New Fields of Research in Celiac Disease

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Research in celiac disease (CD) spans from pathogenesis, through diagnosis of CD, and up to treatment options and follow-up of patients, covering every aspect of this multifaceted disease. While understanding the complex mechanism of CD pathogenesis represents the scientific main interest of research, in clinical practice most efforts are focused on new tools in diagnosis and management. From the perspective of patients living with CD, the main interest is in aspects of gluten tolerance and therapeutic options. Many of these issues are incorporated in the different chapters of this book under each specific topic. In this chapter we present an overview of the main current research fields and challenges in understanding and managing CD. We do not, however, discuss drug treatment for CD as this is covered in a specific dedicated chapter.

New fields in CD pathophysiology:

The strong genetic association of CD to specific human leucocyte antigen (HLA) haplotypes is known for decades, with nearly all patients with CD being positive to HLA DQ2/DQ8 [1, 2]. This genetic susceptibility is the cornerstone of CD, perceived as a mandatory prerequisite to develop CD. While DQ2 is the common haplotype identified in patient with CD worldwide, DQ8 is present in less than 10% [3]. Studies exploring the various susceptible haplotypes reported a gene-dose effect with different magnitude of the risk to develop CD, based on the number of copies of specific allele [4, 5]. Homozygosity for DQ2 alleles poses a much higher (fivefold) risk of developing CD compared to a single DQ2 allele or DQ8 haplotype [4]. Moreover, this DQ2 homozygosity is also associated with earlier disease pre-

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sentation and more severe phenotype [6]. Overall, research on the various associations of HLA haplotypes, with the risk to develop CD and the haplotype-phenotype associations are important for the future development of preventive, diagnostic and therapeutic measures.

An interesting new field is the non-HLA heritability, estimated as responsible for 68% of the heritability of CD in a large recent twins study [7]. The role of genetic factors other than HLA in CD is much less understood and is being currently assessed. Over 40 different loci, other than HLA, have found to be associated with CD so far [8]. The TEDDY international multicentre study has been able to identify dozens of different single-nucleotide polymorphisms (SNPs) in several non-HLA regions, to be associated with CD, using genotype analysis [9]. Most of these involved genes encoded proteins act in activation and regulation of the immune response, and some of them are common with other autoimmune diseases (such as rheumatoid arthritis, type 1 diabetes, and multiple sclerosis [10–12]). These different identified *loci* are associated with T cell activation, inflammatory cytokines and cytokine-receptors regulation, and thymic T-cell selection [13]. Other identified *loci* are noncoding regions, regulating gene expression [14].

These new identified SNPs, along with the already known HLA genes, could be combined to form a more accurate genetic risk score to CD, as shown recently in a pilot study incorporating non-HLA SNPs with HLA gene testing, providing a more accurate discriminating tool for genetic risk assessment [15].

The main challenges in pathogenesis research in CD is understanding the mechanisms linking between the genetic susceptibility and the immune response to gluten exposure. It has been long known that gluten-specific T cells are present in intestinal tissue of patients with CD [1], being activated to produce an inflammatory cascade in response to presentation to deaminated gluten peptides. The specific gluten epitopes, recognized by pathogenic T cells, have been identified in wheat, as well as in rye and barely [16]. The reasons for the loss of tolerance to dietary gluten by a minority of HLA-susceptible individuals are still poorly understood. The role of regulatory T cells (Tregs) in this process is of major interest in contemporary studies, given the effect of Tregs in inhibiting the immune response to digested antigens. Recent comparative studies have found an attenuated inhibitory effect of gluten-specific Tregs on intestinal and circulating T lymphocytes in patients with CD, suggesting Treg dysfunction to play an important role in the pathogenesis of the disease [17, 18]. Gene expression studies in patients with CD further demonstrate upregulation of IFN-γ and reduced expression of the transcription factor BACH2 genes—which is a dominant regulator of T cell differentiation promoting development of Tregs [19]. Further studies are needed to better identify the specific characteristics and behaviour of these Tregs and the role they play in the loss of gluten tolerance and activation of the pathologic response.

The role of environmental factors in the development of CD is detailed in the specific chapter on this topic. A major contemporary focus, now being actively studied, is host-microbiome interaction and the potential role of gut microbiota in CD pathogenesis. This field is of particular interest, due to its potential in exploring prevention strategies in at-risk populations. In the last few years there is plethora of

publications regarding the role of gut microbiota in the pathogenesis of immune-regulated disorders [20], and among these CD and the loss of gluten oral tolerance has an increasing presence. Previous studies have already shown unbalanced microbiota population in the gut of patients with CD, with higher incidence of pro-inflammatory bacteria [21, 22], and reduction in *Bifidobacterium* species that are considered modulators of gut immunity [23, 24]. Most of these dysbiosis patterns were found to be constant in patient with CD regardless of inflammation and status of gluten-free diet (GFD), suggesting their primary prominent role in CD pathogenesis [25]. Interestingly, HLA-DQ2 genotype was found to influence early life gut microbiota pattern in infants at high family risk of developing CD [26]. In this study, infants with the high-risk HLA-DQ2 had reduced gut *Bifidobacterium*, compared to subjects with lower-risk genotype. These findings may suggest that the genetic susceptibility to CD may in part influence early life microbiota colonization, which could be linked to immune-regulation mechanisms that should further be investigated. In addition, many studies currently focus on identifying specific composition of gut microbiota in patients with CD and their association with patterns of disease presentation and symptoms [27, 28]. Large prospective longitudinal studies currently being performed, such as the CDGEMM study of infants at risk of CD [29], could shed more light on the relationship between early patterns of gut microbiota and later development of CD.

Several studies have tried to explore the effects of different strains of probiotics in patients diagnosed with CD, and more trials are currently ongoing. These studies focus on supplementing GFD with probiotics in order to influence the dysbiosis present in CD, or to attenuate intestinal inflammatory response derived by gluten [30]. A 3-months RCT in newly diagnosed patients with CD on GFD, showed favourable outcomes with *Bifidobacterium longum* CECT supplementations, demonstrating reductions in activated T lymphocytes and some inflammatory cytokines, as well as reduction in the *Bacteroides fragilis* group and in the content of IgA in stools [31]. Another RCT has shown a reduction in TNF-α in a small group of patients with CD supplemented with *B. breve* strains [32]. An improvement in celiac symptom index was demonstrated in a subset of patients treated with *B. infantis* NLS-SS (together with GFD) [33]. One published RCT investigated the effect of two strains of *Lactobacillus* in children with CD autoimmunity under gluten-containing diet [34], and demonstrated some modulations in the peripheral immune response, with no effect on CD serology. Additional ongoing trials with different strains of probiotics, are currently active and pending results (ClinicalTrials.gov: NCT04160767; NCT03775499; NCT04014660; NCT03562221).

Hopefully, future studies will focus on prevention of CD development through alternation of gut microbiota. The effects of probiotics on patients at risk to develop CD, with the potential of altering the interaction between gut microbiota and the host immune response to gluten, should further be explored.

New aspects in CD diagnosis:

Diagnosis of CD in adults still relays on the combination of CD serology and duodenal biopsies [35, 36], while in children and adolescents CD serology could be sufficient to diagnose selected cases with very high titres of TTG (above 10 times upper limit of normal), combined with positive endomysium antibodies [37]. In practice, duodenal biopsies are mandatory worldwide for many cases with lower TTG titres and in adult population. There is a great interest in the search for non-invasive novel biomarkers for the diagnosis of CD [38], in order to overcome challenges with false positive and false negative serologic results, challenges with patients already on GFD, better management of potential CD, improved diagnosis accuracy with borderline histologic findings, and future expansion of non-invasive diagnosis to adult population.

One unique clinical challenge in modern era is the diagnosis of CD in patients that are already practicing a GFD. Starting GFD before or without diagnosis of CD has several reasons, including symptoms related to gluten ingestion making the patient reluctant from performing gluten challenge; other family members already on GFD with limited exposure to gluten in their household; non-celiac gluten or wheat sensitivity; and cultural or personal dietetic preferences. As gluten intake influences the diagnostic accuracy of both serologic markers and histologic findings, a main target of research in this field is identifying biomarkers that are constant even in the absence of dietary gluten ingestion. Such potential markers are gluten-specific T cells in peripheral circulation, identified by HLA-DQ-gluten tetramers [39]. Several recent studies have shown that HLA-DQ-gluten tetramers can accurately identify patients with CD, whether they are with or without GFD, compared to healthy controls [40–43]. These findings hold promise in clinical practice, as they may assist in CD diagnosis in specific individuals without the need for gluten challenge and intestinal biopsies. Currently, the assays of HLA-DQ-gluten tetramers are not commercially available and require additional research to better define their diagnostic accuracy and feasibility in clinical practice.

In addition, there are several markers for intestinal mucosal damage, that although not specific for CD-induced enteropathy, they could be combined with positive TTG serology in the diagnostic process. A combination of positive CD serology with non-invasive markers of small intestinal damage, are in the focus of interest as a mean to diminish the need for confirmatory biopsies in patients with lower than needed serological titres for non-biopsy diagnosis of CD. The two main markers for intestinal damage being investigated in CD are citrulline [44, 45] and Fatty Acid Binding Protein 2 (FABP2 or I-FABP) [44, 46–48], both been shown to correlate with the degree of villous atrophy in patients with CD.

Finally, there is a unique cytokines profile in CD, reflecting the immune dysregulation and inflammatory process, that differ from healthy individuals and correlates with TTG levels [49]. Some current studies focus on identifying specific cytokines to differentiate between patient with CD and healthy individuals or subjects with non-celiac gluten sensitivity and searching for better correlation with intestinal mucosal damage [50, 51].

Specific circulating microRNA were also found to be indicative of CD in recent studies [52, 53]. Continuous research is needed to better define their role both in pathogenesis and in diagnostic yield as biomarkers.

Research priorities in the management of CD

Although GFD is the cornerstone of CD treatment, strict adherence is challenging in both paediatric and adult patients [54, 55]. A major focus in current research, derived mainly by real-life challenges of patients' community, is identifying the minimal amounts of gluten that can produce immunogenic-inflammatory reactions, while defining the threshold of maximal tolerated gluten in daily life. Based on previous studies, the safe amount of gluten to be tolerated by patients with CD was first considered as under 50 grams of gluten per day (for adults), although some patients showed worsening of intestinal histology after ingestion of only 10 grams per day [56]. Other smaller studies showed different thresholds with wide variation between studies, with the combined conclusion that daily gluten intake below 10 grams is probably safe to patients with CD [57]. A different question relates to the amount of gluten required to elicit a quick pathologic response or in other words what would be the ideal quantity of gluten and the optimal marker to follow when challenging a patient on a GFD. A recent RCT compared various endpoints and biomarkers after 15 days of micro-challenge with 10 and 3 grams of gluten in 14 adult patients [58]. With 3 grams daily gluten consumption, the only significant change from baseline was in patients' self-reported symptoms. Other markers including duodenal histology, video capsule endoscopy findings, and gluten-specific T-cells, showed significant changes at 10 grams gluten only. This trial provides a framework for future studies to incorporate modern biomarkers in the investigation of gluten challenge, in order to better define the accurate threshold of tolerated gluten in CD. Moreover, specific cut-offs for paediatric population are lacking and merit further studies.

In clinical practice, follow-up of patients with CD includes clinical and dietary assessments, as well as monitoring for CD serology as a surrogate marker for treatment adherence. However, CD antibodies have low sensitivity to detect villous atrophy in patients with CD on self-reported GFD, shown to be as low as below 50% in a large recent meta-analysis [59]. For that reason, better markers are needed in order to monitor intentional and unintentional gluten consumption in patients with CD. Gluten immunogenic peptides (GIP) are novel markers for gluten consumption, based on their resistance to gastric and intestinal degradation. GIP could be detected in faeces of patients, as soon as 3 days after minimal gluten ingestion [60]. Several studies have proved detection of faecal or urine GIP in patients with CD who were reporting good adherence to GFD [61–65], highlighting the gap between patients' perception or reported adherence and real-life exposure to gluten. Furthermore, as these tests provide short term information, exposures a few days before the test (stool GIP) and even testing more than 24 hours after exposure (urine GIP) may miss incidental or non-incidental transgressions. There are ongoing studies aiming to explore further utilization of GIP testing, including a point-of-care home test for patients with CD (NCT03462979, clinicaltrials.gov).

Another interesting arm of research is the genetic manipulation of wheat, aiming to develop wheat with reduced gluten toxicity. Several studies so far have failed to identify sufficient changes in wheat genome that will both preserve wheat gastronomic and technical properties, together with prevention of immunogenic-inflammatory response in CD [66, 67].

Most recently, the International Wheat Genome Sequencing Consortium analyzed and published a detailed annotated reference genome sequence of wheat, covering 94% of its genome [68]. This development could assist in future studies in wheat engineering exploring the production of non-immunogenic gluten to be appropriate for ingestion by patients with CD.

Another approach being investigated in the food industry, is modification of pre-digested wheat in order to lower gluten presence in the product or reduce its immunogenicity. For that purpose several processes are being studied, including fermentation of wheat by microorganisms that release proteases able to digest gliadin peptides [69–71], thermal processing by microwave [72, 73], and the use of microbial transglutaminase [74]. The role of these modifications and their long-term safety in patients with CD are yet to be defined.

Finally, returning from the research arena to the clinical practice, many CD patients are eager to consume a gluten containing diet. As stated above, a specific chapter in this book is dedicated to novel treatment strategies beyond a gluten diet, and thus are not covered here. We hope that the various research priorities delineated in our chapter will pave way to better therapeutic options in pathways that are already studied as well as new therapeutic modalities, that will enable patients to enjoy better quality of life in full remission.

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