilizer. Arrowroot starch also found application in sizing textiles[49].

Hernández-Medina et al.[50], pointed out that in the Yucatan Peninsula (Mexico), the main R&T grown in the 'milpas' (Mesoamerican agro-ecosystem whose main productive components are maize, beans and squash) are of American origin. Four of these R&T were cultivated before the Conquest (makal *Xanthosoma yucatanensis*; sweet potato *Ipomoea batatas*; cassava *Manihot esculenta*; and jícama *Pachyrhizus erosus*) and the others (sagú *Marantha arundinacea* and potato *Solanum tuberosum*), although American, were introduced by the Spanish.

2.6. Andean Grains: Quinoa and Amaranth

Quinoa (*Chenopodium quinoa* Willd.) is an indigenous plant from the Andean region, cultivated by the Incas who called it ''the mother grain'' and considered it a sacred food. Quinoa dates more than 5,000 years ago[51]. In the Inca Empire quinoa occupied a place of prominence next only to maize[52]. However, after the Spanish conquest other crops, such as potato and barley, relegated quinoa to the background.

Mainly, quinoa is grown in the South American region (especially in and around the Andes), including countries like Ecuador, Peru, Chile and northern Argentina (Jujuy and Salta provinces)[53]. The worldwide production in 2012 was 82,510 tonnes and the harvested area was 102,745 ha[11]. Recently, there has been growing interest in a number of countries (especially in Europe), initiating introduction and research work on quinoa[54-56].

The proximate composition of quinoa varies with cultivar, but mostly ranges from 10 to 18% for protein, 4 to 9% for crude fat, 54 to 64% for carbohydrates, 2 to 4% for ash, and 2 to 5% for crude fiber[17]. Quinoa seeds are considered an interesting foodstuff, owing to their high protein content and lack of gluten. The grain has high-protein content with abundance of essential amino acids, and a wide range of vitamins and minerals[57]. The protein content in the grain ranges from 7.47 to 22.08% with an average equal to 13.81%[58]. Albumin and globulins (chenopodin) are the major protein fraction (44-77% of total protein) while the percentage of prolamines is low (0.5-0.7%)[59], which are the toxic proteins for celiac patients. The seeds have a balanced amino acid profile with high lysine (5.1-6.4%), histidine and methionine contents[59-61], being higher than in cereals.

Schoenlechner et al.[62], stressed that the amino acid profile of the proteins of quinoa is comparable to that of caseins. Besides, this pseudocereal has been attracting attention due to its high mineral content[63]. Although, digestible carbohydrates of pseudocereals flours that ranged between 56 and 59%, were inferior to the amount found for rye (64%) and wheat flours (70%)[64].

Peptides obtained by enzymatic hydrolysis from quinoa seed flour protein concentrate exhibited functional and bioactive properties, especially radical scavenging activity, which is dependent on the molecular size of the peptides[65].

Pseudocereals flours exhibit higher qualitative and quantitative lipid profiles than wheat flour. Quinoa lipids are characterized by a high degree of unsaturation, which is desirable from a nutritional point of view[66]. The predominant fatty acid is linoleic acid (50.7-54.3% of the total) followed by oleic acid (20.8-24.9%) and palmitic acid (8.3-8.9%). Likewise, a high ω-3 polyunsaturated fatty acids (PUFAs) level was reported in quinoa seeds, being this a beneficial and healthy feature[7]. Besides, lipids have a significant effect on the quality and texture of baked goods because of their ability to associate with proteins and starch, especially in breadmaking applications[67]. Thus, pseudocereals flour addition to bakery celiac formulations allows improving the textural characteristics of products enhancing their nutritional value.

Amaranth (*Amaranthus sp.*), quinoa (*Chenopodium quinoa Willd.*) and buckwheat (*Fagopyrum esculentum*) are referred to as pseudocereals, as their seeds resemble in function and composition those of the true cereals, although they are dicotyledonous plants.

Amaranth is an ancient crop consumed as vegetable and grain during the Maya and Aztec periods; the Spanish conquerors called amaranth “the Inca wheat”. Seeds more than 2,000 years old have been found in ancient tombs[68]. It was named as *kwicha* and *huautli* in the area between Mexico and Chile by pre-Columbian major civilizations and cultures like Inca, Aztec and Maya, which considered amaranth as their staple food together with maize and beans. Amaranth grains were also found in 2,000 BC graves, and it was mentioned in Bernardino de Sahagun Ribeira "Florentine Codex", listing its wide array of valuable exploitations[69]. Additionally, in a document on history, economy and ethnography of the Aztecs, commissioned by the Spanish viceroy Antonio de Mendoza in around 1541-42, it is written that each year around 8,000,000 kg of *huautli* were brought to Tenochtitlan, as an annual tribute paid to the emperor Montezuma, being this quantity comparable to the tribute in maize and bean[70].

Aztecs used amaranth in beverages, sauces, porridges; they milled it into flour and prepared *tortillas* (also with maize flour), popped grains like maize, and for various medical uses. Likewise, amaranth had an important position also in Indians' religion. The grain was ground, mixed with water, honey, or even human blood and dough was then formed into the shapes of idols (*zoale*). Idols were paraded and consumed in a ritual manner as a symbol of communion with the gods, because of that the Spaniards prohibited the cultivation and use of amaranth by legislative fiat. Besides this, the cause of reduction in amaranth production was the introduction of new crops from Europe.

In the 16th century, amaranth was first introduced as an ornamental plant in Europe. Different species of amaranth spread throughout the world during 17th, 18th and 19th centuries. In India, China and under the harsh conditions of Himalayas this plant became an important grain and/or vegetable crop. It can be used as a high-protein grain or as a leafy vegetable, and it has potential as a forage crop.

Nowadays, there are three species of amaranth grown for grain production: *A. hypochondriacus, A. cruentus* and *A. caudatus*. Although the three species are native to America, they are also currently distributed in Asia and Africa. In the Americas, *A. hypocondriacus* is sited primarily in northern and central Mexico, *A. cruentus* in southern Mexico and Central America and *A. caudatus* in the Andes, though there are cultivated areas in countries such as Argentina[68]. It is a valuable nutritious foodstuff with high production ability; a good yield of amaranth is considered to be above 12,080 kg/ha. It is a very adaptable crop, resistant to drought, to a wide range of temperature, to insects and diseases. It grows well at different altitudes and on soils with variable levels of nutrients[69]. Likewise, both edible and non-edible amaranth species has been used as biomass source because of its high yield under marginal conditions[71].

The chemical composition of the little seeds is 14-19% of protein, 5-8% of lipids, 62-69% of starch, 2-3 % of total carbohydrates and 4-5% of fiber[69]. Its composition is comparable with that of oat. Starch is the major part of

carbohydrates. Starch granules are small (1-3 µm), easily degradable by α-amylases, and resistant to mechanical stress and freezing conditions. Repo-Carrasco-Valencia et al.[72] reported that *A. caudatus* starch showed 31.3-33.4% of digestibility *in vitro*.

The main lipids, composed of linoleic, oleic and palmitic acid, in amaranth seed are placed in the embryo. According to the seed composition, amaranth oil is similar to the ones obtained from cotton or maize but it has lower digestibility. Amaranth oil contains about 8% of squalen, a sterol precursor, used in medicine and cosmetic industry. About 90% of amaranth total lipids correspond to triglycerides and complex lipids (phospholipids and glycolipids). In the three cultivated species of amaranth the ratio of saturated to unsaturated fatty acids is in the range 0.26-0.31[68].

Content of minerals depends on species and growing conditions. Amounts of calcium and magnesium are higher than the amounts in other cereals. Seeds are a good source of vitamins mainly ascorbic acid and those from the B-complex, and the antioxidants α-tocopherol and β- and γ- tocotrienols.

With regard to amaranth proteins, albumin, globulin and glutelin fractions were referred to as the most abundant, with a minor fraction of prolamin (1.5-11%)[68]. Albumins and globulins contain less glutamic acid and proline and more lysine than prolamins[66]. In contrast to cereals, amaranth has higher content of amino acids mainly lysine, methionine, treonin and cysteine. Amaranth seeds are also a source of tryptophan and amino acids containing sulphur. The balanced amino acid composition of amaranth is close to the optimum protein reference pattern in the human diet according to FAO/WHO requirements[70]. This well balanced amino acid composition is the result of the fact that in amaranth 65% of proteins are found in the embryo and only 35% in the perisperm, whereas in other grains amino acids in the endosperm prevail (85% in average) and they are poorer in essential amino acids. Besides, it has been pointed out that the amino acid profile of the proteins of amaranth is comparable to that of egg[62].

The role of proteins as bioactive components has been recognized, either directly or after hydrolysis *in vivo* or *in vitro*, showing some encrypted peptides antihypertensive, antioxidant and positive effects on cholesterolemia[73,74].

Food uses of amaranth include its incorporation as ingredient in bread, pasta, baby's food, instant drinks, etc. The most common product is the flour although whole amaranth seeds can be added in breads, müsli bars, breakfast food and biscuits. Likewise, leaves and stems are and interesting vegetable suitable for soups, salads or other meals.

On the other hand, *Amaranthus australis* L. or *Amaranthus cruentus* L. crops were proposed as a source of raw material for solid biomass-based production processes that could be used to obtain high quality biofuel[71].

2.7. American Legume Grains

Beans (*Phaseolus sp.*) are one of the oldest foods known by man and has been an important part of the human diet since ancient times. Their cultivation began about 7,000 years BC in southern Mexico and Guatemala. Since the Mesoamerican cultures of Mexico were expanded, these beans and farming practices gradually spread throughout South America as they explored and traded with other cultures. Beans were called *etl*, *buul* and *purutu* by the Aztecs, Mayas and Incas respectively[75]. The oldest-known domesticated beans in the Americas were found in Guitarrero Cave, an archaeological site in Peru, and dated to around the second millennium BCE.

Five kinds of *Phaseolus* beans were domesticated by pre-Columbian cultures: common beans (*Phaseolus vulgaris*) grown from Chile to the northern part of the actual territory of the United States, and lima and sieva beans (*Phaseolus lunatus*), as well as the less widely distributed teparies (*Phaseolus acutifolius*), scarlet runner beans (*Phaseolus coccineus*) and polyanthus beans (*Phaseolus polyanthus*). One especially famous use of beans by pre-Columbian people as far north as the Atlantic seaboard is the “Three Sisters” method of companion plant cultivation: where beans are grown together with maize and squash[75]. By the time the Europeans arrived, beans

were cultivated throughout the New World, in North America as well as Central and South America. Since the early 17th century, American bean varieties were already popular in Europe, Africa and Asia.

The worldwide production in 2012 was 23.23 million metric tons, harvested from 29.92 million hectares[11]. These values are overestimated because FAO does not report data for *Phaseolus* and non-*Phaseolus* species separately. India was the leading producer, responsible for 21% of the total production, followed by Brazil, Myanmar, China, the U.S., and Mexico. In Latin America, wild beans are grown in a wide arc stretching from northern Mexico (approximately 30°N) to northwestern Argentina (about 35°S) at altitudes from 500 to 2,000 m and rainfall regimes from 500 to 1,800 mm.

Before domestication, wild *P. vulgaris* had already diverged into two major gene pools, each with its characteristic geographic distribution, in Mesoamerica and the Andes[76]. The Mesoamerican area comprises the southern part of Central America, Colombia and Venezuela; likewise, in the Andean region southern Peru, Bolivia and Argentina are included. These two wild gene pools can be distinguished at the morphological and molecular levels[76].

New cultivars of *P. vulgaris* are continually being developed and released from the research centers. The economic value of a new cultivar depends on its yield, rate of maturity, its resistance to disease, and seed size, color, nutritional quality, cooking time, and the flavor and texture of the cooked food. The criteria for selection are resistance to disease, yields and maturation rate. Nowadays, nutritive quality is also taking into account.

Legumes, considered as poor men's meat, are generally good sources of nutrients. They are an important and relatively inexpensive source of protein, dietary fiber and starch for a large part of the world population, mainly in developing countries. Beans are also one of the best non-meat sources of iron, providing 23-30% of the daily recommended levels from a single serving[77].

With regard to chemical composition, beans and their derivative flours are an important source of proteins, and their contents varied significantly among the botanical origin of the flours. Legume flours are good supplements for cereal-based products. Cereals are deficient in the essential amino acid lysine,

while legumes have a high content. On the other hand, cereal proteins complement legume proteins in the essential amino acid methionine[68]. Beans, as well as lentils, have a specific amino acid composition: high in lysine and low in sulphur amino acids. Pirman et al.[78], demonstrated that the amino acid composition of three cultivars of beans is similar in the uncooked state. In comparison to the lentils, beans contain more methionine, tyrosine and serine, and less arginine[78].

Common beans have been considered as a low glycemic food, mainly because of its dietary fiber and resistant starch content. Ramírez-Jiménez et al.[79], stressed that *P. vulgaris* beans showed low starch digestibility and increased amounts of resistant starch after drying treatments. Starch from legume flour is more slowly digested than those of cereal ones and its ingestion produce less abrupt changes in plasma glucose and insulin. Legume seeds are also valuable sources of dietary fiber, vitamins and minerals including folate, thiamine and riboflavin[7]. Consumption of legumes has been associated with many health benefits, including the reduction of the risk of type 2 diabetes and cardiovascular disease, as well as the prevention of the onset of various types of cancer. Beans have been studied due to bioactive components, such as antioxidants, phenolic compounds, dietary fiber fractions, resistant starch and oligosaccharides present in the seed[79].

Bean flours have been added to foods in order to increase the nutritional value or to provide specific desired functional attributes[80,81]. Despite the nutraceutical or nutritional contribution, incorporation of these flours into functional products is determined by some technological properties such as solubility, water binding capacity and fat absorption.

3. Final Considerations

The American Continent (and particularly South and Central America), as one of the most mega-diverse zones of our planet, has supplied a great quantity of plant foods for the nutrition of the humankind.

Latin-America is already the World largest net food exporting region although it has not achieved its complete potential to expand agricultural production for regional consumption and global export. This part of the planet has been endowed with abundant natural resources, even with a third of the world's fresh water stocks. Likewise, the region has a large number of experienced farmers who have conserved and transmitted their knowledge of agriculture and nourishment.

It has been mentioned that the next decades will offer a critical opportunity to reinforce novel forms of productive and environmentally sustainable agriculture in the region. Experts point out that the challenge is much bigger than just producing more food. It is essential to feed a rapidly growing global population without expanding farming into environmentally susceptible areas, reducing the productive ability of the land already cultivated, and affecting quality.

Despite of the existence of numerous species total or partially domesticated, which goes back to the first American people, the commercial utilization of the autochthonous genetic resources is still incipient in the region.

The domestication of native plants, including those already known and commercialized by local people, with little entrance in the national or international market, is a great opportunity to be developed. In many regions of the continent, this richness is yet under-utilized, particularly due to economic and market pressure, which favor exotic crops and products.

In this context of a clear need for diet diversification, those populations with special nutritional requirements, such as celiac patients, should be benefited with the offer of more balanced, rich and safe diet components. There is an urgent need to explore the possibility of learning to a great extent from traditional foods and spread on local and territorial knowledge for the development and production of innovative gluten-free foods, looking after the local livelihood from which the whole mankind could benefit.

References

1. Charrondière UR, Stadlmayr B, Rittenschober D, Mouille B, Nilsson E, Medhammar E et al. *FAO/INFOODS food composition database for biodiversity.* Food Chem. 2013; 140: 408-12.
   http://dx.doi.org/10.1016/j.foodchem.2012.08.049
   PMid:23601383
2. UNEP. United Nations Environment Programme. *Global Biodiversity Assessment.* Cambridge: Cambridge University Press. 1995; 1140.
3. Savard J-PL, Clergeaub P, Mennechez G. *Biodiversity concepts and urban ecosystems.* Landsc Urban Plan. 2000; 48: 131-42.
   http://dx.doi.org/10.1016/S0169-2046(00)00037-2
4. SOFI. *The State of Food Insecurity in the World 2012.* Rome: FAO. 2012.
   http://www.fao.org/publications/sofi/en/
5. He C. *Opening address. International Scientific Symposium. Biodiversity and Sustainable Diets United against Hunger.* Rome: Food and Agriculture Organization of the United Nations. 2010.
6. CIP. Centro Internacional de la Papa (International Potato Center). 2014.
   http://cipotato.org/es/
7. Dini C, García MA, Viña SZ. *Non-traditional flours: frontiers between ancestral heritage and innovation.* Food Funct. 2012; 3: 606-20.
   http://dx.doi.org/10.1039/c2fo30036b
   PMid:22499487
8. ICC. International Association for Cereal Science and Technology. *Growing interest in gluten-free foods and beverages and important conclusions for the future of gluten-free discussed at the GF13.* The 3rd International Symposium on Gluten-Free Foods and Beverages (Vienna, Austria, 12-14 June 2013) 2014.
   https://www.icc.or.at/news/gf13_report. Accessed on March 2014.
9. Gibson L, Benson G. *Origin, History, and Uses of Corn* (Zea mays). Iowa State University, Department of Agronomy. 2002.
   www.agron.iastate.edu/Courses/agron212/readings/corn_history.htm. Accessed: January 2014.
10. Hannon S. *The Journey of New World Foods.* 2010.
    http://www.pbs.org/when-worlds-collide/. Accessed: March 2014.
11. FAOSTAT. *FAO Statistics Division.* 2014. http://faostat.fao.org/

12. Singh N, Singh S, Shevkani K. *Maize: Composition, Bioactive Constituents, and Unleavened Bread.* In: Preedy VR, Watson RR, Patel VB (Eds.). Flours and Breads and their Fortification in Health and Disease Prevention. USA: Academic Press, Elsevier Inc. 2011; 89-99.
http://dx.doi.org/10.1016/B978-0-12-380886-8.10009-1

13. FAO Maize in human nutrition. *FAO Corporate Document Repository. Produced by Agriculture and Consumer Protection. Food and Agriculture Organization of The United Nations.* Rome, Italy. 1992.
http://www.fao.org/docrep/t0395e/t0395e00.HTM. Accessed: January 2014.

14. Veloso Naves MM, Vieira Leão de Castro M, Luiz de Mendonça A, Gebrim Santos G, Silva MS. *Corn germ with pericarp in relation to whole corn: nutrient contents, food and protein efficiency, and protein digestibility-corrected amino acid score.* Ciênc Tecnol Aliment Campinas. 2011; 31(1): 264-9.
http://dx.doi.org/10.1590/S0101-20612011000100040

15. Vander Zaag P. *One potato, two potato.* Far Eastern Economic Review. 1984; 23: 64-6.

16. Kiple KF, Ornelas KC (Eds.). *The Cambridge World History of Foods* (parts 1 and 2). Cambridge University Press. 2008.
http://www.cambridge.org/us/books/kiple/default.htm. Accessed: February 2014.

17. Rodríguez-Sandoval E, Sandoval G, Cortes-Rodríguez M. *Effect of quinoa and potato flours on the thermomechanical and breadmaking properties of wheat flour.* Brazilian J Chem Eng. 2012; 29(3): 503-10.
http://dx.doi.org/10.1590/S0104-66322012000300007

18. Misra A, Kulshrestha K. *Potato flour incorporation in biscuit manufacture.* Plant Food Hum Nutr. 2003; 58(3): 1-9.
http://dx.doi.org/10.1023/B:QUAL.0000040337.69812.cb

19. Ezekiel R, Singh N. *Use of potato flour in bread and flat bread.* In: Preedy VR, Watson RR, Patel VB (Eds.). Flours and Breads and their Fortification in Health and Disease Prevention. USA: Academic Press, Elsevier Inc. 2011; 247-59.
http://dx.doi.org/10.1016/B978-0-12-380886-8.10023-6

20. USDA (U.S. Department of Agriculture, Agricultural Research Service). *USDA National Nutrient Database for Standard Reference.* 2013. Release 26. Nutrient Data Laboratory Home Page,
http://www.ars.usda.gov/ba/bhnrc/ndl

21. Gahlawat P, Sehgal S. *Protein and starch digestibilities and mineral availability of products developed from potato, soy and corn flour.* Plant Foods Hum. Nutr. 1998; 52: 151-60.
http://dx.doi.org/10.1023/A:1008045023304
PMid:9839814

22. Rodríguez-Galdón B, Ríos-Mesa D, Rodríguez-Rodríguez EM, Díaz-Romero C. *Amino acid content in traditional potato cultivars from the Canary Islands.* J Food Comp Anal. 2010; 23: 148-53.
http://dx.doi.org/10.1016/j.jfca.2009.08.009

23. Ochoa CM. *The potatoes of South America: Bolivia.* Cambridge: Cambridge University Press. 1990; 551.

24. Ispizúa VN, Guma IR, Feingold S, Clausen AM. *Genetic diversity of potato landraces from northwestern Argentina assessed with simple sequence repeats (SSRs).* Genet Resour Crop Evol. 2007; 54: 1833-48.
http://dx.doi.org/10.1007/s10722-007-9207-8

25. Andrade-Mahecha MM, Tapia-Blácido DR, Menegalli FC. *Physical-chemical, thermal, and functional properties of achira (*Canna indica *L.) flour and starch from different geographical origin.* Starch/Stärke. 2012; 64: 348-58.
http://dx.doi.org/10.1002/star.201100149

26. Noman ASM, Hoque MA, Haque MM, Pervin F, Karim MR. *Nutritional and anti-nutritional components in* Pachyrhizus erosus *L. tuber.* Food Chem. 2007; 102: 1112-8.
http://dx.doi.org/10.1016/j.foodchem.2006.06.055

27. Ribeiro RA, Finger FL, Puiatti M, Casali VWD. *Chilling injury sensitivity in arracacha* (Arracacia xanthorrhiza) *roots.* Trop. Sci. 2005; 45: 55-7.
http://dx.doi.org/10.1002/ts.48

28. Wang Y, Wang Y, McNeil B, Harvey LM. *Maca: An Andean crop with multi-pharmacological functions.* Food Research Int. 2007; 40: 783-92.
http://dx.doi.org/10.1016/j.foodres.2007.02.005

29. Puoci F, Malanchin R, Piangiolino C, Restuccia D, Curcio M, Parisi OI et al. *Maca flour: a powerful ingredient for functionally enhanced bread.* Int Food Res J. 2013; 20(3): 1293-300.

30. Zheng BL, He K, Kim CH, Rogers L, Shao Y, Hunag ZY et al. *Effect of lipidic extract from Lepidium meyenii on sexual behavior in mice and rats.* Urology. 2000; 55: 598-602.
http://dx.doi.org/10.1016/S0090-4295(99)00549-X

31. Sandoval M, Okuhama NN, Angeles FM, Melchor VV, Condezo LA, Lao J et al. *Antioxidant activity of the cruciferous vegetable maca* (Lepidium meyenii). Food Chem. 2002; 79: 207-13.
http://dx.doi.org/10.1016/S0308-8146(02)00133-4

32. Lee K-J, Dabrowski K, Sandoval M, Miller MJS. *Activity-guided fractionation of phytochemicals of maca meal, their antioxidant activities and effects on growth, feed utilization, and survival in rainbow trout* (Oncorhynchus mykiss) *juveniles.* Aquaculture. 2005; 244(1-4): 293-301.
http://dx.doi.org/10.1016/j.aquaculture.2004.12.006

33. Zhang YZ, Yu LJ, Ao MZ, Jin WW. *Effect of ethanol extract of Lepidium meyenii Walp on osteoporosis in ovariectomized rat.* J Ethnopharmacol. 2006; 105: 274-9.

http://dx.doi.org/10.1016/j.jep.2005.12.013
PMid:16466876

34. Campos D, Noratto G, Chirinos R, Arbizu C, Roca W, Cisneros-Zevallos L. *Antioxidant capacity and secondary metabolites in four species of Andean tuber crops: native potato* (Solanum sp.), *mashua* (Tropaeolum tuberosum *Ruiz & Pavón), Oca* (Oxalis tuberosa *Molina) and ulluco* (Ullucus tuberosus *Caldas).* J Sci Food Agric. 2006; 86(10): 1481-8.

http://dx.doi.org/10.1002/jsfa.2529

35. Azeredo HMC. *Betalains: properties, sources, applications, and stability - a review.* Int J Food Sci Technol. 2009; 44(12): 2365-76.

http://dx.doi.org/10.1111/j.1365-2621.2007.01668.x

36. Coll-Aráoz MV, Kortsarz-González AM, Mercado MI, Ponessa GI, Grau A, Catalán CAN. *Ontogeny and total sugar content of yacon tuberous roots and other three* Smallanthus *species* (Heliantheae, Asteraceae), *insights on the development of a semi-domesticated crop.* Genet Resour Crop Evol. 2014; 61: 163-72.

http://dx.doi.org/10.1007/s10722-013-0022-0

37. Dempewolf H, Rieseberg LH, Cronk QC. *Crop domestication in the Compositae: a family-wide trait assessment.* Genet Resour Crop Evol. 2008; 55: 1141-57.

http://dx.doi.org/10.1007/s10722-008-9315-0

38. Abera T, Admasu S, Desse G. *Effect of processing on physicochemical composition of cassava. Cyanide and other antinutrients in cassava.* Saarbrücken: VDM Verlag Dr Müller GmbH & Co. 2010.

PMCid:PMC2791881

39. Hillocks RJ. *Cassava in Africa.* In: Hillocks RJ, Thresh JM, Bellotti AC (Eds.). Cassava: Biology, production and utilization. Oxford: CABI Publishing. 2002; 41-54.

http://dx.doi.org/10.1079/9780851995243.0041

40. Allem AC. *The origins and taxonomy of cassava.* In: Hillocks RJ, Thresh JM, Bellotti AC (Eds.). Cassava: Biology, production and utilization. Oxford: CABI Publishing. 2002; 1-16.

http://dx.doi.org/10.1079/9780851995243.0001

41. Malandula Chipeta M, Bokosi JM. *Status of Cassava* (Manihot esculenta) *Production and Utilization in Malawi.* Int J Agron Plant Prod. 2013; 4(S): 3637-44.

42. UNCTAD United Nations Conference on Trade and Development. Sweet Potato Commodity Profile. 2012.
http://www.unctad.info/en/Infocomm/AACP-Products/COMMODITY-PROFILE---Sweet-potato/. Accessed: March 2014.

43. Greene JL, Bovell-Benjamin, AC. *Macroscopic and Sensory Evaluation of Bread Supplemented with Sweet-potato Flour.* J Food Sci. 2004; 69(4): 167-73.
http://dx.doi.org/10.1111/j.1365-2621.2004.tb06359.x

44. Walter Jr WM, Collins WW, Purcell AE. *Sweet Potato Protein: A Review.* J Agric Food Chem. 1984; 32: 695-9.
http://dx.doi.org/10.1021/jf00124a001

45. Sun M, Mu T, Zhang M, Arogundade LA. *Nutritional assessment and effects of heat processing on digestibility of Chinese sweet potato protein.* J Food Comp Anal. 2012; 26: 104-10.
http://dx.doi.org/10.1016/j.jfca.2012.03.008

46. Maeshima M, Sasaki T, Asahi T. *Characterization of major proteins in sweet potato tuberous roots.* Phytochem. 1985; 24: 1899-902.
http://dx.doi.org/10.1016/S0031-9422(00)83088-5

47. Guerrero-Beltrán JA, Estrada-Girón Y, Swanson BG, Barbosa-Cánovas GV. *Pressure and temperature combination for inactivation of soymilk trypsin inhibitors.* Food Chem. 2009; 116(3): 676-9.
http://dx.doi.org/10.1016/j.foodchem.2009.03.001

48. Aquino MU. *BAR (Bureau of Agricultural Research) Chronicle (Philippines).* 2009; 10(8): 13.

49. Kumar CG, Parrack P. *Arrowroot* (Marantha arundinacea) *starch as a new low-cost substrate for alkaline protease production.* World J Microbiol Biotechnol. 2003; 19: 757-62.
http://dx.doi.org/10.1023/A:1025156105148

50. Hernández-Medina M, Torruco-Uco JG, Chel-Guerrero, L., Betancur-Ancona, D. *Caracterización fisicoquímica de almidones de tubérculos cultivados en Yucatán, México.* Ciênc. Tecnol. Aliment. 2008; 28 (3): 718-26. ISSN 1678-457X.
http://dx.doi.org/10.1590/S0101-20612008000300031

51. Tapia M. *The Environment, Crops and Agricultural Systems in the Andes and Southern Peru.* IICA. 1982.

52. Cusack D. *Quinoa: grain of the Incas.* Ecologist. 1984; 14: 21-31.

53. Abugoch L, Castro E, Tapia C, Añon MC, Gajardo P, Villaroel A. *Stability of quinoa flour proteins (*Chenopodium quinoa *Willd.) during storage.* Int. J. Food Sci Technol. 2009; 44: 2013-20.
http://dx.doi.org/10.1111/j.1365-2621.2009.02023.x

54. Galwey NW. *The potential of quinoa as a multipurpose crop for agricultural diversification: a review.* Ind. Crops Prod. 1992; 1(2-4): 101-6.
http://dx.doi.org/10.1016/0926-6690(92)90006-H

55. Jacobsen SE. *The worldwide potential of quinoa (*Chenopodium quinoa *Willd.).* Food Rev Int. 2003; 19(1-2): 167-77.
http://dx.doi.org/10.1081/FRI-120018883

56. Bhargava A, Shukla S, Ohri D. Chenopodium quinoa—*An Indian perspective.* Ind Crops and Prod. 2006; 23: 73-87.
http://dx.doi.org/10.1016/j.indcrop.2005.04.002

57. Repo-Carrasco R, Espinoza C, Jacobsen SE. *Nutritional value and use of the Andean crops quinoa* (Chenopodium quinoa) *and kañiwa* (Chenopodium pallidicaule). Food Rev Int. 2003; 19(1-2): 179-89.
http://dx.doi.org/10.1081/FRI-120018884

58. Cardozo A, Tapia ME. *Valor nutritivo. Quinua y Kaniwa. Cultivos Andinos.* In: Tapia ME (Ed). Serie libros y Materiales educativos.. Bogotá: Instituto Interamericano de Ciencias Agrícolas. 1979. 49: 149-92.

59. Koziol MJ. *Chemical composition and nutritional value of quinoa (*Chenopodium quinoa *Willd.).* J Food Comp Anal. 1992; 5: 35-68.
http://dx.doi.org/10.1016/0889-1575(92)90006-6

60. Van Etten CH, Miller RW, Wolff IA, Jones Q. *Amino acid composition of seeds from 200 angiosperm plants.* J Agric Food Chem. 1963; 11: 399-410.
http://dx.doi.org/10.1021/jf60129a016

61. Peiretti PG, Gaia F, Tassone S. *Fatty acid profile and nutritive value of quinoa* Chenopodium quinoa *Willd. seeds and plants at different growth stages.* Animal Feed Sci Technol. 2013; 183: 56-61.
http://dx.doi.org/10.1016/j.anifeedsci.2013.04.012

62. Schoenlechner R, Siebenhandl S, Berghofer E. *Pseudocereals.* In: Arendt EK, Dal Bello F (Eds.). Gluten-free Cereal Products and Beverages. Ireland: Cork. 2008; 149-76.
http://dx.doi.org/10.1016/B978-012373739-7.50009-5

63. Park SH, Morita N. *Dough and breadmaking properties of wheat flour substituted by 10% with germinated quinoa flour.* Food Sci and Technol Int. 2005; 11(6): 471-6.
http://dx.doi.org/10.1177/1082013205060766

64. Collar C, Angioloni A. *Pseudocereals and teff in complex breadmaking matrices: Impact on lipid dynamics.* J. Cereal Sci. 2014; 59: 145-54.
http://dx.doi.org/10.1016/j.jcs.2013.12.008

65. Aluko RE, Monu E. *Functional and Bioactive Properties of Quinoa Seed Protein Hydrolysates.* J. Food Sci. 2003; 68: 1254-8.
http://dx.doi.org/10.1111/j.1365-2621.2003.tb09635.x

66. Alvarez-Jubete L, Arendt EK, Gallagher E. *Nutritive value of pseudocereals and their increasing use as functional gluten-free ingredients.* Trends Food Sci Technol. 2010; 21: 106-13.
http://dx.doi.org/10.1016/j.tifs.2009.10.014

67. Collar C, Martínez JC, Rosell CM. *Lipid binding of fresh and stored formulated wheat breads. Relationships with dough and bread technological performance.* Food Sci Technol Int. 2001; 7(6): 501-10.

68. Añón MC, Puppo MC, Pedroza-Islas R, Oliete B, Villagómez-Zavala D. *Valor nutricional y saludable de materias primas para la elaboración de productos de panificación.* In: Lutz M, León, AE (Eds.). Aspectos nutricionales y saludables de los productos de panificación. Valparaíso: Ed. Universidad de Valparaiso. 2009; 71-119.

69. Amicarelli V, Camaggio G. Amaranthus*: a crop to rediscover.* Forum Ware International. 2012; 2: 4-11.

70. Grobelnik Mlakar S, Turinek M, Jakop M, Bavec M, Bavec F. *Grain amaranth as an alternative and perspective crop in temperate climate.* Revija za geografijo - Journal for Geography. 2010; 5(1): 135-45.

71. Viglasky J, Andrejcak I, Huska J, Suchomel J. *Amaranth* (Amarantus L.) *is a potential source of raw material for biofuels production.* Agron Researc. 2009; 7(2): 865-73.

72. Repo-Carrasco-Valencia R, Peña J, Kallio H, Salminen S. *Dietary fiber and other functional components in two varieties of crude and extruded kiwicha* (Amaranthus caudatus). J. Cereal Sci. 2009; 49(2): 219-24.
http://dx.doi.org/10.1016/j.jcs.2008.10.003

73. Fritz M, Vecchi B, Rinaldi G, Añón MC. *Amaranth seed protein hydrolysates have in-vivo and in-vitro antihypertensive activity.* Food Chem. 2011; 126: 878-84.
http://dx.doi.org/10.1016/j.foodchem.2010.11.065

74. Orsini Delgado MC, Tironi VA, Añón MC. *Antioxidant activity of amaranth protein or their hydrolysates under simulate gastrointestinal digestion.* LWT Food Sci and Technol. 2011; 44: 1752-60.
http://dx.doi.org/10.1016/j.lwt.2011.04.002

75. Chazan M. *World Prehistory and Archaeology: Pathways through Time.* Pearson Education, Inc. 2008.

76. Gepts P. *Origin and evolution of common bean: past events and recent trends.* Hort Sci. 1998; 33(7): 1124-30.

77. Shimelis EA, Rakshit SK. *Proximate composition and physico-chemical properties of improved dry bean* (Phaseolus vulgaris L.) *varieties grown in Ethiopia.* LWT Food Sci and Technol. 2005; 38: 331-8.
http://dx.doi.org/10.1016/j.lwt.2004.07.002

78. Pirman T, Stibilj V, Stekar JMA, Combe E. *Amino acid composition of beans and lentil.* Zb Bioteh Fak Univ Ljubl, Kmet Zooteh. 2001; 78: 57-68.

79. Ramírez-Jiménez AK, Reynoso-Camacho R, Mendoza-Díaz S, Loarca Piña G. *Functional and technological potential of dehydrated* Phaseolus vulgaris L. *flours.* Food Chem. 2014.
http://dx.doi.org/10.1016/j.foodchem.2014.04.008

80. Anton AA, Gary Fulcher R, Arntfield SD. *Physical and nutritional impact of fortification of corn starch-based extruded snacks with common bean* (Phaseolus vulgaris L.) *flour: Effects of bean addition and extrusion cooking.* Food Chem. 2009; 113(4): 989-96.
http://dx.doi.org/10.1016/j.foodchem.2008.08.050

81. Boye J, Zare F, Pletch A. *Pulse proteins: Processing, characterization, functional properties and applications in food and feed.* Food Res Int. 2010; 43: 414-31.
http://dx.doi.org/10.1016/j.foodres.2009.09.003

Chapter 19

Gluten-Free Spirits and Drinks

Mª Angeles Bustamante, Edurne Simón

Department of Nutrition & Food Science. University of the Basque Country (UPV/EHU), Alava, Spain.

marian.bustamante@ehu.es, edurne.simon@ehu.es

Doi: http://dx.doi.org/10.3926/oms.267

How to cite this chapter

Bustamante MA, Simón E. *Gluten-Free Spirits and Drinks*. In Arranz E, Fernández-Bañares F, Rosell CM, Rodrigo L, Peña AS, editors. *Advances in the Understanding of Gluten Related Pathology and the Evolution of Gluten-Free Foods*. Barcelona, Spain: OmniaScience; 2015. p. 645-673.

Abstract

Alcoholic beverages are those containing more than 0,5% (vol/vol) of alcohol. They can be obtained by various processes (fermentation, addition, distillation, extraction, etc.). As there is no established classification of alcoholic beverages, alcoholic concentration is the most commonly used: 1) fermented alcoholic beverages such as beer, cider and wine, and 2) distilled beverages and spirits (higher in alcoholic concentration).

The manufacture of some fermented alcoholic beverages and spirits sometimes involves the use of gluten-containing raw materials (cereals such as barley, wheat or rye). For this reason, in many cases it has been thought that they should not be included in the diet of celiacs. It is also common to add plant material to clarify alcoholic beverages in order to filter or to remove particles in suspension and sometimes malt proteins or hydrolyzed preparations containing wheat gluten are used. As well as this, the addition of flavorings to some spirits is permitted and most of these are obtained from the fermented raw materials. Despite this, nowadays it is possible to find types of fermented alcoholic beverages and spirits that do not contain gluten and are suitable for the celiac population on the market.

As a result, gluten analysis of fermented alcoholic beverages and spirits is often needed to confirm their claim to be the gluten-free. But as gluten is sometimes hydrolyzed, the sandwich R5 ELISA method is not appropriate when foods and beverages are treated with proteolytic enzymes or when they are fermented. In these cases, other techniques such as mass spectrometry (MS) or competitive R5 ELISA have some advantages over the sandwich method.

Keywords

Gluten-free beverages, beer, spirits, gluten analysis.

1. Introduction

Alcoholic beverages are those containing more than 0,5% (vol/vol) of alcohol and can be obtained by various processes (fermentation, addition, distillation, extraction, etc.). There is no definite classification of alcoholic beverages by alcohol content, development process, carbonic content, etc. So the most commonly used classification is the following one: fermented alcoholic beverages such as beer, cider and wine, and distilled beverages and spirits (with high alcoholic concentration).

Fermented alcoholic beverages have been known since the earliest civilizations. Cereals, fruits and juices were left in containers and fermented spontaneously, producing an alcoholic liquid. With the Greek, Roman, Egyptian and Assyrian civilizations methods of making wine and beer were improved[1]. Beers are made from grains, cider from apples, and wine and wine derived beverages are made from grapes. Distillation processes for the production of spirits were developed later.

The process of making some fermented alcoholic beverages and spirits sometimes involves the use of gluten-containing raw materials (grains such as barley, wheat or rye). For this reason, in many cases it has been thought that they should not be included in the diet of celiacs. But nowadays it is possible to find on the market types of fermented alcoholic beverages and spirits that do not contain gluten and are suitable for celiacs.

Regulation (EU) No 1169/2011 of the European Parliament and of the Council[2], on the provision of food information to consumers, lists in Annex II the substances or products causing allergies or intolerances. The substances and products include those cereals containing gluten, namely: wheat, rye, barley, oats, spelt, kamut or their hybridised strains, and products thereof. But it also includes some exceptions: a) wheat based glucose syrups including dextrose; b) wheat based maltodextrins; c) glucose syrups based on barley and d) cereals containing wheat based glucose syrups. Some of these products are used in the manufacture of some gluten-free spirits and drinks.

2. Fermented Alcoholic Beverages

2.1. Beer

Beer is one of the most frequently consumed alcoholic beverages. It is a very popular beverage in most European and American countries and can constitute an important part of diet and leisure time. The average annual consumption in 2011 was established in about 68,2 kg/capita in Europe and 78,3 kg/capita in Northern America[3].

Beer is a non-distilled alcoholic beverage produced by the fermentation of malted barley grains or other cereals (wheat, rye and oats) with yeast. Most beer is also flavored with hops, which adds a bitter taste and acts as a natural preservative[4]. Other flavorings such as herbs or fruit may occasionally be included.

In traditional beer-making or brewing, barley (*Hordeun vulgare var. distichum*) is the most important raw material. The barley is germinated in a controlled manner to develop the enzyme system responsible for transforming the starch into sugars. This process is known as malting: enzymes are synthesized and mobilized, and the starch granules are mobilized in the endosperm. Malting is halted by drying and the malted barley is then toasted to deactivate the enzymes, denature proteins and produce characteristic colours and aromas.

The wort is prepared by mixing the starch source (normally malted barley) with hot water, is known as "mashing". The malt is ground and made into a paste with brewing water and partially degraded and solubilized with malt enzymes. Wort is boiled with hops or hop products to hydrolyze and dissolve proteins and to dissolve hop ingredients. Boiling also destroys any enzymes remaining from the mashing stage. Next, alcoholic fermentation is produced with the addition of *Saccharomyces cerevisiae* (for ale) or *Saccharomyces uvarum* (for lager). During fermentation, the wort becomes beer in a process which takes from a week to some months, depending on the yeast and beer type. Fermentation is complete when the desired alcohol content has been reached. Nowadays, the majority of beers receive a relatively short

conditioning period after fermentation and before filtration. This conditioning is performed to drop proteins out of solution to prevent cloudiness in the bottled or otherwise packaged product[4,5].

Beer can be classified according to its dry malt extract (DME), which is the total solid organic ingredient that the wort contains before fermentation. It is expressed in g/100g wort (%). Traditional beers have DME ≥ 11%; Special beers: DME ≥ 13%; Extra Special Beers: DME ≥ 15%; and Alcohol free beers have a variable DME between 2-4 %.

Depending on the type of fermentation beer can be classified into top fermented beers and bottom fermented beers. Top fermented beers are fermented at temperatures up to 20ºC and yeasts rise to the surface during fermentation creating a very thick, rich yeast head. Ale, Stout, Porter are the most known. British beers as well as the wheat beers, German Altbier and Kölsch are also top fermented beers.

Bottom fermented beers or lager are made at low temperatures ranging from 7 to 15°C. At these temperatures, lager yeasts grow less rapidly than ale yeasts, and they tend to settle out to the bottom of the fermentation container. They can be stored for a long time (months) and they are commonly identified according to the place where they are from: Munich, Vienna, Pilsner, etc. Some beers are made with spontaneous fermentation with natural or wild yeast strains. Lambic, Gueuze and Faro are some of the latter.

Beer contains an average of 0.2-0.6 g/100 mL of proteins or peptides that mainly come from barley. This quantity is bigger than that found in other alcoholic beverages, such as wine (0.1-0.2 g/100mL). Proteins remain relatively unmodified, but they may suffer proteolysis and other chemical modification events. Prolamin concentration in beer depends on the malt type, the mashing technology, type of fermentation, maturation and stabilization process. Because of the use of malted barley or wheat, the possible presence of toxic proteins in beer has been discussed for a long time, and therefore, beer is excluded from the diet of celiacs.

Although most celiacs could drink one beer with low levels of gluten, each person displays a different level at which his or her autoimmune response will be activated. In addition, the amounts of dietary gluten that each one can ingest without damaging the mucosa of the small intestine is generally unknown and should be kept below 50 mg/day, as suggested by Catassi et al.[6].

In this sense, many adult celiacs are unhappy that this beverage is not permitted in their diet or that they can drink only one glass a week. For this reason, over the past years several beers have been launched in the market advertised as "gluten-free" or "gluten-removed". The availability of safe gluten-free beers would improve celiac patient well-being and perception of a normal social life.

2.1.1. Strategies to Brew Gluten-Free Beers

The most obvious strategy to brew gluten-free beer is the use of raw material without gluten. Gluten-free beers are made from cereals without gluten, such as millet, rice, sorghum, corn and teff. The use of oat in gluten-free beers is controversial because not all people with gluten intolerance can include this cereal in their diet without adverse effects.

The use of pseudocereals like buckwheat, quinoa or amaranth to brew beers for the celiac market is well known[7,8,9,10]. These are taxonomically different from *Poaceae* (grass family) and are considered gluten free[11,12]. Another option is the use of other vegetable products as raw materials (potatoes and sweet potatoes, chestnut chips, or chips made from almonds or hazelnuts, and other fermentable sugar sources and syrups)[12,13].

Nevertheless, rice (*Oryza sativa*) is probably the most commonly used gluten free grain, industrially and for research objectives. However, there are few data concerning malting and brewing with 100% rice.

Some works[14,15] have shown that the rice malts obtained have much lower extract contents and, on the whole, a lower enzymatic activity than barley malt.

Compared to barley malt, rice malts had a lower soluble protein percentage and low soluble/total protein ratio, which implies that they were poorly modified during extraction process.

Using these gluten free alternatives, the brewing process is similar to that of malted barley but obviously parameters such as germination and fermentation conditions, pH of mashing, yeast strain used, temperatures and storage conditions have to be adjusted depending on the raw material used[7,8,9,10].

Commonly, malt is produced from the barley or wheat and some authors had showed important differences in celiac immunotoxicity of barley varieties[16] or wheat varieties which are naturally reduced in celiac disease related gluten epitopes[17].

Thus, a second strategy for brewing low gluten beers is to select cereals with fewer immunogenic epitopes for the process. Therefore, when gluten free beer is produced from traditional raw materials by elimination of toxic proteins and peptides, the right choice of malt facilitates this process.

Furthermore, sometimes it is necessary the use of industrial enzyme preparations and gluten free adjuncts because gluten free malt is not as suitable as barley malt for brewing[11]. For example, the addition of enzymes like beta-amylase and amyloglucosidase increases the amount of fermentable sugars in the sorghum malt worts[18].

Despite of this, most brewers have created different types of beers from gluten free raw material: Ale, Pale Ale, Pilsner, Lager, lemon-flavored beer, etc. However, the colour, flavour and the taste may be quite different from traditional beers made from barley or wheat.

Another method of brewing gluten-free beer is to make gluten-removed beer. Brewers use barley to produce the malt, which gives the traditional flavor of beer. Then they add microbial peptidases or grain endopeptidases[11,19,20]. So it does not actually remove the gluten from the beer. Instead the gluten is broken into small fragments, which are supposed to be too small to be toxic to individuals with celiac disease. But it is difficult to

quantify the amount of gluten in a product, such as beer, when the protein has been hydrolyzed[20,21,22,23,24].

As a result, in some states of the USA manufacturers of gluten-removed beers who label the beer with the claim "gluten levels are below 20 mg/kg", must add that the "product is fermented from grains containing gluten and is processed to remove it"[20].

Proteases from germinated gluten cereals are produced during fermentation and cleave celiac toxic peptides into non-toxic fragments. It is also possible to add a prolyl endopeptidase (e.g. a proline-specific endo-protease) that breaks down gluten molecules and other proteins at the carboxyl end of the amino acid proline. Prolyl endopeptidases from microbial origin has been used in brewing industries to prevent haze and when they are added during fermentation or at the end of the process, produces gluten free beer or with low quantities of gluten[11,19,25].

Sometimes, the use of both types of enzymes (from grain and microbial prolyl endopeptidase) has been used to obtain a beer with lower concentration of gluten (< 20 mg/kg)[25,26].

Another approach to reach gluten free beer is based on precipitation of hordeins. During years haze formation has been considered a defect in beer brewing. The proteins that produce haze are derived from the prolin-rich barley hordeins. To prevent this defect, substances like tannins, unflavored gelatins and silica hydrogels have been used for the beer stabilization. These substances form complexes with the proline present in barley hordein than can be removed by precipitation and/or filtration[11,27,28]. The process eliminates the haze and simultaneously reduces the gluten content. This fourth strategy does not ensure the complete removal of gluten but sometimes the level reached is acceptable for celiac.

Finally, manufacture of all these gluten-free beers has to be carried out in an entirely gluten-free environment.

2.2. Wine

Wine is a product exclusively obtained from the alcoholic fermentation of grapes. Fresh grapes, crushed grapes or grape juice may be used as raw material for further full or partial fermentation[1,4].

White and red grapes are used, but white grapes produce only white wine. Red grapes can make white, rosé or red wine. This depends on the cultivar and the vinification process. There are cultivars of red grapes that yield white juice and, if solids are separated before fermentation begins, white wine is obtained. Red grapes which have white or red juice, yield either rosé or red wine if the fermentation is done with the whole fruit because red pigments or anthocyanins are presented in the skins. A limited period of maceration brings about rosé wines[5].

None of the raw materials required for the production of wine contains gluten so it is considered as a gluten-free fermented beverage. But sometimes, it is necessary to clarify the wine in order to remove particles in suspension because they can affect the appearance and the flavor of the wine. This procedure has to ensure long-term clarity and prevent sediment during storage. The fining process relies on adding substances that induce flocculation and settling in cloudy or in non-stabilized wines. Filtration can remove particles such as grape fragments and dead yeast but fining can remove soluble substances (phenolic compounds such as tannins, and coloring matter in red wine).

Fining is carried out with protein-based products which are often a mixture of denatured or partially denatured proteins that trap undesirable substances, resulting in a precipitate. Proteins used for fining have been typically of animal origin. In red wine the most commonly used are egg albumin, serum albumin, casein, isinglass or gelatin from fish. These protein-phenol complexes are then removed by decanting, centrifugation or filtration. Nowadays winemakers are seeking a substitute for these animal proteins due to the restrictions imposed because of bovine spongiform encephalopathy (BSE) in animals and its possible transmission to humans. In this regard, the

legislation in several countries of the European Union has been adopted to avoid the use of blood powder and serum albumin[29,30].

Plant proteins have proved to show potential in this context. Malt proteins, sorghum prolamins and legume proteins have been used to clarify wines. Wheat gluten, especially hydrolyzed preparations, allows a very efficient clarification of the wine, with a selective precipitation of condensed tannins from red wine.

Since proteins derived from plants are considered good wine fining agents, it seems to be important to quantify their residual amount in the fined wine, as some plant proteins could cause severe immunological responses or chronic intolerance. The possibility that gluten proteins remain in the wine after treatment cannot be excluded, representing a potential hazard for persons who have celiac disease[29,30].

Labeling legislation in European Union, Canada, USA and Australia requires that potentially allergenic compounds must be stated on the labels but there are few data about whether any plant proteins derived from fining agents are present in the finished wine. Despite this fact, the published results provide evidence that the gluten concentration in the treated wines is by far below even the most restrictive legal threshold for gluten-free drinks.

2.3. Cider

Cider is a fermented alcoholic beverage made from apple juice[1]. Cider alcohol strength varies from 1.2% to 8.5% (vol/vol).

This is the general process for making cider. Apples are crushed and the pulp obtained is then transferred to the cider press where it is pressed until all the 'must' or juice is squeezed from the apple pulp. Then the fermentation takes place in casks or barrels. The proportion of alcohol content depends directly of the total sugar content in its must, because during the fermentation process the sugar is transformed into carbonic anhydride and alcohol. Then the cider is transferred to another cask to maintain the quality and after some months the process is completed and it is ready for bottling.

As in the case of wine, all raw materials used in the manufacture of cider are gluten free. Therefore, if the whole process is carried out in an entirely gluten-free environment, cider is in general considered to be a gluten-free beverage.

However, low quantities of malted barley are added to some special ciders which are then filtered to guarantee gluten removal.

3. Spirits

The *Official Journal of the European Union*[31] *gives the following definition:* spirit drinks are alcoholic drinks intended for human consumption. By definition, spirit drinks possess particular organoleptic qualities and have a minimum alcoholic strength of 15% vol. But some of them reach 40% vol.

Spirit drinks are produced by two ways. The first one is directly, a) by distillation, with or without added flavorings, of naturally fermented products; b) by maceration of plant materials in ethyl alcohol of agricultural origin and/or distillates of agricultural origin, and/or spirit drinks; c) by the addition of flavorings, sugars or other sweetening products and/or other agricultural products and/or foodstuffs to ethyl alcohol of agricultural origin and/or to distillates of agricultural origin and/or to spirit drinks. The second way is by the mixture of a spirit drink with one or more of: a) other spirit drinks; b) ethyl alcohol of agricultural origin or distillates of agricultural origin; c) other alcoholic beverages; and/or d) drinks.

The spirit drinks are classified by category (rum, whisky vodka, etc.).

3.1. Rum

Rum is a spirit drink produced exclusively by alcoholic fermentation and distillation, either from molasses or syrup produced in the manufacture of cane sugar or from sugar-cane juice itself[31]. Addition of alcohol or flavorings is forbidden and rum may only contain added caramel for coloring. The minimum alcoholic strength by volume of rum is 37.5 %.

Rum is considered a gluten-free beverage because in the manufacturing process cereals containing gluten are not used.

However, pre-made drink mixes with rum, such as those intended for piña colada, mojito, daiquiri, etc. may contain gluten ingredients as flavorings.

3.2. Whisky or Whiskey

Whisky or whiskey is a spirit drink produced exclusively by distillation of a mash made from malted cereals with or without whole grains of other cereals, which has been saccharified by the diastase of the malt contained therein, with or without other natural enzymes and fermented by the action of yeast[31,32]. The distillation is carried out one or more times so that the distillate has an aroma and taste derived from the raw materials used. At the end, the final distillate is matured for at least three years in wooden casks. This distillate, to which only water and plain caramel (for coloring) may be added, retains its color, aroma and taste derived from the production process. The minimum alcoholic strength by volume of whisky is 40 %.

Although some experts as the American Dietetic Association and Dieticians of Canada considered whisky as a gluten-free beverage[33], the fact that distillation removes the harmful gluten proteins should be demonstrated. In this sense, the whisky industry has undertaken a program of study that has showed the absence of gluten or other allergenic materials in distillates that use wheat and barley as raw materials[34].

But some celiacs associations advise against consuming whisky if the consumer is particularly sensitive. They claim that it is possible for distillation not to remove 100% of the gluten, or that a small amount of gluten is added back in as part of processing after distillation. In some cases, whisky manufacturers add caramel coloring (which may contain gluten) or even a small amount of the undistilled grain mash after the distilling process.

3.3. Vodka

Vodka is a spirit drink produced from ethyl alcohol of agricultural origin obtained following fermentation with yeast of either potatoes and/or cereals; or other agricultural raw materials[31]. Then, it is distilled and/or rectified to reduce selectively the organoleptic characteristics of the raw materials used and the products formed during fermentation. This process may be followed by redistillation and/or treatment with appropriate processing aids to give it special sensory qualities. The only flavorings that might be added are natural flavoring compounds present in distillate obtained from the fermented raw materials. In addition, the product may be given special organoleptic characteristics, other than a predominant flavor. The minimum alcoholic strength of vodka is 37.5 % vol.

There are plenty of vodkas made from non-gluten sources, such as potato vodka, corn vodka or grape vodka and they are usually gluten-free. Most of them are labelled as being gluten-free.

3.4. Gin

Gin is a juniper-flavored spirit drink produced by flavouring organoleptically suitable ethyl alcohol of agricultural origin with juniper berries (*Juniperus communis* L.). The minimum alcoholic strength by volume of *gin* shall be 37.5 %[31]. Natural and natural-identical flavouring substances from material of vegetable or animal origin can be added for the production of gin.

Due to the fact that gin is a distilled spirit, some experts consider it as a gluten-free beverage. But it is a controversial subject because other experts do not recommend this alcoholic beverage for celiac since the agricultural alcohol used is made from cereals which may include wheat, barley or rye and flavorings are used.

3.5. Grain, Wine, Fruit, Honey, Cider, Perry, Grape Marc Spirits

There is a wide range of alcoholic beverages produced exclusively by alcoholic fermentation and/or distillation of the raw materials (grains, wine, fruit, honey, cider, perry, etc). The sales denomination is different depending on the type of raw materials used in the manufacture[31]. For example, the sale denomination of fruit spirit shall be 'spirit' preceded by the name of the fruit, berry or vegetable, such as: cherry spirit or *kirsch*, mirabelle, peach, apple, pear, apricot, fig, citrus or grape spirit or other fruit spirits. In the case of marc spirits, the sales denomination consists of the name of the fruit followed by "marc spirit". If marcs of several different fruits are used, the sales denomination shall be "fruit marc spirit". The Table 1 shows some characteristics of this kind of beverages.

Table 1. Characteristics of spirits made from different raw materials.

Product	Raw material	Process	Alcoholic strength
Grain Spirit	Fermented mash of whole grain cereals	Distillation	≥ 35.0%
Wine Spirit	Wine	Distillation	≥ 37.5%
Grape marc Spirit	Grape marc	Fermentation and Distillation	≥ 37.5%
Fruit marc Spirit	Fruit marc (except grape)	Fermentation and Distillation	≥ 37.5%
Fruit Spirit	Fleshy fruit or must of such fruit, berries or vegetables	Fermentation and Distillation	≥ 37.5%
Cider or Perry Spirit	Cider or Perry	Distillation	≥ 37.5%
Honey Spirit	Honey mash	Fermentation and Distillation	≥ 35.0%

In this kind of spirits the key is the raw material used in the manufacture. Most of them are clearly gluten-free if they are handled in a gluten-free environment, but other, such as grain spirits, may be made from gluten-containing cereal grains and are not required to indicate the type of cereal is used in the manufacture. Despite this, as in the case of other foods, it is necessary to indicate the allergens at the label.

4. Problems in the Quantification of Gluten in Alcoholic Fermented Beverages and Spirits

The assessment of gluten content in beers and other beverages should take into account two important aspects. Firstly, as malt beer is usually produced from barley and wheat cereals, in addition to gliadins, the techniques used have to be able to accurately detect and quantify barley prolamins. The use of a single wheat gliadin standard could be unsuitable for the accurate determination of gluten from cereals which consist of a complex mix of proteins that have different responses to the antibodies used[22,23,35]. It seems that accurate determination of hordein requires that the hordein standard used to calibrate the assay be similar in composition to the hordeins present in the beverages[22,35]. Depending on the standard used, the quantification may over- or under-estimate by several orders of magnitude.

Secondly, the assay ought to accurately quantify partially hydrolized prolamins (gliadins and/ or hordeins), though there is no suitable hydrolyzed hordein standard for beer[11]. Measuring the quantity of hydrolyzed prolamins in these types of products is one of the main problems in gluten analysis because prolamins have been broken into smaller fragments[20,21,36]. This is what happens, for example, during the brewing process.

ELISA is considered the method type I by Codex Alimentarius for the analysis of gluten-free foods. It is also recommended by the Working Group on Prolamin Analysis and Toxicity (WGPAT) and the Food and Drug Administration (FDA)[37].

Sandwich R5 ELISA Méndez method is used to analyse intact prolamins. R5 antibody is capable of recognizing several small repetitive coeliac toxic epitopes and, as the epitope QQPFP (glutamine-glutamine-proline-phenylalanine-prolin) is present in wheat gliadin, barley hordein and rye secalin, R5 could recognize all fractions of all three grains. This method is based on the requirement that at least two specific epitopes are recognized by the antibody. However, it is not appropriate when foods and beverages are treated with proteolytic enzymes or when they are fermented because there may not be two of this sequence. This is due to prolamins being partially hydrolyzed into fragments containing two or more epitopes and small fragments having only one epitope. Consequently, small hydrolyzed products with a single epitope cannot be reliable determined by using sandwich R5 ELISA[22,36,38].

As competitive R5 ELISA requires only one antibody binding epitope, it is more suitable for the detection of hydrolyzed gluten than sandwich R5 ELISA. Both methods have been validated in multi-lab international trials[38].

The second FAO accepted sandwich ELISA kit is based on the Skerritt antibody[39,40]. This antibody was one of the first monoclonal antibodies raised against wheat gliadin[39]. It recognizes w-gliadins, a subfraction that differs both in their presence and levels within the cereals. In this sense, the Skerritt antibody only has a weak response to the hordeins found in barley and thus may underestimate the gluten content[23,26,41]. Tanner et al.[23] suggested that this antibody does not seem to be appropriate for the gluten analysis of beers and the use of ELISA sandwich based on it should be discarded to avoid falses negatives and dissenting results.

Second generation competitive ELISA methods have been developed using antibodies raised against the dominant immuno-reactive peptides involved in the biological response of celiac disease, e.g. G12 and A1 monoclonal antibodies have been raised against the toxic 33-mer of α-gliadin[42]. These antibodies are able to recognize peptides (besides the 33-mer peptide) from wheat, barley, rye, and varieties of oats which showed immunogenicity in T-cells from celiac patients[43,44].

Other competitive ELISA kit has been tested through an interlaboratory study in accordance with AOAC guidelines[45]. The Gluten Tec kit uses a monoclonal antibody that detects a well characterized T cell stimulatory epitope of toxic prolamins. Both intact and small protein fragments, resulting from the hydrolysation of intact proteins, could be detected and, indeed, beer was selected as a food matrix for validation trials.

Competitive ELISA showed some advantages of repeatability and accuracy when analysing alcoholic beverages obtained by hydrolysis processes[46]. However, there are still some unresolved questions concerning competitive ELISA and the analysis of these beverages is summarized in the following paragraphs.

One concern is to establish how prolamin fragmentation into smaller peptides occurs because the relation between prolamin and the fragments can vary from sample to sample. As competitive ELISA relates the total gluten amount in food with possible toxic peptides, a reliable conversion factor into gliadin cannot be given[36].

As mentioned, during alting and fermentation endogenous proteolytic enzymes break down the barley/wheat prolamins into short peptide fragments, and even into amino acids. Nevertheless, the heterogeneous mixture of peptides obtained are quite water soluble so they remain in the final product. Many of these peptide fragments contain high proline and glutamine levels. This may suggest that potentially immunogenic epitopes for celiac population may still remain in this beer[16,43].

A limitation of the competitive ELISA technique is that the fragment size recognized by the antibodies is not always established as a whole. It would be possible that the antibodies in ELISA competitive assay would also recognize smaller fragments that do not trigger the disease. Therefore, the gluten content is overestimated and products that could be suitable for celiac population would not be labelled as gluten-free.

By contrast, another aspect to consider is that the enzyme (proline endoprotease) used to produce gluten-removed beer may also destroy the recognized epitope sequence at the amino acid proline[19,25]. Thus, competitive

ELISA does not detect gluten peptides. Since this enzyme breaks peptides at the prolines and the extent of this breakdown is high, toxic peptides will be broken down too and the gluten remaining in beer will be low. Nevertheless, underestimation of toxic prolamins by antibodies that not discriminate the immunoactivity of the peptide might endanger celiac safety.

In order to avoid these problems, antibodies should be specific and correlated with the potential immunotoxicity of the beer[16,43,44].

As well as this, some alcoholic beverages, such as wine or beer contain phenolic compounds. As a result it is necessary to use a protein (e.g. fish gelatin or skimmed milk powder) in the extraction procedure to prevent the phenolic-rich matrix interfering with the ELISA assay. In R5 competitive ELISA kits it is well established that beers are extracted with ethanol containing 10% fish gelatin.

Indeed, when proteins are denatured due to fermentation or by using proteolytic enzymes, a simple ethanol solution is not capable of extracting all the prolamins. Consequently reducing and disaggregating agents such as 2-mercaptoethanol are added to ethanol in the extraction processes by ELISA sandwich[38]. However, these types of reagents are not compatible with the competitive assay because mercaptoethanol interferes with the specific binding of the antibody, obtaining false results. Some authors[46] have assayed a cocktail extraction, called UPEX, containing the reducing agent Tris (2-carboxyethyl)-phosphine (TCEP) and the surfactant N-lauroylsarcosine. Other study focusing on hordeins[23] suggest that an alcohol extraction with urea/ dithiothreitol (DTT) successfully extracts the majority of hordeins from barley flour and malt.

MALDI-TOF mass spectrometry was the first non-immunological technique employed to identify prolamins in flours and real complex food samples[47]. Nevertheless, this system did not detect prolamins levels below 20-25 mg/kg and so is not appropriate in food samples with low prolamin levels.

Recently, mass spectrometry (MS) methods for the direct and absolute identification and quantification of food allergens and gluten have been developed[23,35,41]. Thanks to its high sensitivity, LC-MS allows the detection of

allergenic proteins in trace amounts. In this sense, some authors confirmed that the ELISA sandwich results did not correlate with the relative content of individual hordein peptides as determined by MS, with all barley based beers containing hordein[22,23,41]. Tanner et al.[23] found that 20% of ELISA results for beers were false negatives compared to results obtained by relative mass spectrometry. They suggested that mass spectrometry could be more reliable than ELISA, as ELISA enumerates only the concentration of particular amino-acid epitopes which may vary between different hordeins and may not be related to the absolute hordein concentration[35,41].

Although LC-MS/MS could offer analytical specificity which is superior to that of immunoassays or conventional high performance/pressure liquid chromatography (HPLC), the high initial cost of the equipment is not easily affordable and its throughput is lower than of immunoassays[48].

Other techniques such as those based on DNA detection have been also developed. Polymerase Chain Reaction (PCR) is useful to confirm other methods but they give only partial information in routine analysis of beers. Mujico et al[49,50] observed a certain degree of positive correlation between protein and DNA in some hydrolysed food samples. Nevertheless, in food matrixes submitted to an intensive hydrolysis process, as syrups and malt extracts, the DNA was practically undetectable due to massive DNA degradation, and amplification by Q-PCR was not possible. Our Laboratory of gluten analysis UPV/EHU have also tested several techniques to analyze gluten content in alcoholic drinks using sandwich and competitive ELISA, compared with PRC technique.

We reported that despite the detection of gluten traces (5-40 mg/kg) by ELISA methods in some beers, it was not possible to find any WBR-DNA amounts, with an optimized design of a quantitative Real Time WBR-PCR protocol[51]. It must be pointed out that DNA extraction in these samples was difficult, and an improvement of this step may be enough to enhance detection of gluten-DNA. When DNA qualitative detection was used, by means of the SureFood® Allergen Gluten Real Time PCR kit, it was possible to detect DNA in some gluten containing beers.

In order to establish the amount of gluten detected in alcoholic beverages it is necessary to point out that gluten detection in spirits or distilled beverages is fairly unusual. Many of the beers consumed contained very low gluten levels, but frequently more than one serving of beer is consumed, which results in a toxic prolamin storage[19,52,53].

As mentioned before, when gluten content of beers is measured, several authors described that when using ELISA sandwich some of the negative or very low gluten results could not be considered gluten free when analyzed with competitive ELISA or other techniques[23,41,43].

5. Gluten-Content in Beers and Spirits

The studies reviewed showed a high diversity of gluten content in the different beers. This is due, at least in part, to the changes in the brewing processes. There are many differences in filtration processes, enzymatic processes, and/or the use of different varieties of malt barley that modify final content of gluten in beers[12,19,25,28,52] (Table 2). Moreover, beers often contain significant quantities of gluten free adjuncts, which help to 'dilute' the initial raw material gluten content. Also the use of silica gel for removal of proteins may reduce the level of gluten in stabilized beer[28].

In addition, nutritional composition of beers showed that final product contains about 0.2-0.6% protein. Dostálek et al.[28] found that prolamin content decreased from 100 % in malt to less than 0.2% in beer during the mashing process, fermentation and stabilization process. Which is to say that anti-gliadin antibodies concentration was reduced by at least three orders of magnitude in beer compared with raw malt (on average, malt contained 18780 mg/kg of gluten, wort had 48 mg/kg and beer, 6.0 mg/kg of gluten).

Several studies agree with the fact that beers sold as "gluten free" contained gliadin levels below the detection limit of 6 mg/kg gluten[11,40,52,54]. Most of the beers analyzed contain relatively low amounts of gluten because the quantity detected is usually between 10 and 50 mg / kg of gluten[28,26,52].

Table 2. Factors influencing gluten levels during the brewing process.

Brewing Parameters	Gluten Levels
Grain varieties for malt	Vary
Use of wheat	Highly Increase
Malt process	Vary
Dry Malt extract (DME)	Increase
Original gravity or beer density	Increase
Addition or use of stabilizers (e.g. prolyl or proline-specific endoprotease; Silica gel)	Decrease
Use of specific process equipment (centrifuge, filters)	Decrease

When comparing various types of beer, alcohol free beers usually have a very low content of protein and gluten content is below the detection limit or under the definition of "gluten free" (20 mg/kg)[28,52]. Comino et al.[43] found that 59% of the one hundred analyzed beers contained more than 100 mg/kg of gluten, but other studies showed that many of the lager and ale samples were below 20 mg/kg[11,26,28,52,53].

Taking into account the relationship between cereal composition and gluten content, most of the studies reveal that type of cereal is a major element. Beers made of barley tend to produce low gluten levels[52] whereas wheat or malted wheat beers contain very large quantities of gluten (more than 100 mg/kg and even higher than 500 mg/kg) and, thus wheat beers cannot be included in the diet of celiacs[23,53,54].

As suggested before, other factors related to the brewing process affect final content of gluten. Some studies[25,53] described that many of the barley beer with highest gluten levels (> 100 mg/kg) were not clarified by filtration,

so the level of gluten detected may also correlate with turbidity, which could explain the higher prolamin concentration in some of them. For example, very high gluten content (> 800 mg/kg) is found in the Hefeweizen beer. This German style of wheat beer is a top fermented, unfiltered beer with a noticeable yeast sediment and a cloudy appearance[23,53,54].

From the point of view of labelling, beers producers should closely monitor the manufacturing process to ensure consistently low gluten levels before they label beers made from barley, wheat or rye as "gluten free". With respect to spirits, celiac patients should be aware of the risks of the consumption of these types of highly alcoholic drinks and they should always check the label information and any suspect added ingredients[55,56].

6. Conclusion

Beer is the most popular fermented alcoholic beverage and the most likely to contain small amounts of gluten.

The accurate quantification of gluten in beers and other beverages is a challenging problem due to the hydrolysis of gluten and potential immunotoxicity modifications that occur as a result of the processing steps.

Although it is necessary to consider the origin and type of grain used, it is usual that special wheat-beers contain very significant amounts of gluten while other beers brewed with barley rarely exceed 50 mg/kg of gluten.

The remaining fermented beverages, such as wine or cider, rarely contain gluten but it is important to confirm that no gluten-containing additional ingredients are added after brewing.

The manufacturing of distilled drinks or spirits implies that the end product does not usually contain gluten. Nevertheless, it is necessary to check that additional gluten-containing ingredients are not then added.

Analytical techniques used to gluten detection in these alcoholic drinks are not as useful as for other matrices. The competitive ELISA technique has some advantages over other methods but it still needs to be improved.

References

1. Gil A. *Tratado de Nutrición.* 2nd ed., vol.2. Madrid: Editorial Médica Panamericana. 2010.
2. Regulation (EU) No. 1169/2011. *Provision of food information to consumers, amending Regulations (EC) No. 1924/2006 and (EC) No. 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No. 608/2004. Regulation (EU) No. 1169/2011 of the European Parliament and of the Council of 25 October 2011.* Official Journal of the European Union, L 304/18 (22.11.2011).
3. Food and Drug Administration (Internet). Silver Spring: U.S. Food and Drug Administration. 2014.

   http://www.fda.gov/RegulatoryInformation/Guidances. Accessed: February 2015.
4. Belitz H-D, Grosch W, Schieberle P. *Food Chemistry.* 4th revised and extended ed. Berlin Heidelberg: Springer-Verlag. 2009.
5. Alais C, Linden G. *Food biochemistry.* New York: Ellis Horwood. 1991.

   http://dx.doi.org/10.1007/978-1-4615-2119-8
6. Catassi C, Fabiani E, Iacono G, D'Agate C, Francavilla R, Biagi F et al. *A prospective, double-blind, placebo-controlled trial to establish a safe gluten threshold for patients with celiac disease.* Am J Clin Nutr. 2007; 85: 160-6.

   PMid:17209192
7. Ceppi ELM, Brenna OV. *Brewing with Rice Malt - A Gluten-free Alternative.* J Inst Brewing. 2010; 116(3): 275-9.

   http://dx.doi.org/10.1002/j.2050-0416.2010.tb00431.x
8. Chiba Y, Bryce JH, Goodfellow V, MacKinlay J, Agu RC, Brosnan JM et al. *Effect of Germination Temperatures on Proteolysis of the Gluten-Free Grains Sorghum and Millet during Malting and Mashing.* J Agric Food Chem. 2012; 60: 3745-53.

   http://dx.doi.org/10.1021/jf300965b
   PMid:22440185
9. Agu RC, Chiba Y, Goodfellow V, MacKinlay J, Brosnan JM, Bringhurst TA et al. *Effect of Germination Temperatures on Proteolysis of the Gluten-Free Grains Rice and Buckwheat during Malting and Mashing.* J Agric Food Chem. 2012; 60: 10147-54.

   http://dx.doi.org/10.1021/jf3028039
   PMid:22950683

10. De Meo B, Freeman G, Marconi O, Booer C, Fantozzi P. *Behaviour of Malted Cereals and Pseudo-Cereals for Gluten-Free Beer Production.* J Inst Brewing. 2011; 117(4): 541-6.

http://dx.doi.org/10.1002/j.2050-0416.2011.tb00502.x

11. Hager A, Taylor JP, Waters DM, Arendt EK. *Gluten free beer - A review.* Trends Food Sci Technol. 2014; 36: 44-54.

http://dx.doi.org/10.1016/j.tifs.2014.01.001

12. Zannini E, Jones JM, Renzetti S, Arendt EK. *Functional replacements for gluten.* Annu Rev Food Sci Technol. 2012; 3: 227-34.

http://dx.doi.org/10.1146/annurev-food-022811-101203
PMid:22385166

13. O'Shea N, Arendt E, Gallagher E. *State of the art in gluten-free research.* J Food Sci. 2014; 79: R1067-76.

http://dx.doi.org/10.1111/1750-3841.12479
PMid:24784553

14. Usansa U, Burberg F, Geiger E, Back W, Wanapu C, Arendt KE et al. *Optimization of Malting Conditions for Two Black Rice Varieties, Black Non- Waxy Rice and Black Waxy Rice (Oryza sativa L. Indica).* J Inst Brew. 2011; 117: 39-46.

http://dx.doi.org/10.1002/j.2050-0416.2011.tb00441.x

15. Ceppi, ELM, Brenna, OV. *Experimental studies to obtain rice malt.* J Agric Food Chem. 2010; 58: 7701-07.

http://dx.doi.org/10.1021/jf904534q
PMid:20524666

16. Comino I, Real A, Gil-Humanes J, Pistón F, de Lorenzo L, Moreno ML et al. *Significant differences in coeliac immunotoxicity of barley varieties.* Mol Nutr Food Res. 2012; 56: 1697-707.

http://dx.doi.org/10.1002/mnfr.201200358
PMid:22968973

17. van den Broeck H, Hongbing C, Lacaze X, Dusautoir JC, Gilissen L, Smulders M et al. *In search of tetraploid wheat accessions reduced in celiac disease-related gluten epitopes.* Mol Biosyst. 2010; 11: 2206-13.

http://dx.doi.org/10.1039/c0mb00046a
PMid:20714643

18. Espinosa-Ramírez J, Pérez-Carrillo E, Serna-Saldívar SO. *Production of Lager Beers from Different Types of Sorghum Malts and Adjuncts Supplemented with beta-Amylase or Amyloglucosidase.* J Am Soc Brew Chem. 2013; 71(4): 208-13.

19. Guerdrum LJ, Bamforth CW. *Prolamin Levels Through Brewing and the Impact of Prolyl Endoproteinase.* J Am Soc Brew Chem. 2012; 70(1): 35-8.

20. Thompson T. *Is Barley-Based "Gluten-Removed" Beer Safe for People with Celiac Disease?* 2014.

http://www.glutenfreewatchdog.org. Accessed: April 2015.

21. Diaz-Amigo C, Popping B. *Accuracy of ELISA Detection Methods for Gluten and Reference Materials: A Realistic Assessment.* J Agric Food Chem. 2013; 61, 24: 5681-8.
http://dx.doi.org/10.1021/jf3046736
PMid:23713744

22. Tanner GJ, Blundell MJ, Colgrave ML, Howitt CA. *Quantification of Hordeins by ELISA: The Correct Standard Makes a Magnitude of Difference.* PLoS ONE. 2013; 8(2): e56456.
http://dx.doi.org/10.1371/journal.pone.0056456
PMid:23509607 PMCid:PMC3585327

23. Tanner GJ, Colgrave ML, Blundell MJ, Goswami HP, Howitt CA. *Measuring Hordein (Gluten) in Beer – A Comparison of ELISA and Mass Spectrometry.* PLoS ONE. 2013; 8(2): e56452.
http://dx.doi.org/10.1371/journal.pone.0056452
PMid:23509606 PMCid:PMC3585340

24. Ferre S, García E, Méndez E. *Measurement of hydrolysed gliadins by a competitive ELISA based on monoclonal antibody R5: analysis of syrups and beers.* Proceedings of the 18th Meeting Working Group on Prolamin Analysis and Toxicity. 2003; 65-70.

25. Tanner GJ, Colgrave L., Howitt CA. *Gluten, Celiac Disease, and Gluten Intolerance and the Impact of Gluten Minimization Treatments with Prolylendopeptidase on the Measurement of Gluten in Beer.* J Am Soc Brew Chem. 2014; 72: 36-50.
http://dx.doi.org/10.1094/ASBCJ-2014-0129-01

26. Van Landschoot A. *Gluten-free barley malt beers.* Cerevisia. 2011; 36: 93-7.
http://dx.doi.org/10.1016/j.cervis.2011.09.001

27. Siebert KJ. *Effects of proteinepolyphenol interactions on beverage haze, stabilization, and analysis.* J Agric Food Chem. 1999; 47: 353-62.
http://dx.doi.org/10.1021/jf980703o
PMid:10563900

28. Dostalek P, Hochel I, Mendez E, Hernando A, Gabrovska D *Immunochemical determination of gluten in malts and beers.* Food Addit Contam. 2006; 23: 1074-8.
http://dx.doi.org/10.1080/02652030600740637
PMid:17071509

29. Granato TM, Nasi A, Ferranti P, Iametti S, Bonomi F. *Fining white wine with plant proteins: effects of fining on proanthocyanidins and aroma components.* Eur Food Res Technol. 2014; 238: 265-74.
http://dx.doi.org/10.1007/s00217-013-2108-5

30. Simonato B, Mainente F, Tolin S, Pasini G. *Immunochemical and Mass Spectrometry Detection of Residual Proteins in Gluten Fined Red Wine.* J Agric Food Chem. 2011; 59: 3101-10.

http://dx.doi.org/10.1021/jf104490z
PMid:21375303

31. Regulation (EC) No 110/2008. *Definition, description, presentation, labelling and the protection of geographical indications of spirit drinks and repealing Council Regulation (EEC) No 1576/89. REGULATION (EC) No 110/2008 of the European Parliament and of the Council of 15 January 2008.* Official Journal of the European Union, L 39/16 (13.2.2008).

32. Agu RC, Bringhurst TA and Brosnan JM. *Production of Grain Whisky and Ethanol from Wheat, Maize and Other Cereals.* J Inst Brew. 2006; 112: 314-23.

http://dx.doi.org/10.1002/j.2050-0416.2006.tb00737.x

33. Martin S. *Against the grain: An overview of celiac disease.* J Am Acad Nurse Prac. 2008; 20: 243-50.

http://dx.doi.org/10.1111/j.1745-7599.2008.00314.x
PMid:18460164

34. Aylott, R. *Whisky analysis. In: Inge Russell and Graham Stewart editors. Whisky. Technology, Production and Marketing.* Second Edition. Elsevier, 2014: 243-70.

http://dx.doi.org/10.1016/B978-0-12-401735-1.00014-3

35. Colgrave ML, Goswami H, Howitt CA, Tanner GJ. *What is in a Beer? Proteomic Characterization and Relative Quantification of Hordein (Gluten) in Beer.* J Proteome Res. 2012; 11: 386-96.

http://dx.doi.org/10.1021/pr2008434
PMid:21999962

36. Thompson T, Mendez E. *Commercial assays to assess gluten content of gluten-free foods: why they are not created equal.* J Am Diet Assoc. 2008; 108: 1682-7.

http://dx.doi.org/10.1016/j.jada.2008.07.012
PMid:18926134

37. Codex Alimentarius (Internet). *Roma: Codex Alimentarius International Food Standards.* 2014 July.

http://www.codexalimentarius.org/standards. Accessed: February 2015.

38. Immer U, Göbwein Ch, Lauterbach SH. *Ridascreen® Gliadin competitive-New assay format for the R5 antibody.* Proceedings of the 22th Meeting Working Group on Prolamin Analysis and Toxicity. Dublin, Ireland. 2007; 45-52.

39. Skerritt JH, Hill AS. *Enzyme-immunoasay for determination of gluten in foods - collaborative study.* J AOAC Int. 1991; 74: 257-64.

40. Allred LK, Sealey Voyksner JA, Voyksner RD. *Evaluation of Qualitative and Quantitative Immunoassays To Detect Barley Contamination in Gluten-Free Beer with Confirmation Using LC/MS/MS.* J AOAC Int. 2014; 97(6): 1615-25.
http://dx.doi.org/10.5740/jaoacint.14-058
PMid:25313640

41. Colgrave ML, Hareshwar Goswamia H, Blundellb M, Howitt CA, Tanner GJ. *Using mass spectrometry to detect hydrolysed gluten in beer that is responsible for false negatives by ELISA.* J Chromatogr A. 2014; 1370: 105-14.
http://dx.doi.org/10.1016/j.chroma.2014.10.033
PMid:25454134

42. Morón B, Bethune MT, Comino I, Manyani H, Ferragud M, López MC et al. *Toward the assessment of food toxicity for celiac patients: characterization of monoclonal antibodies to a main immunogenic gluten peptide.* PLoS One: 2008; 3(5): e2294.
http://dx.doi.org/10.1371/journal.pone.0002294
PMid:18509534 PMCid:PMC2386552

43. Comino I, Real A, Moreno ML, Montes R, Cebolla A, Sousa C. *Immunological determination of gliadin 33-mer equivalent peptides in beers as a specific and practical analytical method to assess safety for celiac patients.* J Sci Food Agric. 2013; 93(4): 933-43.
http://dx.doi.org/10.1002/jsfa.5830
PMid:22886585

44. Real A, Comino I, Moreno ML, López-Casado MA, Lorite P, Torres I et al. *Identification and In Vitro Reactivity of Celiac Immunoactive Peptides in an Apparent Gluten-Free Beer.* PLoS ONE 2014; 9(6): e100917.
http://dx.doi.org/10.1371/journal.pone.0100917
PMid:24963630 PMCid:PMC4071002

45. Mujico JR, Dekking L, Kooy-Winkelaar Y, Verheijen R, van Wichen P, Streppel L et al. *Validation of a new enzyme-linked immunosorbent assay to detect the triggering proteins and peptides for celiac disease: interlaboratory study.* J AOAC Int. 2012; 95: 206-15.
http://dx.doi.org/10.5740/jaoacint.11-042
PMid:22468361

46. Mena MC, Lombardía M, Hernando A, Méndez E, Albar JP. *Comprehensive analysis of gluten in processed foods using a new extraction method and a competitive ELISA based on the R5 antibody.* Talanta. 2012; 91: 33-40.
http://dx.doi.org/10.1016/j.talanta.2011.12.073
PMid:22365676

47. Méndez E, Valdés I, Camafeita E. *Analysis of gluten in foods by MALDI- TOFMS.* Methods Mol Biol. 2000; 146: 355-67.
http://dx.doi.org/10.1385/1-59259-045-4:355

48. Grebe SKG, Singh RJ. *LC-MS/MS in the Clinical Laboratory – Where to From Here?* Clin Biochem Rev. 2011; 32: 5-31.

PMid:21451775 PMCid:PMC3052391

49. Mujico JR, Lombardia M, Mendez E. *Detection of wheat DNA in foods by a quantitative real-time* PCR *system: can the measurement of wheat DNA be used as a non-immunological and complementary tool in gluten technology?* Proceedings of the 18th Meeting of the WG on Prolamin Analysis and Toxicity. 2004: 91-8.

http://dx.doi.org/10.1016/j.foodchem.2011.03.061

50. Mujico JR, Lombardía M, Mena MC, Méndez E, Albar JP. *A highly sensitive real-time PCR system for quantification of wheat contamination in gluten-free food for celiac patients.* Food Chem. 2011; 128: 795-801.

http://dx.doi.org/10.1016/j.foodchem.2011.03.061

51. Churruca I, Lasa A, Miranda J, Fernández-Gil P, Simón E. *The usefulness of a complementary real-time polymerase chain reaction technique in the determination of gluten in foods.* Proceedings of the 23th Meeting of the WG on Prolamin Analysis and Toxicity. 2009: 59-67.

52. Guerdrum LJ, Bamforth CW. *Levels of gliadin in commercial beers.* Food Chem 2011; 129: 1783-4.

http://dx.doi.org/10.1016/j.foodchem.2011.06.021

53. Van Zandycke S. (Internet) *Gluten-reduced beers made with barley. The New Brewer.* 2013; 78-84.

http://www.brewersassociation.org/. Accessed: February 2015.

54. Kanerva P, Sontag-Strohm T. Lehtonen P. *Determination of Prolamins in Beers by ELISA and SDS-PAGE.* J Inst Brew. 2005; 111: 61-4.

http://dx.doi.org/10.1002/j.2050-0416.2005.tb00649.x

55. Alcohol and Tobacco Tax and Trade Bureau. *Interim Policy on Gluten Content Statements in the Labeling and Advertising of Wines, Distilled Spirits, and Malt Beverages. TTB Ruling.* Department of the treasury. Number 2-2012 (May 24, 2012).

56. Thompson T. *The Gluten-Free Labeling Rule: What Registered Dietitian Nutritionists Need to Know to Help Clients with Gluten-Related Disorders.* J Acad Nutr Diet. 2015; 115: 13-6.

http://dx.doi.org/10.1016/j.jand.2014.10.001
PMid:25534893

Chapter 20

Market and Nutrition Issues of Gluten-Free Foodstuff

Cristina M. Rosell[1], María Estela Matos[2]

[1] Institute of Agrochemistry and Food Technology (IATA-CSIC), Spain.

[2] Instituto de Ciencia y Tecnología de Alimentos (ICTA). Universidad Central de Venezuela, Venezuela.

crosell@iata.csic.es, mariaestelams@gmail.com

Doi: http://dx.doi.org/10.3926/oms.268

How to cite this chapter

Rosell CM, Matos ME. *Market and Nutrition Issues of Gluten-Free Foodstuff.* In Arranz E, Fernández-Bañares F, Rosell CM, Rodrigo L, Peña AS, editors. *Advances in the Understanding of Gluten Related Pathology and the Evolution of Gluten-Free Foods.* Barcelona, Spain: OmniaScience; 2015. p. 675-713.

Abstract

Nutritional therapy is currently the unique treatment for gluten intolerance. However, food technologists have been developing gluten free foods without having in mind both the nutritional status and nutrients' needs. It is important to consider that gluten intolerant patients do not have the same requirements when diagnosed than when they are fulfilling a long-life gluten free diet. Their needs are different at each stage, because of that diet might respond to their nutritional demands and be adapted. This chapter gives an overview of the nutritional pattern of gluten free intolerants at diagnosis, their requirements and how the currently marketed gluten free products meets those needs. In addition, this chapter reviews the tools that food technologist have for enriching the gluten free products, particularly bakery products, in macro- and micronutrients giving response to the consumers. It is also highlighted the role that nutritionist must play in this picture giving proper advice to consumers.

Keywords

Nutrition, gluten free, food, bread, bakery products, market, enrichment, micronutrients.

1. Introduction

Gluten free foodstuff development has attracted in the last decade great attention due to better diagnoses of celiac disease and common chatters about the relationship of gluten free products with healthiness. A few years ago, gluten-free products were virtually unheard of except in specialty health food stores. Whatever is the real motivation to consume gluten free foodstuff, nowadays a rising demand for gluten-free products is observed in the market trends. The market for gluten-free foods and beverages has continued to grow even faster than anticipated. 'Gluten-free' has become an identity for the tens of millions of Americans who have reduced or eliminated their consumption of wheat, barley, rye, and oats. While growth rates will be moderate over the next five years in the wake of market expansion, Packaged Facts projects that U.S. sales of gluten-free foods and beverages will exceed $6.6 billion by 2017 (Packaged Facts, 2011). Trend data shows the gluten-free target audience to be 44 million strong. North America is the largest market for gluten-free products accounting nearly 59% of the market share in 2012. Major demand in the market is anticipated to come from countries such as U.K., Italy, U.S., Spain, Germany, Australia, Brazil, Canada, India, etc. (http://www.marketsandmarkets.com). The increasing interest has promoted the launching of hundredth of gluten free foodstuff, being a niche market with steady growing shares.

Those trends have been accomplished by numerous research studies on the topic of developing gluten free breads, as recent reviews pointed out. Very recently, even a breeding strategy has shown very successful results obtaining reduced gliadin wheat breads with 97% lower gliadin content than wheat breads[1]. It has been estimated that celiac patients could safely consume 67 grams of low-gliadin bread per day.

Additionally, analytical methods for gluten detection have been an active area of debate pertaining immunochemical and non-immunochemical assays developed for gluten quantification, their sensitivity, specificity, cross-reaction

and their feasibility for testing gluten-free food consumed by patients with celiac disease[2,3].

Previous reviews showed that much research has been conducted on gluten free foods from different angles to obtain good quality gluten free foods. Nevertheless, nutrition quality of those products has been of interest only recently. In the last couple of years, the driven force of the gluten free research has been the nutrition quality. Very recently, Matos and Rosell[4] reviewed the different available strategies for improving the nutritional quality of gluten free breads.

This chapter will be focused primarily on presenting nutritional status of gluten intolerant population to define their nutritional requirements, and secondly on the nutritional quality of the existing gluten free food at scientific and commercial levels. Only by knowing the real needs of consumers it would be possible to design tailored made gluten free products for improving health status of gluten intolerant individuals.

2. Motivation to Consume Gluten Free Foodstuff

Increasing diagnoses of celiac disease and food allergies; growing awareness of these ailments among patients, healthcare practitioners, and the general public; the availability of more products, and better ones; and a trend that has friends and family members eating gluten-free to support loved ones are among the factors stimulating continuing expansion in this market (http://thegluten-freeagency.com/gluten-free-market-trends/). In recent years, an increasing number of individuals are suffering from celiac disease (CD). CD not only affects the gut, but is a systemic disease that may cause injury to the skin, liver, joints, brain, heart, and other organs. It is a complex genetic disorder, and human leukocyte antigen (HLA) status appears to be the strongest genetic determinant of risk for celiac autoimmunity[5].

Recently, Worosz & Wilson[6] described different types of consumers of gluten-free products, which are: persons who claim a gluten sensitivity or CD medical diagnostic, persons with perceived gluten-sensitivity, and consumers

who do not have CD but who express interest in gluten-free products as a lifestyle. Additionally, these authors defined two types of gluten-free consumerism: Ethical Consumerism describes consumption driven by the ways in which a product is perceived to fit into an individual's overall lifestyle, to benefit the environment, and /or to meet social goals; and Non-CD Health Consciousness are those who are "motivated to improve and/or maintain their health and quality of life". Consumers who do not have CD may express interest in gluten-free products as a lifestyle choice because it evokes a cultural-ecological-, civic-, historical-, ethical-, or health-based interest or quality[6]. Gluten-free has been described by consumers as: "a mainstream sensation, embraced by both out of necessity and as a personal choice toward achieving a healthier way to live".

3. Special Nutritional Requirements of the Celiac Patients

In CD patients, ingestion of gluten leads to inflammation and mucosal damage of the small intestine. The typical lesion in the small intestinal epithelium is villous atrophy with crypt hyperplasia, leading to malabsorption of most nutrients including iron, folic acid, calcium, and fat-soluble vitamins[7]. This can lead to associated diseases such as osteoporosis, anemia and type I diabetes and skin disorders. Individuals with celiac disease are more susceptible to pancreatic insufficiencies, dysbiosis, lactase insufficiencies, and folic acid, vitamin B12, iron, and vitamin D deficiencies, besides accelerated bone loss[8].

CD patients might show an alteration in lipid metabolism, for instance low serum total and high-density lipoprotein-cholesterol derived from lipid malabsorption and decreased intake[9]. Moreover, the exclusion of wheat, rye and barley, important vitamin and mineral sources, from the diet might provoke deficiencies in iron, vitamin B and dietary fiber. In fact, common nutrient deficiencies in celiac subjects at diagnosis are calorie/protein, fiber, iron, calcium, magnesium, vitamin D, zinc, folate, niacin, vitamin B12 and riboflavin[10].

Therefore, somewhat that might be surprising is that nutritional deficiencies are not only associated with poverty and developing countries, but also to population in developed countries who suffer from undiagnosed disease and those that must adhere to restricted diets like occurs with CD patients. Moreover, it has been identified the high frequency of underweight at diagnosis thus CD individuals might be in need of careful personalized nutritional management[11]. Health care counselors must be monitoring both growth and feeding patterns to identify unbalanced diets that may lead to nutritional deficiencies.

Recently, nutritional status of newly diagnosed adult CD-patients was analyzed in Netherlands[12]. Serum concentrations of folic acid, vitamin A, B6, B12, and D, zinc, haemoglobin and ferritin were determined and results showed that CD patients before gluten free diet compliance had at least one value below the lower limit of reference. The most frequent deficiencies were observed in zinc followed by iron, folic acid, vitamin B12, B6 and A.

4. Importance of Nutrition for Gluten Intolerant Patients

When following a gluten free diet does not respond to therapeutical counselling, nutritional unbalance might not be a problem. However, it is widely accepted that gluten intolerance therapy is restricted to gluten removal from the diet and uncertainty remains as to whether this gives a nutritionally balanced diet. In addition to that, it must be taken into account that individuals with CD may require additional nutritional supplementation to assist in regulation of several of these complications. Untreated CD individuals show reduced levels of iron, folate, vitamin B12, vitamin D, zinc, and magnesium; those deficiencies usually revert after gluten removal from the diet[13.] Nevertheless, folate and vitamin B12 deficiencies, and even vitamin D and calcium, may persist, being recommended the vitamin supplementation to meet healthy intake recommendations.

Lifelong adherence to gluten free diet as a treatment for gluten intolerant patients means complete exclusion of wheat and wheat containing products

from the diet, which poses huge challenges in terms of compliance. Gluten free diet is very effective and greatly improves nutritional status, inducing an increase in fat and bone compartments, but does not completely normalize body composition, and it might be very difficult to maintain. It is paramount that health care providers have a deep understanding of CD and the gluten free diet in order to educate patients and their families.

Primarily, it was set up that treatment of patients with a gluten-free diet was enough to treat them and keep them healthy. That measure involved selecting appropriate foods by omitting gluten-containing products. In general, clinical studies were only focused on the recovery of intestinal mucosa after removing gluten from the diet but no long dietary studies were conducted. The intestine heals with removal of gluten from the diet but the intolerance is permanent and the injury recurs if gluten is introduced again. Several evaluations of the dietary intake of CD individuals on gluten free diet have been reported to estimate the convenience of the nutrient intakes. In children with diagnosed CD and on gluten free diet similar pattern to healthy children were observed pertaining dietary intakes of energy and nutrients, differences were observed in the lower intake of vitamin D, riboflavin, niacin, thiamine, magnesium and selenium among CD children and their higher intake of iron and calcium[14]. In spite of caution is necessary when analyzing dietary registrations in teenagers, results suggested that adolescents on gluten free diet have higher intake of saturated fatty acids and sucrose and lower intake of dietary fiber than healthy adolescents on a gluten containing diet. In general, CD individuals have a tendency to compensate for the restrictions of a gluten-free diet by eating foods containing high levels of fat, sugar and calories, because of that they may show an excessive consumption of total fats and saturated fats. Mariani et al.[15] reported that diet of CD adolescent patients was hyperproteic and hyperlipidic and contained low amounts of carbohydrates, iron, calcium, and fiber.

A decade ago, Hallert et al.[16] investigated the vitamin status in celiac patients on a gluten-free diet for 10 years by using a 4-day food record. Results showed that the daily intakes of folate and vitamin B12 were

significantly lower in celiac patients, which may have clinical implications considering the linkage between vitamin deficiency, elevated total plasma homocysteine levels and cardiovascular disease. Haapalahti et al.[17] reported that one third of screen-detected CD adolescent had lower median values of blood folic acid besides low iron status (transferrin receptor-ferritin index) and although no association was found between the nutritional status and the markers of mucosal injury (villous-crypt measures), the level of transglutaminase was associated with whole blood folic acid and with transferrin receptor-ferritin index.

Later on, Shepherd and Gibson[18] analyzed a seven-day prospective food intake in 55 patients adhered to gluten free diet for more than 2 years and concomitantly in 50 newly-diagnosed, revealing similar nutritional intake between groups. However, differences were observed in the macronutrients intake, for instance starch intake decreased after 12 months under the diet, and fiber intake was inadequate for all CD individuals except males with long term under the diet. Newly-diagnosed and experienced patients showed deficiencies in thiamin, folate, magnesium, calcium and iron (females) or zinc (males). According to these authors, dietary deficiencies after short-time adhered to gluten free diet were similar to those after long term adherence to this diet.

A study carried out in Germany with 1,000 patients by recording a prospective 7-day food diary and a questionnaire revealed that male celiac patients showed no significant difference for the intake of energy and macronutrients compared to healthy individuals, although lower fiber intake was detected[19]. Regarding female patients, they showed higher fat intake and lower carbohydrate consumption. Both genders evidenced deficiencies of vitamin B1, B2, B6, folic acid, magnesium and iron. Similarly, in Sweden a study was conducted among 13-year old diagnosed with CD in early childhood with those of a non-celiac to compare their energy and nutrient intakes[20]. Dietary intake was assessed using a food-frequency questionnaire during 4 weeks. Most adolescents recorded an intake above requirements for most nutrients, with the exception of vitamin C, and thiamine, but the later only in

the CD boys. Regarding fatty acids, they showed a high intake of saturated fats and a low intake of unsaturated fats. Girls and boys in the CD-group had an overall lower nutrient density compared to the healthy group.

During the first few years of treatment it seems that celiac patients have a nutritionally adequate diet. But after several years of dietary compliance some deficiencies have been detected, that should force food processors and nutritionist to adequate food composition and diet, respectively, to prevent those deficiencies and in consequence the risk to suffer some ailments associated to them.

4.1. Nutritional Therapy Facing Daily Shopping

Commercial gluten free foods are available in all the countries but they are very expensive and sometimes difficult to find. In fact, a qualitative study carried out in 2007 with 15 households confirmed the additional domestic costs for households with a member suffering from celiac disease[21]. Later on, Singh and Whelan[22] confirmed the fact that cost and availability of gluten-free products might be the main cause of incomplete dietary compliance. Those authors investigated the availability and cost of 20 gluten-free foods (including branded gluten-free and cheapest gluten-free) across 30 different stores; results indicated limited availability of gluten-free foods (41% foods being available in a gluten-free version per store, and no gluten free products were present in convenience store) and gluten-free foods were more costly than their gluten containing counterparts. Increasing availability and affordability of gluten-free foods may improve diet compliance.

Other aspect is the quality of the marketed gluten free products perceived by consumers. In Latvia, a survey was conducted from December 2010 till the end of July 2011 to find the opinion about quality of gluten-free products in this country, showing that the quality of gluten-free flour, flour blends and pasta was acceptable but no the quality of bread and confectionery, which required considerable technological improvement[23,24].

In some countries that fact limits the accessibility to those products leading to celiac children being anorexic and malnourished. Those states will need high calorie and high protein diets.

On the other hand, up to a few years ago gluten free products were somewhat limited in the market, thus CD patients had limited choice of food products and they consumed excessively packaged gluten-free products, such as snacks and biscuits with a high intake of lipids[10].

4.2. A Unique Nutritional Therapy or Adapted to Age Range?

It is clear that each age range or special human states might have specific nutritional requirements thus a tailored meal plan would be advisable, which consider the likes and dislikes of the person, the socio-economic condition, and the life style to ensure adequate intake of all nutrients. Studies conducted in adults and children show that approximately 20%-38% of patients with CD have nutritional complications, such as calorie/protein imbalance, dietary fiber, mineral and vitamin deficiencies likely to be caused either by the poor nutritional quality of the gluten free products or the incorrect alimentary choices of CD patients.

Children should be aware that according to dietary reference intake values recommended distribution of daily calorie intake for a healthy and balanced diet should be 55% from complex and simple carbohydrates, 15% from dietary protein and 25%–30% or less from lipids[25]. The adequacy of the diet is of really significant importance in children, because it is the age of maximal energy and nutrient requirements for growth, development and activity.

Moreover, compliance with the gluten free diet becomes difficult for adolescents. In fact, Altobelli et al.[26] reported that at least one third interviewed teenagers reported feeling angry “always” or “most of the time” about having to follow the gluten free diet. Therefore, health professionals must take special care to identify adolescents with major disease-related problems.

Lately, a very interesting study was reported for confirming the adherence to a gluten free diet by measuring the exhaled breath of healthy individuals after being adhered to gluten free diet for four weeks[27]. Twelve volatile compounds were associated to gluten-free diet and only seven could be chemically identified as 2-butanol, octane, 2-propyl-1pentanol, nonanal, dihydro-4-methyl-2(3H)-furanone, nonanoic acid and dodecanal.

5. Nutritional Quality of Gluten Free Products

Medical nutrition therapy is defined as specially processed or formulated foods that are used for the dietary management of patients. Amongst the medical foods, low-protein/protein-free foods have improved the physical manifestation of metabolic disorders in patients with amino acid or protein-related diseases, such as Phenylketonuria, Tyrosinaemia type I, as well as celiac. Most of the cereal-based gluten free foods currently marketed are a blend of refined or chemically-based food ingredients with unpalatable, frequently artificial flavors. Despite the numerous advances in the development of gluten free products resembling the quality of their gluten containing counterparts, to date there is no one raw material or defined ingredient to effectively replaced gluten. Scientific papers reached the conclusion that the combination of modified or functional starches or flours, with hydrocolloids and supplemented with fibers, proteins and co-texturizers is the best alternative to obtain gluten free products[28].

The production of protein free cereal foods is a technological challenge. Studies on gluten free products, particularly bread, have been concentrated on improving technological parameters (volume, crumb hardness, and so on) besides sensorial perception. However, the nutritional concept of the gluten free baked goods has been scarcely addressed. Historically, nutrition counselling for celiac disease has focused on the foods to avoid gluten in the diet. But some bells of alarm sounded after[29] survey showing the nutrient intakes and food consumption patterns of adults with celiac disease who adhere to a strict gluten-free diet. These authors compiled the three-day

estimated self-reported food records of forty-seven volunteers to assess daily intakes of calories, percent daily calories from carbohydrates, dietary fiber, iron, calcium and grain food servings. Recommended daily amounts of fiber, iron and calcium were met by 46, 44 and 31% of women and 88, 100 and 63% of men, respectively.

There is growing concern over the nutritional adequacy of the GF dietary pattern because it is often characterized by an excessive consumption of fats and reduced intake of complex carbohydrates, dietary fiber, vitamins and minerals[30,31].

Some nutritional aspects of selected commercial gluten-free products including breads have pointed out the nutritionally variability of gluten free products. Matos and Rosell[31] evaluate the nutritional pattern of gluten-free breads representative of the Spanish market for this type of products (Table 1). In general, authors found that the protein, fat and mineral content of the gluten-free breads showed great variation, ranging from 0.90 to 15.5 g/100g, 2.00 to 26.1 g/100g and 1.10 to 5.43 g/100g, respectively; and as consequence had very low contribution to the recommended daily protein intake, and a high contribution to the carbohydrate dietary reference intake. Additionally, dietary fiber content showed great variation (1.30 to 7.20 g/100 g). Mentioned authors suggested that gluten-free breads showed great variation in the nutrient composition, being starchy based foods low in proteins and high in fat content. Fat composition of gluten free products is of great concern because they contained trans fatty acids that may provoke metabolic imbalance when combined with inadequate intake of essential fatty acids[10]. A high intake of dietary lipids is a risk factor in the development of coronary heart disease and obesity[32].

Table 1. Proximate composition of different gluten free breads reported in the scientific literature.

Reference	Raw material	Chemical composition (g/100g, dm)					Dietary fiber (g/100g, dm)		
		Protein	Fat	Minerals (Ash)	Fibers	Total carbohydrate (*)	TDF	SDF	IDF
Pagliarini et al.[33] (1)	Maize starch	2.3	5.4	3.9	7.4	81.0	nr	nr	nr
	Maize starch, rice flour	3.5	14.0	Nr	nr	81.1	nr	nr	nr
	Rice flour, rice starch, tapioca starch	4.2	11.4	1.9	4.9	77.6	nr	nr	nr
	Maize starch, rice flour	5.3	9.1	2.4	10.9	72.3	nr	nr	nr
	Maize starch, rice flour	6.0	9.1	Nr	nr	66.4	nr	nr	nr
	Maize starch, rice starch, rice flour	4.3	8.3	2.2	4.5	80.7	nr	nr	nr
Matos and Rosell[31] (2)	Maize starch; egg	3.16	8.51	2.12	nr	86.21	9.69	5.79	3.9
	Maize starch; egg	6.94	16.91	1.10	nr	75.05	5.00	3.08	1.92
	Maize starch; egg	7.31	16.56	1.66	nr	74.47	1.83	0.65	1.18
	Potato starch, maize starch; casein, soy protein	15.05	7.33	1.85	nr	75.76	6.72	1.14	5.58
	Maize starch; egg	5.13	10.64	2.01	nr	82.22	5.1	3.62	1.49
	Maize starch, rice flour; lupine protein	4.92	4.86	2.03	nr	88.18	5.32	3.09	2.22
	Maize starch; egg	3.96	8.28	4.53	nr	83.22	9.37	5.2	4.17
	Maize starch	1.01	2.00	4.03	nr	92.96	2.33	1.07	1.26
	Maize starch	0.91	2.03	5.43	nr	91.63	6.96	2.02	4.94
	Maize starch	1.91	26.10	3.57	nr	68.42	8.22	6.1	2.11
	Maize starch	2.08	18.32	3.98	nr	74.91	8.53	6.94	1.59

Reference	Raw material	Chemical composition (g/100g, dm)					Dietary fiber (g/100g, dm)		
		Protein	Fat	Minerals (Ash)	Fibers	Total carbohydrate (*)	TDF	SDF	IDF
Matos and Rosell[34]	Commercial gf blend	3.30	0.97	1.37	nr	94.36	nr	nr	nr
	Rice flour	7.57	3.40	1.13	nr	87.90	nr	nr	nr
	Rice flour	7.10	3.70	1.31	nr	87.89	nr	nr	nr
	Rice flour, maize starch, potato starch	14.97	0.20	1.47	nr	83.36	nr	nr	nr
	Rice flour, maize starch, potato starch	3.63	1.87	1.03	nr	93.47	nr	nr	nr
	Rice flour, maize starch, potato starch	12.33	9.57	1.46	nr	76.54	nr	nr	nr
	Rice flour, potato starch	7.43	4.77	1.41	nr	86.39	nr	nr	nr
Phimolsiripol et al.[35]	GF-wheat starch, rice flour (control)	5.54	nr	Nr	nr	nr	2.29	0.10	2.19
	GF-wheat starch, rice flour+ rice bran	6.08	nr	Nr	nr	nr	2.46	0.66	1.80
	GF-wheat starch, rice flour+ rice bran	7.58	nr	Nr	nr	nr	5.97	0.58	5.39
	GF-wheat starch, rice flour+ rice bran	6.87	nr	Nr	nr	nr	4.44	0.40	4.05
	GF-wheat starch, rice flour+ rice bran	6.73	nr	Nr	nr	nr	5.03	0.71	4.32
Krupa-Kozak et al.[36]	GF-blend	2.52	1.90	3.03	nr	nr	2.29	0.10	2.19
	GF-blend + calcium caseinate	14.23	1.37	3.52	nr	nr	2.46	0.66	1.80
	GF-blend+ sodium caseinate	14.68	0.67	3.12	nr	nr	5.97	0.58	5.39
	GF-blend + whey proteins hydrolyzate	13.65	1.38	3.28	nr	nr	4.44	0.40	4.05
	GF-blend + whey protein isolate	13.47	13.47	1.74	nr	nr	5.03	0.71	4.32

dm: dry matter; nr: not reported.
(*) Total Carbohydrate (d.b) by difference: 100 – (weight in grams [protein + fat + ash] in 100 g of food) (FAO, 2003).
(1) Nutritional value reported on label (Commercial gluten-free breads samples, according to suppliers' informations).
(2) Commercial gluten free samples.

5.1. Alternatives for Improving Nutritionally Gluten Free Breads

Lately, gluten free formulations are being set up considering the nutritional quality of the final gluten-free baked products. The most common strategy to increase the nutritional value of gluten-free breads is to include nutritionally valued raw flours. Although those flours are often presented as new crops and raw material, they have been used by local populations in traditional ways for many centuries. Consequently, their innovation is rather related to the ways in which old and new uses are being readdressed[37]. Non-traditional flours such as pseudocereals flours (amaranth, quinoa and buckwheat), root and tubers flours (such as potato, cassava, sweet potato and edible aroids: taro and yams), and leguminous flours (chickpeas, lentils, dry beans, peas, and soybean)[38] are gaining popularity in the production of gluten-free foodstuff with major nutritional quality (Table 2). Amaranth is rich in lipids, proteins, carbohydrates, and dietary fiber and other constituents, such as squalene, tocopherols, phenolic compounds, phytates, and vitamins[39].

Vitali et al.[43] compared the nutrient composition of eleven raw materials (carob, soy flour, amaranth, orange sweet potato, red sweet potato, red quinoa, buckwheat, maize, rice flour and chickpea) (Table 2) apt to be used in gluten free diet. Those authors reported that fat content ranged from 0.53 g/100 g dry mater (d.m.) in red sweet potato up to 24.19 g/100 g dm in soy flour, being specially high in red quinoa, amaranth, and chickpea (6.39, 6.28, and 5.84 g/100 g dm, respectively). Protein content of the flours ranged from 5.38 g/100 g dm in carob up to 41.47 g/100 g dm in soy flour. Regarding carbohydrate content, flours could be clustered in three groups comprising low carbohydrate content (soy flour –10.64 g/100 g dm), moderate carbohydrate content ranging from 48.12 g/100 g dm to 68.56 g/100 g dm (wheat flour, carob, amaranth, buckwheat, maize, rice, and chickpea), and very high carbohydrate content (> 80g/100 g dm) observed in sweet potato flours and red quinoa. Essential mineral and dietary fiber contents were significantly higher than those in wheat flour[43]. Additionally, chickpea, rice flour, maize, quinoa, and different types of sweet potato flour are sources of resistant starch

and carob, soy, buckwheat, and sweet potato contain antioxidants that have been related to numerous health benefits in humans. Established the different proximate composition of those flours, Hager et al.[41] compared the nutritional quality of commercial gluten free flours made from teff, sorghum, maize, quinoa, buckwheat, oat and rice, showing that maize and rice flour are poor regarding their nutritional value (low protein, fibre, folate contents), whereas teff and pseudocereals like quinoa and buckwheat based flours presented favorable fatty acid composition and are high in protein and folate. Quinoa and teff gluten free based blends had the additional benefit of having high fiber and mineral (calcium, magnesium and iron) content.

Table 2. Nutritional composition of different gluten free breads reported in the scientific literature.

Reference	Raw material	Chemical composition (g/100g, dm)				
		Protein	Fat	Minerals (Ash)	Fibers	Total carbohydrate
	Wheat flour	9.00-13.00	1.00-1.05	0.5	0.40-1.20	
	Corn flour	6.90-13.00	4	1.00-1.40	3.00-4.00	65.00-80.00
	Rice flour	6.14-7.30	0.45-2.44	0.40-0.60	0.70-0.80	68.00-90.00
Vitali et al.[40]	Carob	5.38	0.56		43.45	
	Soy flour	41.47	24.19		26.07	10.45
	Amaranth	12	6.28		17.08	55.83
	Red quinoa	14.32	6.39		18.26	83.38
	Buckwheat	11.6	2.25		23.42	57.5
	Orange sweet potato	12.25	1.1		21.89	
	Red sweet potato	7.79	0.53		19.8	
Hager et al.[41]	Oat	6.91	6.74	0.82	4.05	
	Quinoa	13.48	8.59	2.43	7.14	
	Buckwheat	12.19	4.21	1.65	2.18	
	Sorghum	4.68	3.50	0.97	4.51	
	Teff	12.84	4.39	2.15	4.54	
Rai et al.[42]	Sorghum flour	13.34	4.56	2.51	2.62	79.59
	Pearl millet	13.11	5.13	1.76	1.37	80.00

Particularly, pseudocereal flours such as buckwheat[41,44-48], amaranth[49], and quinoa[41,50] have been used in several formulations. Pseudocereals like amaranth, quinoa and buckwheat have been used as healthy ingredients for improving the nutritional quality of gluten-free breads, leading to high levels of protein, fat, fiber and minerals[51]. In order to have a better picture about the nutritional quality of the numerous bread formulations proposed in the scientific literature a comparison table is included Table 1.

Cabrera-Chavez et al.[52] obtained a significant increase in protein, fat, minerals and dietary fiber content when rice based pasta was enriched with amaranth flour (Table 3). Gluten free cookies made with blends of the following alternate flours, rice, maize, sorghum and pearl millet had higher nutritional value than the ones obtained with wheat[42].

Table 3. Nutritional composition of other gluten free products reported in the scientific literature.

Reference	**GF products**	**Raw materials**	**Chemical composition (g/100g, dm)**					
			Protein	**Fat**	**Ash**	**Fiber**	**Available carbohydrate**	**Total carbohydrates (*)**
Gularte et al.[53]	Layer cake (control)	Rice flour	6.2	13.0	1.7	1.51	54.3	nr
	Layer cake+ fiber	Rice flour+ oat fiber+ guar gum	5.4	13.5	1.7	7.90	48.1	nr
	Layer cake+ fiber	Rice flour + oat fiber+ inulin	5.5	13.6	1.8	7.90	48.0	nr
	Layer cake+ fiber	Rice flour + oat fiber	5.5	13.2	1.8	8.60	47.6	nr
	Layer cake+ fiber	Rice flour + inulin	5.4	12.8	1.4	2.50	54.5	nr
Gularte et al.[54]	Layer cake + chickpea	Rice flour+ chickpea flour	9.3	14.3	2.2	1.4	45.6	
	Layer cake + pea	Rice flour+ pea flour	8.7	13.7	2.0	2.3	46.2	
	Layer cake + lentil	Rice flour+ lentil flour	9.1	13.8	2.0	2.8	46.0	

Reference	GF products	Raw materials	Chemical composition (g/100g, dm)					
			Protein	Fat	Ash	Fiber	Available carbohydrate	Total carbohydrates (*)
	Layer cake + bean	Rice flour+ bean flour	9.4	13.5	2.2	2.5	45.5	
Cabrera-Chávez et al.[52]	Pasta (control)	Rice flour, amataranth flour	12.9	2.9	1.3	5.5	nr	82.9
	Pasta (flour treatment)	Rice flour, amataranth flour	12.9	3.0	1.3	5.9	nr	82.7
	Pasta (flour treatment)	Rice flour, amataranth flour	12.6	2.9	1.3	5.9	nr	83.1
	Pasta	Rice flour	10.7	0.4	0.9	3.2	nr	87.9
	Pasta	Rice flour	10.0	0.4	1.0	3.0	nr	88.7

dm: dry matter; nr: not reported.
(*)Total Carbohydrate (d.m) by difference: 100 – (weight in grams [protein + fat + ash] in 100 g of food) (FAO, 2003).

Amaranth has been used for producing other convenience gluten free foods like snack bars[55]. Those bars have been also enriched with fructans like inulin and oligofructose for being considered prebiotic ingredients. Amaranth based bars had very good acceptance besides the nutritional advantages of caloric reduction and higher levels of dietary fiber as compared to commercial cereal bars.

Lee et al.[56] set up an alternative gluten free dietary pattern that replaced grains and starches in a 'standard' gluten-free dietary pattern defined from a retrospective review of diet history records of celiac patients. The proposed alternative diet contained oats, high fiber gluten-free bread and quinoa and by that way a significant increase in the protein content, iron, calcium and fiber, besides the B vitamin content (riboflavin, niacin and folate).

Other raw materials such as sorghum flour[41,57-59], carob germ flour[60], chestnut flour[61,62], tigernut flour[63] and teff flour[41,57] have also been used as innovative gluten-free raw materials; and generally, gluten-free breads of good quality have been obtained when optimized breadmaking recipe. The nutritional quality of flour made from pseudocereals or teff is better than that

of wheat flour, but their breadmaking properties and sensory characteristic compromise their suitability for the production of gluten-free bread somewhat.

Gambus et al.[64] tested the nutritional effect of flaxseed (also known as linseed) meal, amaranth and/or buckwheat on the quality of gluten free confectionery products obtaining an increase in the protein content, although considering the amino acid composition amaranth would be the elected commodity. Those flours also gave gluten free products with higher dietary fiber and in the case of linseed meal also an enhancement in the alpha-linolenic acid. Besides the effect on the macronutrients content, products contained more microelements (potassium, phosphorus, magnesium, calcium, iron, manganese, zinc and copper). Gluten free rolls were also prepared containing 10% of ground flaxseed without affecting the technological quality of the rolls, but they significantly increased the content of proteins, fat (including alpha-linolenic acid), mineral compounds, dietary fiber and phytates[65].

Gluten-free cakes made of maize starch and rice flour (1:1) of acceptable sensory quality have been obtained when replaced by 30% debittered lupin flour with the additional benefit that lupin increases the protein, calcium, iron, manganese, phosphorus and zinc contents of the cakes[66]. Other alternative for making healthy gluten free products is the use of green banana, sub-product of low commercial value and little industrial use, which has been revealed as an innovative raw material with many benefits to the food industry and consumers who are on a gluten-free diet[67]. When gluten free pasta was made with green banana flour the resulting product was greatly accepted by CD consumers and this type of pasta had 98% less lipids[68]. In addition, some by-products from the agri-industry have been also used as nutrients sources. For instance cassava generates high volume of waste like cassava hull, which after being dehydrated and milled has been incorporated in the formulation of gluten free cakes replacing rice flour[69]. A progressive increasing amount of substitution up to 100% increased the contents of ash (3.1 to 4.8 g/100g), lipids (8.6 to 16.7 g/100g) and total (4.1 to 19.3 g/100g)

and insoluble dietary fibers (3.5 to 17.3 g/100g). Even acceptable sensory cakes were obtained with 100% cassava peel flour.

Next to the use of new raw materials, protein enrichment has gained interest and with that aim soy protein isolates and also legume flours or legume protein isolates have been incorporated (Marco & Rosell, 2008a, b; Matos & Rosell, 2012b; Ziobro et. al., 2013a; Storck et al., 2013). Generally, the enrichment of gluten free bread in proteins leads to a decrease in both the specific volume and the crumb softness, but despite the detrimental effect on the instrumental quality parameters the nutritional impact was readily evident. For instance, gluten free cakes, when enriched with legume flours (rice flour/legume flour, 50:50) like chickpea, pea, lentil and bean increased their protein content in 30% (Table 3), and in less extension the fat, minerals and dietary fiber content, with except in the case of chickpeas[38].

Gluten free cracker snacks made of pulse fractions (chickpea, green and red lentil, yellow pea, pinto and navy bean flours and pea protein, starch and fiber isolates) giving similar physical characteristics and consumer acceptance to marketed products[70]. Interestingly, the nutritional composition of the crackers was also similar to the commercial cracker with the exception of the % daily values per serving of iron in the chickpea crackers that were 3-6 times higher.

Also protein enrichment has been carried out with proteins from animal sources, like dairy or eggs proteins. Krupa-Kozak et al.[36] tested the effect of different low-lactose dairy proteins (12%) on the quality of gluten free breads (Table 1). Those authors obtained gluten-free breads rich in proteins, and, regarding the energy value delivered by proteins, they could be considered as a source of proteins or high in proteins, because they provided around 15% of the energy. Considering the European Parliament regulation on nutrition and health claims made on foods, a claim that a food is a source of protein may only be applied to food product where at least 12% of the energy value of the food is provided by protein, thus those breads could be labeled as source of proteins.

In addition, gluten free foodstuff has not been immune to the new trends in baked products pertaining fiber enrichment. According to the American Dietetic Association the recommended fiber intakes for adults range from 25 to 30 g/day and the ratio of insoluble dietary fiber and soluble dietary fiber should be 3[71]. Physiological effects of soluble and insoluble fibers are different, while insoluble dietary fiber health benefits are related to intestinal regulation and water absorption, soluble dietary fiber benefits are associated with cholesterol lowering and improved diabetic control and to moderate postprandial glycaemic responses. Matos et al.[31] reported that the fiber content of commercial gluten free breads ranged from 1.30 to 7.20 g/100g, which indicates the great variability in nutritional composition of those products. Cereal fibers from wheat, maize, oat and barley have been used for enriching gluten-free bread formulation based on maize starch, rice flour and hydroxypropylmethylcellulose (HPMC)[72]. Adding those fibers at 3, 6 and 9 g/100 g level led to breads with higher fiber content. At the 9 g/100 g level of inclusion breads contained 7g/100 g dietary fiber, thus they can be labeled as rich in fiber, but acceptance decreased significantly due to their powdery taste. Even different fractions of rice bran, especially those with greater proportion of soluble dietary fiber, have been supplemented up to 10% to gluten free breads improving the quality, particularly darker color of crust, higher specific volume and softer crumb firmness was obtained[35] (Table 1). Similarly, gluten free layer cakes have been enriched with soluble and insoluble fibers like inulin and oat fiber, respectively (Table 3)[53]. Fibers significantly affected the *in vitro* hydrolysis of starch fractions, being the most pronounced effect the decrease in the slowly digestible starch. Overall, combination of oat fiber–inulin resulted in better gluten-free cakes.

Pre-gelatinized flour made from cassava starch and cassava bagasse (70:30), cassava starch and amaranth flour have been blended in a proportion 10:60:30 respectively, to obtain fiber enriched gluten-free pasta containing 9.37 g/100 g dietary fiber[73].

Fiber sources such as rice bran (Phimolsiripol et al., 2012) and inulin (Krupa-Kozak et al., 2012; Phimolsiripol et al., 2013), have been considered in

gluten-free breads development with the consequent improvement in the nutritional quality. Very recently, Psillium gum and sugar beet fibers have been added to gluten free breads (Cappa et al., 2013), and water adsorption must be adapted due to the fibers water binding ability. Those fibers improved the workability of the doughs, but mainly Psillium thanks to its film forming ability contributed to bread development and had more effective anti-staling result.

Nevertheless, although numerous scientific studies have reported the alternatives for improving nutritionally the gluten free products, industry has not really incorporated that new knowledge into marketed products. In fact, do Nascimento et al.[74] analyzed the labels of 324 products including gluten free products and their gluten containing counterparts. They confirmed the short variety of gluten free products, and that raw materials used were reduced to five types of flours: rice, cassava, maize, soy, and potato; but the presence of pseudocereals, suggested in scientific literature, was not evident.

5.2. Enrichment of Gluten Free Products with Minerals and Vitamins

The level of micronutrients in gluten free breads has been also a point of attention. Suliburska et al.[75] determined the content and release of minerals (Ca, Mg, Fe, Zn and Cu) from selected gluten-free products (bread, biscuits, pasta, maize porridge and peas puff) of the Poland market (Table 4). Results showed that the content of minerals varied considerably among the types of products, and it was relatively low. Among the analyzed products bread was characterized by a high content of calcium and zinc, and relative high content of magnesium. However, bread showed the lowest content of iron and copper. Moreover, the potential bioavailability of minerals from gluten-free products was in the range 10-70%, and it depended on the element and the composition of the analyzed product. Authors concluded that it should be consider the enrichment of gluten-free products in minerals.

Table 4. Content of minerals in gluten free food products formulations reported in the scientific literature.

Reference	GF products	Main raw material used	Content of minerals (mg/100g, md)				
			Ca	Mg	Fe	Zn	Cu
Krupa-Kozak et al.[76] (*)	GF Bread (control)	Maize starch, potato starch; inulin	15	nr	nr	nr	nr
	Calcium carbonate	Maize starch, potato starch; inulin + calcium carbonate	1.085	nr	nr	nr	nr
	Calcium chloride	Maize starch, potato starch; inulin + calcium chloride	1.052	nr	nr	nr	nr
	Calcium citrate	Maize starch, potato starch; inulin + calcium citrate	1.088	nr	nr	nr	nr
	Calcium lactate	Maize starch, potato starch; inulin + calcium lactate	1.121	nr	nr	nr	nr
Cabrera-Chávez et al.[52]	Pasta (control)	Rice flour, amaranth flour	29.6	nr	7.5	7.1	
	Pasta (flour treatment)	Rice flour, amaranth flour	29.9	nr	7.6	7.3	
	Pasta (flour treatment)	Rice flour, amaranth flour	28.8	nr	7.6	7.2	
	Pasta	Rice flour	3.6	nr	1.6	0.7	
	Pasta	Rice flour	3.6	nr	1.7	0.7	
Suliburska et al.[75] (*)	Bread	Maize starch	44.62	31.40	1.14	2.46	0.07
	Pasta	Maize starch, pea protein isolate	18.96	19.70	2.66	1.75	0.41
	Corn porridge	Maize porridge	3.43	33.10	1.29	1.63	0.09
	Peas puff	Maize starch, g-f wheat starch, egg	45.8	13.61	1.85	6.37	0.18
	Biscuits	Maize starch, potato starch, g-f wheat starch	25.70	15.73	1.40	0.83	0.08

dm: dry matter; nr: not reported.
(*) selected commercial gluten-free products.

In fact, that point has been addressed in several researches dealing with the supplementation of vitamins and minerals in gluten free breads. Kiskini et al.[77] studied the feasibility of produced gluten-free bread fortified with iron using selected iron compounds. The most acceptable products were those fortified with ferric pyrophosphate, which showed satisfactory sensory and

nutritional characteristics. Buckwheat flour incorporation (10-40%) is also a way to enrich gluten free breads especially in copper and manganese[78]. A more recent research carried out by Krupa-Kozak et al.[76] was focused on the fortification of gluten-free bread containing inulin, with different organic and non-organic calcium sources (Table 4). All experimental breads were significantly richer in calcium compared to the control, confirming the fortification. Additionally, sensory evaluation of the calcium-fortified breads confirmed that calcium carbonate was the most recommended salt for obtaining calcium fortification of gluten-free breads.

It must be stressed that even folate enrichment should be strongly considered after being concluded in different studies that celiac patients adhering to gluten-free diet show low folate intake and suboptimal folate and vitamin B12, possibly due to low folate content in gluten-free products[79].

Good glycemic control is particularly important in celiac disease, as there appears to be a higher incidence of type I diabetes among CD patients[80]. However, limited studies have been focused on assessing the glycemic index (GI) of the gluten-free products[31,80,81]. Overall, pseudocereals such as quinoa and amaranth have shown some hypoglycaemic effects, and have been recommended as an alternative to traditional ingredients in the formulation of cereal-based gluten-free products with low GI[80] 2004; Alvarez-Jubete et al., 2010a). Contrarily, starch-based gluten-free breads have shown estimated glycaemic index values between 83.3 and 96.1, thus this type of breads could be considered as food with high glycaemic index[31]. Therefore, it is necessary to choose suitable materials when formulating gluten-free products to reduce their GI. For instance, fresh egg pasta when made of oat or teff decreased significantly the GI compared to that of wheat pasta, showing similar sensory properties, although taste of pasta made with teff required some additional improvement[82].

The inclusion of prebiotic inulin-type fructans has been reported as one alternative for decreasing the glycaemic index of gluten free breads, even one-third of these fructans is lost during baking[83]. The addition of 12% of these fructans give breads enriched with 8% dietary fiber (4 g of fructans per

50 g bread serving size), and with a glycaemic index and glycaemic load of 48 and 8, respectively, in front of 71 GI and 12 glycaemic load obtained in the absence of this prebiotic. Similarly, dietary fiber supplementation to gluten free layer cakes affected the *in vitro* hydrolysis of starch fractions (related to glycaemic index), inducing mainly a decrease in the slowly digestible starch[53].

Besides the recipes modification for controlling the glycaemic index, other researchers propose to control the raw materials particle size or in the case of the gluten free cereals to control the varieties[84]. In the case of rice flour, particle size heterogeneity is responsible of different pattern in starch enzymatic hydrolysis, and also this effect is grain type dependent. Flour from long grain rice undergoes lower enzymatic hydrolysis[84]. Even breadmaking process can effectively modulate the starch digestibility of the baked rice based gluten free breads[85]. Coarse rice flour united to low hydration during mixing was the most suitable combination to limit starch gelatinization and hindered the *in vitro* starch digestibility[85].

5.3. Other Alternatives for Nutritionally Improving Gluten Free Foods

Additionally to different ingredients and additives, an interesting way to improve the nutritional quality of gluten free foods is to use sourdoughs. This is an ancient practice in breadmaking of wheat based products, where sourdough has been used to help fermentation and also to improve texture, palatability, aroma, nutritional properties and shelf life. Sourdough fermentation promotes mainly acidification and proteolysis releasing multiple microbial metabolites, which are responsible of the bread quality improvement. Nevertheless, this practice is lately being extended to gluten free products because besides the before mentioned benefits, sourdoughs are natural products that can also increase the nutritional value. It is widely known the role of sourdough in acidification, production of exopolysaccharides, and activation of enzymes like proteases, amylases and phytases, as well as the production of antimicrobial substances like propionate. Other reported benefits include a decrease of the glycaemic

response, increase the bioavailability of dietary fiber and phytochemicals, and the production of nutritionally active compounds, such as peptides and amino acid derivatives and potentially prebiotic exo-polysaccharides[86,87]. However, scarce information exists about the use of sourdough in gluten free baked goods[88]. Microbial fermentation by means of lactic acid bacteria and yeast is one of the most ecological/economical methods for improving the quality of gluten free foods with health-promoting characteristics[89].

Some attempts have been carried out with pseudocereals fermentation to obtain nutritionally improved gluten free products. For instance, quinoa fermentation in slurry was possible using Lactobacillus plantarum CRL 778, yielding greater lactic acid production than in wheat[90]. This type of fermentation stimulated flour protein hydrolysis by endogenous proteases, which proceeded faster in quinoa than in wheat (reaching 40-100 % in quinoa at 8 h of incubation vs only 0-20% in wheat). Protein hydrolysis was parallel to peptides and amino acids release, besides the synthesis of greater concentrations of the antifungal compounds (phenyllactic and hydroxyphenyllactic acids) synthesized from phenylalanine and tyrosine[90].

Also pseudocereals, although good sources of vitamins, minerals, fiber, can be improved nutritionally by germination. Those germinated seeds can be added for fortifying gluten free foods[91]. Germination of amaranth, buckwheat, maize, millet, rice, sorghum, and quinoa can reduce their anti-nutrients content. Their use to naturally fortify and enrich gluten-free foods has great potential. For instance, oat and quinoa malts (obtained after germination) were incorporated in rice and potato based gluten free bread obtaining better crumb due to the amylase activity, and protein hydrolysis[92].

A relatively new current is the detoxification of dietary gluten in those cereals containing gluten by enzymatic cleavage of gliadin fragment with the action of prolyl endopeptidases (PEPs) from different organisms, which can be used to produce gluten free foods from gluten containing cereals or they can be ingested as oral therapy[93]. In addition, the degradation of toxic peptides can be made by germinating cereal enzymes and by transamidation of cereal flours[94]. These treatments may lead to flours with baking and nutritional

qualities of toxic cereals. Microbial transglutaminase modifies selectively the glutamine residues of gluten by transamidation with lysine methyl ester or crosslinking gluten peptide chains that can be removed by filtration leading to gluten detoxification[93].

Wheat flour digestion by fungal proteases and selected sourdough lactobacilli has been reported as an alternative to obtain safe foods for celiac patients[86]. The combination of sourdough lactic acid bacteria fermentation and fungal proteases has been applied in the manufacture of experimental gluten-free pasta by Curiel et al.[95]. Those authors formulated the gluten free pasta with pre-gelatinized rice flour: wheat flour (1:1), optimizing the protocol for hydrolyzing completely the wheat flour. Detoxified wheat flour led to pasta with better sensory properties, digestibility, and nutritional quality.

A study carried on in a mouse model to test the antigenicity of a germinated rye sourdough with extensive prolamin hydrolysis. The quantitation of gluten using competitive R5 ELISA confirmed extensive degradation of the gluten R5 epitope but hydrolysis of secalins in germinated rye sourdough remains incomplete, although this open new alternative for CD with diverse grade of intolerance[96].

6. Conclusion

Medical nutrition therapy is crucial for the dietary management of CD individuals. However, numerous studies have stated that although gluten removal is the solely effective measurement for ameliorating CD symptoms, nutritional deficiencies presents previously to diagnose are not completely mitigated and some others might appeared after long-term gluten free diet compliance.

On the other side, gluten free products are made of complex combinations of ingredients, which significantly differed from gluten containing foods in consequence, their composition is rather diverse. Therefore, the gluten free diet compliance might result in CD individuals with nutritional unbalance.

Overall, current marketed gluten free foods, although meet consumer's expectation regarding quality and availability, often their composition does not completely meet dietary requirements of CD consumers, which drives to reconsider the formulation of gluten free foods having in mind the target consumers and even their age range.

Currently, research is moving fast to answer CD individuals' needs and numerous gluten free foods are yearly launched. Strategies like cereal breeding, design of balanced and enriched formulations, food processing and gluten detoxification are among the most interesting alternatives. Extensive research has been carried out in CD and gluten free food technology but still there is no a near date for having high quality gluten free food products nutritionally equivalent to gluten containing products.

Acknowledgment

The authors acknowledge the financial support of Spanish Scientific Research Council (CSIC), the Spanish Ministry of Economy and Sustainability (Project IPT-2012-0487-060000 and Project AGL2011-23802), the European Regional Development Fund (FEDER) and the Generalitat Valenciana (Project Prometeo 2012/064).

References

1. Gil-Humanes J, Piston F, Altamirano-Fortoul R, Real A, Comino I, Sousa C et al. *Reduced-Gliadin Wheat Bread: An Alternative to the Gluten-Free Diet for Consumers Suffering Gluten-Related Pathologies.* PloS one. 2014; 9(3).
   http://dx.doi.org/10.1371/journal.pone.0090898
2. Denery-Papini S, Nicolas Y, Popineau Y. *Efficiency and limitations of immunochemical assays for the testing of gluten-free foods.* Journal of Cereal Science. 1999; 30(2): 121-31.
   http://dx.doi.org/10.1006/jcrs.1999.0268
3. Rosell CM, Barro F, Sousa C, Mena MC. *Cereals for developing gluten-free products and analytical tools for gluten detection.* Journal of Cereal Science. 2014; 59(3): 354-64.
   http://dx.doi.org/10.1016/j.jcs.2013.10.001
4. Matos ME, Rosell CM. *Understanding gluten-free dough for reaching breads with physical quality and nutritional balance.* Journal of the science of food and agriculture. 2014.
   http://dx.doi.org/10.1002/jsfa.6732
5. Fasano A, Catassi C. *Celiac Disease.* New England Journal of Medicine. 2012; 367(25): 2419-26.
   http://dx.doi.org/10.1056/NEJMcp1113994
   PMid:23252527
6. Worosz MR, Wilson NLW. *A Cautionary Tale of Purity, Labeling and Product Literacy in the Gluten-Free Market.* J Consum Aff. 2012; 46(2): 288-318.
   http://dx.doi.org/10.1111/j.1745-6606.2012.01230.x
7. Catassi C, Fasano A. *Celiac disease.* Current Opinion in Gastroenterology. 2008; 24(6): 687-91.
   http://dx.doi.org/10.1097/MOG.0b013e32830edc1e
   PMid:19122516
8. Malterre T. *Digestive and Nutritional Considerations in Celiac Disease. Could Supplementation Help?* Alternative Medicine Review. 2009; 14(3): 247-57.
   PMid:19803549
9. Malandrino N, Capristo E, Farnetti S, Leggio L, Abenavoli L, Addolorato G et al. *Metabolic and nutritional features in adult celiac patients.* Digestive Diseases. 2008; 26(2): 128-33.
   http://dx.doi.org/10.1159/000116770
   PMid:18431062
10. Saturni L, Ferretti G, Bacchetti T. *The Gluten-Free Diet: Safety and Nutritional Quality.* Nutrients. 2010; 2(1): 16-34.
    http://dx.doi.org/10.3390/nu2010016
    PMid:22253989 PMCid:PMC3257612

11. Brambilla P, Picca M, Dilillo D, Meneghin F, Cravidi C, Tischer MC et al. *Changes of body mass index in celiac children on a gluten-free diet.* Nutrition Metabolism and Cardiovascular Diseases. 2013; 23(3): 177-82.
http://dx.doi.org/10.1016/j.numecd.2011.10.002
PMid:22209739
12. Wierdsma NJ, van Bokhorst-de van der Schueren MAE, Berkenpas M, Mulder CJJ, van Bodegraven AA. *Vitamin and Mineral Deficiencies Are Highly Prevalent in Newly Diagnosed Celiac Disease Patients.* Nutrients. 2013; 5(10): 3975-92.
http://dx.doi.org/10.3390/nu5103975
PMid:24084055 PMCid:PMC3820055
13. Caruso R, Pallone F, Stasi E, Romeo S, Monteleone G. *Appropriate nutrient supplementation in celiac disease.* Annals of Medicine. 2013; 45(8): 522-31.
http://dx.doi.org/10.3109/07853890.2013.849383
PMid:24195595
14. Ohlund K, Olsson C, Hernell O, Ohlund I. *Dietary shortcomings in children on a gluten-free diet.* Journal of Human Nutrition and Dietetics. 2010; 23(3): 294-300.
http://dx.doi.org/10.1111/j.1365-277X.2010.01060.x
PMid:20337845
15. Mariani P, Viti MG, Montuori M, La Vecchia A, Cipolletta E, Calvani L et al. *The gluten-free diet: A nutritional risk factor for adolescents with celiac disease?* Journal of pediatric gastroenterology and nutrition. 1998; 27(5): 519-23.
http://dx.doi.org/10.1097/00005176-199811000-00004
PMid:9822315
16. Hallert C, Grant C, Grehn S, Granno C, Hulten S, Midhagen G et al. *Evidence of poor vitamin status in coeliac patients on a gluten-free diet for 10 years.* Alimentary pharmacology & therapeutics. 2002; 16(7): 1333-9.
http://dx.doi.org/10.1046/j.1365-2036.2002.01283.x
17. Haapalahti M, Kulmala P, Karttunen TJ, Paajanen L, Laurila K, Maki M et al. *Nutritional status in adolescents and young adults with screen-detected celiac disease.* Journal of pediatric gastroenterology and nutrition. 2005; 40(5): 566-70.
http://dx.doi.org/10.1097/01.MPG.0000154658.16618.F9
PMid:15861017
18. Shepherd SJ, Gibson PR. *Nutritional inadequacies of the gluten-free diet in both recently-diagnosed and long-term patients with coeliac disease.* Journal of Human Nutrition and Dietetics. 2013; 26(4): 349-58.
http://dx.doi.org/10.1111/jhn.12018
PMid:23198728

19. Martin J, Geisel T, Maresch C, Krieger K, Stein J. *Inadequate Nutrient Intake in Patients with Celiac Disease: Results from a German Dietary Survey.* Digestion. 2013; 87(4): 240-6.

http://dx.doi.org/10.1159/000348850
PMid:23751356

20. Kautto E, Ivarsson A, Norstrom F, Hogberg L, Carlsson A, Hornell A. *Nutrient intake in adolescent girls and boys diagnosed with coeliac disease at an early age is mostly comparable to their non-coeliac contemporaries.* Journal of Human Nutrition and Dietetics. 2014; 27(1): 41-53.

http://dx.doi.org/10.1111/jhn.12125
PMid:23701396

21. Preusse H, Glaser G. *Additional diet-related nutritional costs for patients with celiac disease.* Ernahrungs-Umschau. 2009; 56(10): 554-9.

PMid:24299846

22. Singh J, Whelan K. *Limited availability and higher cost of gluten-free foods.* Journal of Human Nutrition and Dietetics. 2011; 24(5): 479-86.

http://dx.doi.org/10.1111/j.1365-277X.2011.01160.x
PMid:21605198

23. Dura A, Błaszczak W, Rosell CM. *Functionality of porous starch obtained by amylase or amyloglucosidase treatments.* Carbohydrate Polymers. 2014; 101(1): 837-45.

http://dx.doi.org/10.1016/j.carbpol.2013.10.013
PMid:24299846

24. Ozola L, Straumite E (Eds.). *Consumers' attitude towards availability and quality of gluten-free products in the latvian market.* 18th Annual International Scientific Conference - Research for Rural Development 2012. Jelgava. 2012.

25. Penagini F, Dilillo D, Meneghin F, Mameli C, Fabiano V, Zuccotti GV. *Gluten-Free Diet in Children: An Approach to a Nutritionally Adequate and Balanced Diet.* Nutrients. 2013; 5(11): 4553-65.

http://dx.doi.org/10.3390/nu5114553
PMid:24253052 PMCid:PMC3847748

26. Altobelli E, Paduano R, Gentile T, Caloisi C, Marziliano C, Necozione S et al. *Health-related quality of life in children and adolescents with celiac disease: survey of a population from central Italy.* Health and Quality of Life Outcomes. 2013; 11.

http://dx.doi.org/10.1186/1477-7525-11-204

27. Baranska A, Tigchelaar E, Smolinska A, Dallinga JW, Moonen EJC, Dekens JAM et al. *Profile of volatile organic compounds in exhaled breath changes as a result of gluten-free diet.* Journal of Breath Research. 2013; 7(3).

http://dx.doi.org/10.1088/1752-7155/7/3/037104
PMid:23774130

28. Dar YL. *Advances and ongoing challenges in the development of gluten-free baked goods.* Cereal Foods World. 2013; 58(6): 298-304.
http://dx.doi.org/10.1094/CFW-58-6-0298
29. Thompson T, Dennis M, Higgins LA, Lee AR, Sharrett MK. *Gluten-free diet survey: are Americans with coeliac disease consuming recommended amounts of fibre, iron, calcium and grain foods?* Journal of Human Nutrition and Dietetics. 2005; 18(3): 163-9.
http://dx.doi.org/10.1111/j.1365-277X.2005.00607.x
PMid:15882378
30. Thompson T. *Folate, iron, and dietary fiber contents of the gluten-free diet.* Journal of the American Dietetic Association. 2000; 100(11): 1389-96.
http://dx.doi.org/10.1016/S0002-8223(00)00386-2
31. Matos ME, Rosell CM. *Chemical composition and starch digestibility of different gluten-free breads.* Plant Foods for Human Nutrition. 2011; 66(3): 224-30.
http://dx.doi.org/10.1007/s11130-011-0244-2
PMid:21769691
32. Valletta E, Fornaro M, Cipolli M, Conte S, Bissolo F, Danchielli C. *Celiac disease and obesity: need for nutritional follow-up after diagnosis.* European journal of clinical nutrition. 2010; 64(11): 1371-2.
http://dx.doi.org/10.1038/ejcn.2010.161
PMid:20717130
33. Pagliarini E, Laureati M, Lavelli V. *Sensory evaluation of gluten-free breads assessed by a trained panel of celiac assessors.* European Food Research and Technology. 2010; 231(1): 37-46.
http://dx.doi.org/10.1007/s00217-010-1249-z
34. Matos ME, Rosell CM. *Quality Indicators of Rice-Based Gluten-Free Bread-Like Products: Relationships Between Dough Rheology and Quality Characteristics.* Food and Bioprocess Technology. 2013; 6(9): 2331-41.
http://dx.doi.org/10.1007/s11947-012-0903-9
35. Phimolsiripol Y, Mukprasirt A, Schoenlechner R. *Quality improvement of rice-based gluten-free bread using different dietary fibre fractions of rice bran.* Journal of Cereal Science. 2012; 56(2): 389-95.
http://dx.doi.org/10.1016/j.jcs.2012.06.001
36. Krupa-Kozak U, Baczek N, Rosell CM. Application of Dairy Proteins as Technological and Nutritional Improvers of Calcium-Supplemented Gluten-Free Bread. Nutrients. 2013 Nov;5(11):4503-20.
http://dx.doi.org/10.3390/nu5114503
PMid:24241213 PMCid:PMC3847745
37. Dini C, Garcia MA, Vina SZ. *Non-traditional flours: frontiers between ancestral heritage and innovation.* Food & Function. 2012; 3(6): 606-20.
http://dx.doi.org/10.1039/c2fo30036b
PMid:22499487

38. Gularte MA, Gomez M, Rosell CM. *Impact of Legume Flours on Quality and In Vitro Digestibility of Starch and Protein from Gluten-Free Cakes.* Food and Bioprocess Technology. 2012; 5(8): 3142-50.
http://dx.doi.org/10.1007/s11947-011-0642-3

39. Venskutonis PR, Kraujalis P. *Nutritional Components of Amaranth Seeds and Vegetables: A Review on Composition, Properties, and Uses.* Comprehensive Reviews in Food Science and Food Safety. 2013; 12(4): 381-412.
http://dx.doi.org/10.1111/1541-4337.12021

40. Vitali D, Klarić DA, Dragojević IV. *Nutritional and functional properties of certain gluten-free raw materials.* Czech Journal of Food Sciences. 2010; 28(6): 495-505.

41. Hager AS, Wolter A, Jacob F, Zannini E, Arendt EK. *Nutritional properties and ultra-structure of commercial gluten free flours from different botanical sources compared to wheat flours.* Journal of Cereal Science. 2012; 56(2): 239-47.
http://dx.doi.org/10.1016/j.jcs.2012.06.005

42. Rai S, Kaur A, Singh B. *Quality characteristics of gluten free cookies prepared from different flour combinations.* Journal of Food Science and Technology-Mysore. 2014; 51(4): 785-9.
http://dx.doi.org/10.1007/s13197-011-0547-1
PMid:24741176 PMCid:PMC3982011

43. Vitali D, Klaric DA, Dragojevic IV. *Nutritional and Functional Properties of Certain Gluten-Free Raw Materials.* Czech Journal of Food Sciences. 2010; 28(6): 495-505.

44. Renzetti S, Behr J, Vogel RF, Arendt EK. *Transglutaminase polymerisation of buckwheat (Fagopyrum esculentum Moench) proteins.* Journal of Cereal Science. 2008; 48(3): 747-54.
http://dx.doi.org/10.1016/j.jcs.2008.04.005

45. Mezaize S, Chevallier S, Le Bail A, de Lamballerie M. *Optimization of Gluten-Free Formulations for French-Style Breads.* Journal of Food Science. 2009; 74(3): E140-E6.
http://dx.doi.org/10.1111/j.1750-3841.2009.01096.x
PMid:19397719

46. Torbica A, Hadnadev M, Dapcevic T. *Rheological, textural and sensory properties of gluten-free bread formulations based on rice and buckwheat flour.* Food Hydrocolloids. 2010; 24(6-7): 626-32.
http://dx.doi.org/10.1016/j.foodhyd.2010.03.004

47. Mariotti M, Pagani MA, Lucisano M. *The role of buckwheat and HPMC on the breadmaking properties of some commercial gluten-free bread mixtures.* Food Hydrocolloids. 2013; 30(1): 393-400.
http://dx.doi.org/10.1016/j.foodhyd.2012.07.005

48. Krupa-Kozak U, Wronkowska M, Soral-Smietana M. *Effect of Buckwheat Flour on Microelements and Proteins Contents in Gluten-Free Bread.* Czech Journal of Food Sciences. 2011; 29(2): 103-8.

49. de la Barca AMC, Rojas-Martinez ME, Islas-Rubio AR, Cabrera-Chavez F. *Gluten-Free Breads and Cookies of Raw and Popped Amaranth Flours with Attractive Technological and Nutritional Qualities.* Plant Foods for Human Nutrition. 2010; 65(3): 241-6.
http://dx.doi.org/10.1007/s11130-010-0187-z
PMid:20734143

50. Alvarez-Jubete L, Arendt EK, Gallagher E. *Nutritive value of pseudocereals and their increasing use as functional gluten-free ingredients.* Trends in Food Science & Technology. 2010; 21(2): 106-13.
http://dx.doi.org/10.1016/j.tifs.2009.10.014

51. Alvarez-Jubete L, Arendt EK, Gallagher E. *Nutritive value and chemical composition of pseudocereals as gluten-free ingredients.* International Journal of Food Sciences and Nutrition. 2009; 60: 240-57.
http://dx.doi.org/10.1080/09637480902950597
PMid:19462323

52. Cabrera-Chávez F, Calderón de la Barca AM, Islas-Rubio AR, Marti A, Marengo M, Pagani MA et al. *Molecular rearrangements in extrusion processes for the production of amaranth-enriched, gluten-free rice pasta.* LWT - Food Science and Technology. 2012; 47(2): 421-6.
http://dx.doi.org/10.1016/j.lwt.2012.01.040

53. Gularte MA, de la Hera E, Gómez M, Rosell CM. *Effect of different fibers on batter and gluten-free layer cake properties.* LWT - Food Science and Technology. 2012; 48(2): 209-14.
http://dx.doi.org/10.1016/j.lwt.2012.03.015

54. Gularte MA, Gómez M, Rosell CM. *Impact of Legume Flours on Quality and In Vitro Digestibility of Starch and Protein from Gluten-Free Cakes.* Food and Bioprocess Technology. 2012; 5(8): 3142-50.
http://dx.doi.org/10.1007/s11947-011-0642-3

55. Capriles VD, Areas JAG. *Amaranth bars enriched with fructans: acceptability and nutritional value.* Archivos Latinoamericanos De Nutricion. 2010; 60(3): 291-7.

56. Lee AR, Ng DL, Dave E, Ciaccio EJ, Green PHR. *The effect of substituting alternative grains in the diet on the nutritional profile of the gluten-free diet.* Journal of Human Nutrition and Dietetics. 2009; 22(4): 359-63.
http://dx.doi.org/10.1111/j.1365-277X.2009.00970.x
PMid:19519750

57. Renzetti S, Dal Bello F, Arendt EK. *Microstructure, fundamental rheology and baking characteristics of batters and breads from different gluten-free flours treated with a microbial transglutaminase.* Journal of Cereal Science. 2008; 48(1): 33-45.

http://dx.doi.org/10.1016/j.jcs.2007.07.011

58. Onyango C, Mutungi C, Unbehend G, Lindhauer MG. *Modification of gluten-free sorghum batter and bread using maize, potato, cassava or rice starch.* Lwt-Food Science and Technology. 2011; 44(3): 681-6.

http://dx.doi.org/10.1016/j.lwt.2010.09.006

59. Onyango C, Mutungi C, Unbehend G, Lindhauer MG. *Rheological and textural properties of sorghum-based formulations modified with variable amounts of native or pregelatinised cassava starch.* Lwt-Food Science and Technology. 2011; 44(3): 687-93.

http://dx.doi.org/10.1016/j.lwt.2010.08.019

60. Smith BM, Bean SR, Herald TJ, Aramouni FM. *Effect of HPMC on the Quality of Wheat-Free Bread Made from Carob Germ Flour-Starch Mixtures.* Journal of Food Science. 2012; 77(6): C684-C9.

http://dx.doi.org/10.1111/j.1750-3841.2012.02739.x
PMid:22671523

61. Moreira R, Chenlo F, Torres MD. *Rheology of Gluten-Free Doughs from Blends of Chestnut and Rice Flours.* Food and Bioprocess Technology. 2013; 6(6): 1476-85.

http://dx.doi.org/10.1007/s11947-012-0927-1

62. Moreira R, Chenlo F, Torres MD, Prieto DM. *Technological Assessment of Chestnut Flour Doughs Regarding to Doughs from Other Commercial Flours and Formulations.* Food and Bioprocess Technology. 2012; 5(6): 2301-10.

http://dx.doi.org/10.1007/s11947-011-0524-8

63. Demirkesen I, Sumnu G, Sahin S. *Quality of Gluten-Free Bread Formulations Baked in Different Ovens.* Food and Bioprocess Technology. 2013; 6(3): 746-53.

http://dx.doi.org/10.1007/s11947-011-0712-6

64. Gambus H, Gambus F, Pastuszka D, Wrona P, Ziobro R, Sabat R et al. *Quality of gluten-free supplemented cakes and biscuits.* International Journal of Food Sciences and Nutrition. 2009; 60: 31-50.

http://dx.doi.org/10.1080/09637480802375523
PMid:19330630

65. Pastuszka D, Gambus H, Sikora M. *Nutritional and dietary value of gluten-free rolls with flaxseeds added.* Zywnosc-Nauka Technologia Jakosc. 2012; 19(3): 155-67.

http://dx.doi.org/10.15193/zntj/2012/82/155-167

66. Levent H, Bilgicli N. *Enrichment of gluten-free cakes with lupin (Lupinus albus L.) or buckwheat (Fagopyrum esculentum M.) flours.* International Journal of Food Sciences and Nutrition. 2011; 62(7): 725-8.
http://dx.doi.org/10.3109/09637486.2011.572546
PMid:21568822

67. De Gouveia PF, Zandonadi RP. *Green banana: new alternative for gluten-free products.* Agro Food Industry Hi-Tech. 2013; 24(3): 49-52.

68. Zandonadi RP, Botelho RBA, Gandolfi L, Ginani JS, Montenegro FM, Pratesi R. Green Banana Pasta: An Alternative for Gluten-Free Diets. J Acad Nutri Diet. 2012;112(7):1068-72.
http://dx.doi.org/10.1016/j.jand.2012.04.002
PMid:22889636

69. Souza TAC, Soares MS, Campos MRH, Souza TSC, Dias T, Fiorda FA. *Gluten free cakes made with broken rice and cassava peel.* Semina-Ciencias Agrarias. 2013; 34(2): 717-27.
http://dx.doi.org/10.5433/1679-0359.2013v34n2p717

70. Han J, Janz JAM, Gerlat M. *Development of gluten-free cracker snacks using pulse flours and fractions.* Food Research International. 2010; 43(2): 627-33.
http://dx.doi.org/10.1016/j.foodres.2009.07.015

71. Borderias AJ, Sanchez-Alonso I, Perez-Mateos M. *New applications of fibres in foods: Addition to fishery products.* Trends in Food Science & Technology. 2005; 16(10): 458-65.
http://dx.doi.org/10.1016/j.tifs.2005.03.011

72. Sabanis D, Lebesi D, Tzia C. *Effect of dietary fibre enrichment on selected properties of gluten-free bread.* Lwt-Food Science and Technology. 2009; 42(8): 1380-9.
http://dx.doi.org/10.1016/j.lwt.2009.03.010

73. Fiorda FA, Soares MS, da Silva FA, Grosmann MVE, Souto LRF. *Microestructure, texture and colour of gluten-free pasta made with amaranth flour, cassava starch and cassava bagasse.* Lwt-Food Science and Technology. 2013; 54(1): 132-8.
http://dx.doi.org/10.1016/j.lwt.2013.04.020

74. do Nascimento AB, Fiates GMR, dos Anjos A, Teixeira E. *Analysis of ingredient lists of commercially available gluten-free and gluten-containing food products using the text mining technique.* International Journal of Food Sciences and Nutrition. 2013; 64(2): 217-22.
http://dx.doi.org/10.3109/09637486.2012.718744
PMid:22946669

75. Sulibursk a J, Krejpcio Z, Regula J, Grochowicz A. *Evaluation of the content and the potential bioavailability of minerals from gluten-free products.* Acta Scientiarum Polonorum - Technologia Alimentaria. 2013; 12(1): 75-9.

76. Krupa-Kozak U, Altamirano-Fortoul R, Wronkowska M, Rosell CM. *Breadmaking performance and technological characteristic of gluten-free bread with inulin supplemented with calcium salts.* European Food Research and Technology. 2012; 235(3): 545-54.

http://dx.doi.org/10.1007/s00217-012-1782-z

77. Kiskini A, Mandala I, Argiri K, Kapsokefalou M, Komaitis M, Kostaropoulos A. *Iron-fortified gluten-fee products. Functional and nutritional characteristics.* Annals of Nutrition and Metabolism. 2007; 51: 299.

78. Krupa-Kozak U, Wronkowska M, Soral-Śmietana M. *Effect of buckwheat flour on microelements and proteins contents in gluten-free bread.* Czech Journal of Food Sciences. 2011; 29(2): 103-8.

79. Bituh M, Zizic V, Krbavcic IP, Zadro Z, Baric IC. *Gluten-Free Products Are Insufficient Source of Folate and Vitamin B(12) for Coeliac Patients.* Food Technology and Biotechnology. 2011; 49(4): 511-6.

80. Berti C, Riso P, Monti LD, Porrini M. *In vitro starch digestibility and in vivo glucose response of gluten-free foods and their gluten counterparts.* European Journal of Nutrition. 2004; 43(4): 198-204.

http://dx.doi.org/10.1007/s00394-004-0459-1
PMid:15309439

81. Novotni D, Cukelj N, Smerdel B, Bituh M, Dujmic F, Curic D. *Glycemic index and firming kinetics of partially baked frozen gluten-free bread with sourdough.* Journal of Cereal Science. 2012; 55(2): 120-5.

http://dx.doi.org/10.1016/j.jcs.2011.10.008

82. Hager AS, Czerny M, Bez J, Zannini E, Arendt EK. *Starch properties, in vitro digestibility and sensory evaluation of fresh egg pasta produced from oat, teff and wheat flour.* Journal of Cereal Science. 2013; 58(1): 156-63.

http://dx.doi.org/10.1016/j.jcs.2013.03.004

83. Capriles VD, Areas JAG. *Effects of prebiotic inulin-type fructans on structure, quality, sensory acceptance and glycemic response of gluten-free breads.* Food & Function. 2013; 4(1): 104-10.

http://dx.doi.org/10.1039/C2FO10283H
PMid:23032642

84. De La Hera E, Gomez M, Rosell CM. *Particle size distribution of rice flour affecting the starch enzymatic hydrolysis and hydration properties.* Carbohydrate Polymers. 2013; 98(1): 421-7.

http://dx.doi.org/10.1016/j.carbpol.2013.06.002
PMid:23987363

85. de la Hera E, Rosell CM, Gomez M. *Effect of water content and flour particle size on gluten-free bread quality and digestibility.* Food Chemistry. 2014; 151: 526-31.

http://dx.doi.org/10.1016/j.foodchem.2013.11.115
PMid:24423566

86. Gobbetti M, Rizzello CG, Di Cagno R, De Angelis M. *How the sourdough may affect the functional features of leavened baked goods.* Food microbiology. 2014; 37: 30-40.
http://dx.doi.org/10.1016/j.fm.2013.04.012
PMid:24230470

87. Poutanen K, Flander L, Katina K. *Sourdough and cereal fermentation in a nutritional perspective.* Food microbiology. 2009; 26(7): 693-9.
http://dx.doi.org/10.1016/j.fm.2009.07.011
PMid:19747602

88. Moroni AV, Dal Bello F, Arendt EK. *Sourdough in gluten-free bread-making: An ancient technology to solve a novel issue?* Food microbiology. 2009; 26(7): 676-84.
http://dx.doi.org/10.1016/j.fm.2009.07.001
PMid:19747600

89. Zannini E, Pontonio E, Waters DM, Arendt EK. *Applications of microbial fermentations for production of gluten-free products and perspectives.* Applied microbiology and biotechnology. 2012; 93(2): 473-85.
http://dx.doi.org/10.1007/s00253-011-3707-3
PMid:22094979

90. Dallagnol AM, Pescuma M, De Valdez GF, Rollan G. *Fermentation of quinoa and wheat slurries by Lactobacillus plantarum CRL 778: proteolytic activity.* Applied microbiology and biotechnology. 2013; 97(7): 3129-40.
http://dx.doi.org/10.1007/s00253-012-4520-3
PMid:23129182

91. Omary MB, Fong C, Rothschild J, Finney P. *Effects of Germination on the Nutritional Profile of Gluten-Free Cereals and Pseudocereals: A Review.* Cereal Chemistry. 2012; 89(1): 1-14.
http://dx.doi.org/10.1094/CCHEM-01-11-0008

92. Makinen OE, Zannini E, Arendt EK. *Germination of Oat and Quinoa and Evaluation of the Malts as Gluten Free Baking Ingredients.* Plant Foods for Human Nutrition. 2013; 68(1): 90-5.
http://dx.doi.org/10.1007/s11130-013-0335-3
PMid:23386201

93. Wieser H, Koehler P. *Detoxification of Gluten by Means of Enzymatic Treatment.* Journal of Aoac International. 2012; 95(2): 356-63.
http://dx.doi.org/10.5740/jaoacint.SGE_Wieser
PMid:22649919

94. Comino I, Moreno ML, Real A, Rodríguez-Herrera A, Barro F, Sousa C. *The gluten-free diet: Testing alternative cereals tolerated by celiac patients.* Nutrients. 2013; 5(10): 4250-68.
http://dx.doi.org/10.3390/nu5104250
PMid:24152755 PMCid:PMC3820072

95. Curiel JA, Coda R, Limitone A, Katina K, Raulio M, Giuliani G et al. *Manufacture and characterization of pasta made with wheat flour rendered gluten-free using fungal proteases and selected sourdough lactic acid bacteria.* Journal of Cereal Science. 2014; 59(1): 79-87.
http://dx.doi.org/10.1016/j.jcs.2013.09.011

96. Freitag TL, Loponen J, Messing M, Zevallos V, Andersson LC, Sontag-Strohm T et al. *Testing safety of germinated rye sourdough in a celiac disease model based on the adoptive transfer of prolamin-primed memory T cells into lymphopenic mice.* American Journal of Physiology-Gastrointestinal and Liver Physiology. 2014; 306(6): G526-G34.

This book will give you up-to-date information on the basic and clinical aspects of gluten-related disorders, the evolution of grains, bread and gluten-free products covering the necessary integration of the different fields which until recently were considered separate.

To achieve a common platform, this book has three sections.

· The first section deals with chapters explaining the immune response to the toxic peptides of gluten as well as new approaches in treatment.

· The second section revises the advances made in the clinical spectrum of the gluten-related disorders.

· The third section explores the evolution of gluten, bread products and the great challenge to elaborate gluten-free products of high quality. The technological improvements in this field will produce gluten-free foods, less expensive than at present available in food stores.

The authors are recognized investigators, active in the field of gluten-related disorders and the evolution of gluten-free products.

The information provided is essential for celiacs, non-celiac gluten- sensitive persons, Patient Associations of gluten-related disorders, physicians and the food industry. It will be of help to clinical and research scientists in clinical medicine, immunology and pathology, to professionals in nutrition and gluten-free products, to the regulatory authorities, to food chemists and their technologists.

CSIC
Consejo Superior de Investigaciones Científicas
Asociación de Celíacos y Sensibles al Gluten
C/ Lanuza, 19 Bajo - 28028 Madrid
T. 917 130 147 - F. 917 258 059
www.celiacosmadrid.org