The molecular investigation of celiac disease

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ABSTRACT

Celiac disease (CD) is an immune-mediated disorder resulting in nutrient malabsorption now thought to have a prevalence of 1:100 in the Iranian population.

Symptoms of CD are included diarrhea, abdominal pain, steatorrhea, bloating, cramps, flatulence, weight loss, weakness and short stature. In addition to presenting symptoms, patients are also at increased risk of metabolic bone disease, lymphoma (enteropathy-associated with T-cell) and other malignancies in different parts of the body such as gastric, esophageal, bladder, breast and brain. There appears to be a strong genetic component to this disease. In this short review we provided the historical, clinical and genetic aspects of this disease and highlight numerous findings from recent molecular immunology studies.

Keyword: Celiac disease; Molecular investigations; Symptoms

INTRODUCTION

Until recently, CD was considered a comparatively uncommon disorder, with a prevalence rate quoted as 1 in 1000 or lower and considered a disease of essentially European origin but now thought to have a prevalence of between 1:100 to 1:200 in Europe as well as Iranian population [1]. CD develops as a result of an interaction between genetic, environmental, and immunological factors. The clinical presentation of CD is highly variable and ranged from typical symptoms include diarrhea, steatorrhea, bloating, Osteoporosis, anemia, vomiting, delayed puberty, flatulence, weight loss and fatigue to atypical symptoms included abdominal pain, dyspepsia, infertility, neurological disorders and constipation [2].

In addition to the incidence of presenting symptoms, celiac patients are also at increased risk of osteoporosis, non-Hodgkin and enteropathy associated T-cell lymphomas (NHL and EATL) and other malignancies [3]. Celiac disease also is an association with a variety of diseases with an autoimmune etiology such as autoimmune cholangitis, hepatitis, myocarditis, neuropathy dermatitis herpetiformis, primary biliary cirrhosis, Down's syndrome, sarcoidosis, epilepsy, Sjogren's syndrome, diabetes mellitus type I and, thyroid disease [4-14].

Celiac disease may develop in only genetically susceptible individuals. The strongest genetic factor that is associated with CD is human leukocyte antigen (HLA)-DQ2 and -DQ8 and is found in virtually all CD patients and the absence of HLA-DQ2 or -DQ8 virtually excludes the diagnosis of CD [15].

In this review I investigated the recent immunological, genetic and molecular evidence from the literature to suggest a mechanism underlying the pathophysiology of this disease.

Gene Identification in Celiac Disease

Genetic linkage and genetic association studies are complementary advances which used in the search for genetic susceptibility genes in CD. Genetic linkage studies make use of families with affected sibling pairs to identify chromosomal regions shared between the affected siblings above the mean of what is statistically expected (Figure 1). Linkage regions

usually encompass 10 to 100 genes. Once linkage is identified, the next step is a genetic association study to identify the specific disease gene from the candidate gene locus [16].
Candidate gene association studies search for differences in the frequencies of genetic variants in patients compared to control individuals. Such association studies can focus on positional candidate genes from a linkage region, or on functional candidate genes selected from Hypothesized disease pathology [17]. More recently, it has become feasible to perform genome wide association studies which can test thousands of SNPs across the whole genome for association.

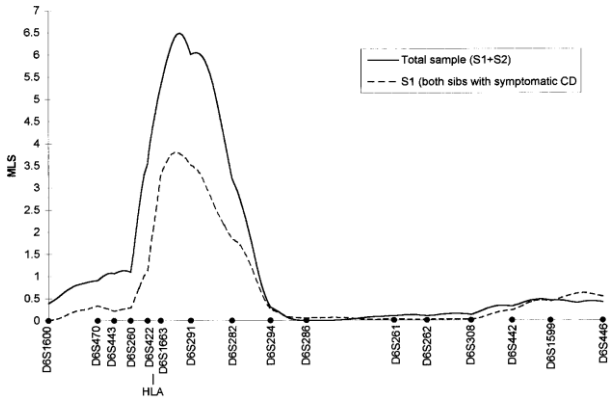

Figure 1. Distribution of the MLS scores for chromosome 6.

Native Immunity Correlates with Celiac Disease

Different investigators cleared the role of HLA-DQ2 in the pathogenesis of CD, but none of them deal with why wheat is a harmless food in most people with HLA-DQ2 or HLA-DQ8 [18, 19]. Since the immunogenic gluten epitopes to the adaptive immune system in a pro-inflammatory circumstance released; therefore, the lack of immunological tolerance to gluten can be understood. Any loss of the entirety of gut epithelium would allow the activation of the resident immune system to any "nonself" antigens leaking in from the gut lumen [19].
The identification of the triggers for loss of epithelial cells is related to the presence of gluten antigens in the lamina propria, and the beginning of HLA-DQ2/DQ8-restricted adaptive immune response.
Undoubtedly the key to explaining the presence of gluten antigens in the lamina propria, and the initiation of the HLA-DQ2 and/or -DQ8-restricted adaptive immune response, is then to identify the trigger for such a loss of epithelial integrity.
Hue et al. in 2004 showed that toxic p31–49 peptide induced the term of the atypical MHC molecule MICA on the cell surface of the villus epithelium in treating celiac patients but not general controls [20]. Also high levels of MICA surface expression detected in untreated celiac patients and that the surface levels appeared to associate with the clinical severity of disease. MICA is induced by cellular stress and serves as a ligand for the NKG2D receptor. The NKG2D receptor is found on the surface of CD8+ αβ T cells, γδ T cells and most NK cells [21]. Compatible to these findings Meresse et al. demonstrated that resident intraepithelial lymphocytes in the small intestine of celiac patients are capable of lysing epithelial cells solely through the NKG2D-MICA interaction and express the NKG2D receptor [22].

Genetics of Coeliac Disease

The higher prevalence of CD in sibs of CD patients has shown in several studies compared with the general population [23, 24].
In Bourgey et al. Study CD status was determined and HLADQ genotyping performed in the cohort of 188 Italian families with CD. The result of this study showed the overall risk a sib of a CD patient will develop the disease was estimated at 10%. These results make it possible to provide more accurate information to parents with a child with CD about the real risk for another child [2].
Genetic component was found in around 87% of a large cohort of 73 Italian twins with at least one affected with CD [25]. As expected, concordance rates were significantly observed in monozygotic than dizygotic twins with an adjusted hazard ratio for developing CD in those with an affected monozygotic twin of 14.3%.

Fasano et al. screened 13 145 people using modern serology techniques and histopathological biopsy confirmation in a large-scale multicentre study in the USA [26]. The result of this study showed that the prevalence of CD in first-degree relatives was 1:22, in second-degree relatives was 1:39 and in symptomatic patients was 1:56. The overall risk for individuals without these risk factors was 1:133.

Celiac disease susceptibility has been strongly associated with HLA-DQ2 and HLA-DQ8. The HLA DQ2 (DQA1*05/DQB1*02) heterodimer is present in approximately 95% of patients with CD, and the remaining patients (5–10%) have HLA-DQ8 (DQA1*0301/DQB1*0302). HLA-DQ2 is common in Europeans and is expressed by 25-30% of the normal European population. Consequently, the estimated HLA contribution to the development of the CD is estimated to be approx. 35% [27-35]. Most of the CD patients who do not carry DQ2.5 or DQ8, suggesting that carrying part of the risk molecules has functional implications for the risk of CD [35].

The lack of these alleles has a high negative predictive value in general and therefore, HLA-DQ2 and/or DQ8 are best thought of as necessary but not sufficient for the development of celiac disease.

Several studies on Iranian population healthy population using PCR/RFLP method in different parts of Iran show that the most common haplotype in different ethnic groups like Turk, Kurd, Lur, Arab, Turkmen and Baloch is DQ7 and DQ2 [36-39] (table 1).

Table 1. HLA-DRB1, DQA1, and DQB1 allele and haplotypes frequencies in different ethics of Iran

Ethic	DR (%)	DQ A1(%)	DQ B1(%)	Haplotype (%)
Shiraz	B1*11 (25)	*0501 (39)	*0301 (31)	DRB1*11-DQA1*0501-DQB1*0301 (25)
Kurd	B1*1103/04	*0501	*0301	DRB1*1103/04-DQA1*0501-DQB1*0301
Azari	B1*1103/04	*0501	*0301	DRB1*1103/04-DQA1*0501-DQB1*0301
Baloch	B1*0301 (29)	*0101/2 (42.5)	*0201 (32)	DRB1*0301-DQA1*0501-DQB1*0201 (22.1)
Zoroastrian	B1*0701	*0501	*0201	DRB1*0701-DQA1*0201-DQB1*0201

These results conducted significant similarities in HLA class II haplotype distributions with European countries.

These days diverse techniques are carried out to detect the HLA risk alleles in different populations. Koskinen et al. [35] and Monsuur et al. [36] used tagging single nucleotide polymorphisms (SNPs) method for detecting the HLA risk alleles. The results of showed that the sensitivity and specificity of HLA-SNP to recognize DQ2.2, DQ2.5, DQ7, and DQ8 haplotypes in the different European population was higher than >98% (figure 2). Also using this method Rostami Nejad et al. evaluate the distribution of HLA DQ2 and DQ8 in Iranian CD patients compared to healthy controls [41]. The results of this study showed that the frequency of DQ2 was higher in CD patients than controls and also the prevalence of DQ8 was higher than that reported in other populations.

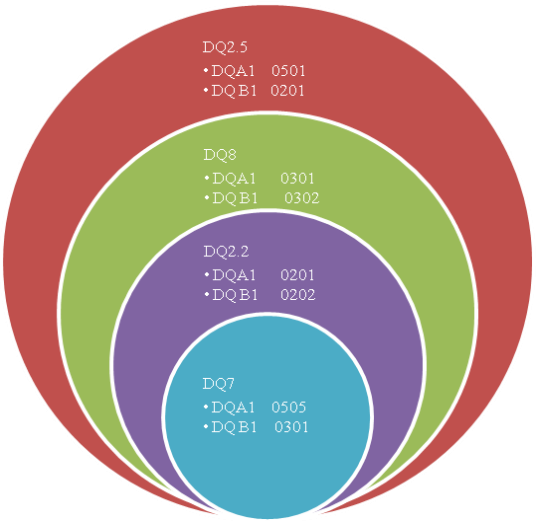

Figure 2. Genetic risk associated with the different HLA-DQ molecules.

HLA-DQA1* and -DQB1* together form heterodimers of which DQ2.5 and DQ8, either in homozygous or heterozygous state, confer risk to CD due to their ability to present gluten to T cells. DQ2.2 and DQ7 can only confer risk to CD when both are present together or with DQ2.5 (trans effect). Patients who carry DQ7 haplotype are low risk for CD, an intermediate risk if they were

homozygous for HLA-DQ2.2, DQ8, heterozygous for DQ2.5 or for DQ2.2, a high risk for those homozygous for DQ2.5and DQ2.5/DQ2.2

Trynka et al. in their large sample collection study genotyped 183 non-*HLA* risk loci in 12,041 patients with celiac disease and 12,228 controls using variables from the 1000 Genomes Project pilot European dataset [42]. They identified 13 new CD risk loci and found multiple independent association signals at over one-third of these loci. In particular, 29 of the 54 fine-mapped signals seemed to be localized to single genes and, in some instances, to gene regulatory elements.

During 2010, 27 seronegarive children with celiac disease and 9 of their brothers were evaluated by PCR, DQ2/DQ8 haplotypes [43]. The result of this investigation confirmed the diagnosis of CD in 22 children and 7 of their brothers. The outcome of this study is underlying the importance of the genetic testing in seronegative patients and also in identifying family risk for people with celiac disease.

CONCLUSION

Celiac disease is a genetically determined pathological condition associated with HLA genes that code for the DQ2 and DQ8 molecules. Various studied suggested that the HLA-DQB1*02 allele (coding HLA DQ2) in addition to having an important role in the predilection of the CD, also it is in contribution with the severity of the intestinal mucosa damage [44-48].

Based on these findings, Biagi et al. reported that patients with homozygous for HLA DQ2 not only have more severe clinical symptoms, also develop more severe intestinal lesions than is normally observed which take longer to heal after gluten free diet [49].

The previous studies confirmed that HLA-DQ genes code for proteins responsible for the histologic, immunological, and clinical heterogeneity of the celiac disease [50, 51]. In contrast, even if there is a correlation between the risk of CD and the dose of the DQB1*02 allele, it is certainly not possible to predict whether and when these patients will develop the disease, if there is any risk of complications and if the degree of mucosal atrophy will be more severe. Also we should attention to this point that this condition depending on environmental factors such as the time of exposure to gluten and the patient's compliance to a gluten-free diet.

Rashtak, Murray in 2007 suggests that when there is a high suspicion of CD, HLA typing is a high-sensitivity rule-out test and when the probability is low serological testing is a high-specific rule-in test [50]. This strategy might be helpful in encouraging health professionals to use serology because the index of suspicion is generally low for atypical presentations [2]. In contrast, using serology alone might result in missing those patients with negative serology even when the suspicion is low [52-54]. Perhaps performing HLA typing in seronegatives would give some more degree of reassurance in ruling it out and finally I suggest that the performance of readily available serological tests in combination with the genetic risk study, significantly reduce the burden of celiac disease in at risk population.

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