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Review

Genetic susceptibilty and celiac disease: what role do HLA haplotypes play?

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Summary. Celiac disease is a chronic immune-mediated enteropathy triggered by exposure to dietary gluten in genetically predisposed individuals. Many genes involved in the pathogenesis have been identified and a crucial role is known to be played by the Human Leukocyte Antigen (HLA) system. The main determinants for genetic susceptibility are HLA-DQA1 and HLA-DQB1 genes encoding for HLA-DQ2 and HLA-DQ8 molecules, carried by almost all patients affected. However, since HLA-DQ2 and HLA-DQ8 heterodimers explain almost 40% of the disease heritability, HLA typing should not be applied in diagnosis, but exclusively to clarify uncertain diagnoses, considering its negative predictive value. (www.actabiomedica.it)

Key words: celiac disease, HLA typing, diagnostics, genetic predisposition

Introduction

Celiac disease is a chronic immune-mediated enteropathy triggered by exposure to dietary gluten in genetically predisposed individuals (1). In celiac patients, the ingestion of gluten leads to the activation of both the innate and adaptive response of the immune system, with a subsequent chronic inflammation that determines changes in the mucosal structure including villous atrophy, crypt hyperplasia and lymphocyte infiltration. These changes in structure cause subsequent loss of function by the intestinal mucosa and the onset of symptoms brought by nutrient malabsorption.

The range of clinical manifestations in celiac disease varies widely, with a high prevalence of asymptomatic individuals. For these reasons, the disease itself has been represented as an iceberg: the tip is associated with classical symptoms of nutrient malabsorption; the largest part of the iceberg corresponds to atypical manifestations, as well as silent and latent phenotypes (2).

Diagnosis is based on serological tests, whose aim is to search for auto-antibodies produced by the activation of B lymphocytes following gluten ingestion, and small bowel biopsy.

According to the European Society of Pediatric Gastroenterology Hepatology and Nutrition (ESPGHAN), the diagnosis of celiac disease can be confirmed by an elevation in the specific antibody titer associated to an alteration of the duodenal mucosa seen at the histologic analysis, or if the antibody titer exceeds 10 times the upper limits in association to typical symptoms of malabsorption (1).

The main auto-antibodies used in the diagnostic process are anti-tissue transglutaminase (anti-TG2) and anti-endomysium (EMA) antibodies. Deamidated Gliadin Peptide (AGA-DPG) antibodies are used along with anti-TG2 in diagnosing celiac disease in the pediatric population younger than 2 years of age.

Small intestine biopsy is performed during esophagogastroduodenoscopy (EGDS) and it is followed by

histological analysis. The disease activity grade according to the histological findings on the biopsy is classified based on Marsh-Oberhüber criteria.

So far, the only available and effective treatment for celiac disease is the adoption of a life-long gluten-free diet, that could lead to the normalization of serological parameters and the regression of mucosal damage.

Celiac disease and genetics

Celiac disease has a multifactorial etiology, linked to the contribution of both the genetic predisposition and various environmental factors, including gluten and the timing of its introduction in infant diet. Other possible environmental factors are under study as possible concurring element in etiology, such as an alteration in the intestinal microbiota, an alteration in the intestinal mucosa permeability, and infections.

CD development depends on the presence of key genes that orchestrate the immunological response to dietary gluten. Genetic risk genes are searched with the help of two complementary methods: genetic linkage and genetic association studies. Genetic linkage studies identify common chromosomal regions shared by affected siblings using Single Nucleotide Polymorphisms (SNPs) as genetic markers. After linkage has been identified, association studies are used to identify the disease-specific gene from the candidate gene locus. This type of study compares frequencies of genetic variants in patients with those in controls (3).

Many genes involved in the pathogenesis have been identified and a crucial role is known to be played by the Human Leukocyte Antigen (HLA) system. The HLA super-locus is a genomic region placed on the short arm of chromosome 6 (6p21), where it encompasses approximately 4000 kb. This super-locus can be separated into five HLA regions: the extended class I, class I, class III, class II and extended class II regions. It contains hundreds of genes with immunological functions and it is characterized by a high gene density and variability and an extensive linkage disequilibrium. This phenomenon, where certain combinations of alleles are passed on to the offspring more often than it is usually expected, makes it very difficult to determine whether a specific gene is involved directly in the disease susceptibility as a causal genetic variant or whether it marks the effect of linked genes. The term "haplotype" is used to refer to the set of alleles on a single chromosome that are inherited together (4, 6).

In the case of Celiac Disease specific alleles of the HLA system are involved in the pathogenesis of the disease.

HLA class I and II regions comprise genes encoding for proteins that have important roles in the regulation of the immune system, such as glycoproteins for antigen presentation to the immune cells, as well as some other fundamental molecular and cellular processes (4, 5).

Glycoproteins for antigen presentation encoded by HLA class II are heterodimers constituted by an alpha-heavy chain and a small beta2-microglobulin chain whose genes map on the HLA-D region, comprising HLA-DP, HLA-DQ and HLA-DR genes (6). These molecules present exogenous antigens to CD4+ lymphocytes, which activate the humoral response.

Glycoproteins encoded by HLA class I (A, B and C) are instead involved in the presentation of endogenous antigens to CD8+ lymphocytes that trigger an immune cytotoxic response.

In particular, HLA-DQA1 and HLA-DQB1 genes are the main determinants for genetic susceptibility, referred to as CELIAC1 by the HUGO Gene Nomenclature Committee (http://www.genenames.org/) and encoding for HLA-DQ2 and HLA-DQ8 molecules, carried by almost all patients presenting the disease (7).

Almost 95% of patients with CD express HLA-DQ2 and the rest of them usually carry the HLA-DQ8 heterodimer, encoded by DQA1*0301-DQB1*0201 alleles.

HLA-DQ2 heterodimers are encoded by DQA1*05 and DQB1*02 alleles, which are involved in the formation respectively of the α and β chains of the heterodimer. They could be inherited in one out of two different configurations: DQ2.5*cis,* on the same chromosome, or *DQ2.5trans*, where each allele is encoded on one of the two homologous chromosomes, one chromosome from each parent (6). The DQ2.5*cis* very frequently appears in linkage disequilibrium with DRB1*03:01 allele, which was first associated with CD risk (8).

However, the presence of these alleles is necessary but not sufficient for disease development: in fact, although HLA-DQ2 allele is common in the white population (30% of people are carriers), almost 3% of them will develop CD (9, 10). Risk of developing CD for people carrying the risk alleles is estimated being between 36-53% (11). Furthermore, risk of developing CD depends on gene dose, so far only demonstrated for HLA-DQ2: homozygous individuals have a risk at least 5 times higher than heterozygous individuals (12). A familiar aggregation has been found in 5-15% of patients as well as a higher concordance rate of celiac disease in monozygotic than in dizygotic twins (83-86% vs. 11%) (3).

Genetic linkage studies have identified other three chromosomal regions officially recognized as genetic predisposing factors for celiac disease so far: 5q31-q33 (CELIAC2), 2q33 (CELIAC3) and 19p13.1 (CELIAC4).

HLA influence on CD susceptibility shows a dose effect. CD risk can be classified according to the number of DQA1*05 and DQB1*02 alleles carried by the individual. Homozygosity for DQ2.5*cis* and heterozygosity for DQ2.5*cis* with a chromosome possessing a second DQB1*02 allele (DQ2.2) confer the highest risk to develop CD. Heterozygosity for DQ2.5*cis* in individuals with a single copy of DQB1*02 (non-DQ2.2) or presence of DQ2.5*trans* confer intermediate risk. DQ2 negativity suggests an extremely low chance of developing CD (13).

Since HLA-DQ2 and HLA-DQ8 heterodimers explain almost 40% of the disease heritability, the remaining 60% is estimated to be shared between an unknown number of non-HLA genes. Recently, genome-wide association studies identified many non-HLA genes that may be involved in the risk of developing CD. They are involved in controlling the immune response and among them we can find genes encoding for IL2, IL21, CTLA4, CCR3, IL 12A, AH2B3 and TAGAP (14).

Although their contribution to the onset of the disease is weak (almost 15%) it has been shown that they could aid to identify those individuals who are at higher risk for CD (14, 15).

Interestingly, it seems that the presence of the haplotype AH 8.1 could be an additional factor risk to CD. Together with AH 18.2, they are called "ancestral haplotypes" and are made up of DQA1*05, DQB1*02 and DRB1*03:01 alleles. These alleles can also be found within non-specific allelic combinations and constitute other less frequent haplotypes. DRB1*03:01 haplotypes have been associated to numerous immune-mediated disorders, as type 1 diabetes, multiple sclerosis or selective IgA deficiency. In some cases, different DRB1*03:01 haplotypes showed a different contribution to the risk of developing the disease (13).

A study conducted in the Saharawi population demonstrated how the genes located in the previously mentioned haplotype (here mentioned as B8/DR3/DQ2) could also be related to specific clinical manifestations of the disease. The study showed how its presence was more related to atypical forms than typical ones and was not significantly implicated in the susceptibility of CD (16). This could be considered a probable future implication of HLA-DQ2 in the clinical practice: not only used as a risk-predictor, but also as a parameter to foresee the possible clinical manifestations of the disease.

HLA typing in clinical practice

Up to now, genetic testing in the clinical practice of celiac disease has been proved to be useful only for HLA-DQA1 and HLA-DQB1 genes, which are strongly associated with the risk of disease onset.

However, HLA typing should not be applied in diagnosis, but exclusively used to clarify uncertain diagnoses, considering its negative predictive value: its positivity does not necessarily predict the certain onset of the disease, but indicates a genetic predisposition to develop the disease. On the other hand, when predisposing genes are not present, it is very unlikely for the patient to develop celiac disease in the future (6, 17).

According to ESPGHAN 2012 guidelines, HLA typing should be performed in patients with uncertain diagnosis of CD: this category includes patients with negative serology and mild infiltrative changes in small intestinal biopsy specimens (1).

In pediatric age, HLA typing should be performed to add strength to diagnosis in presence of clinical symptoms referred to CD and positive serol-

ogy (anti-TG2 antibodies higher than 10 times the upper limit threshold and positive EMA). In this case small bowel biopsy may be omitted.

Furthermore, in asymptomatic patients at risk for developing CD, HLA typing could be used to select those who will need a strict follow-up based on periodical (usually annual) antibody testing. This group of patients includes those people who are more likely to develop CD than the general population, such as patients affected by another autoimmune-mediated disease such as Diabetes Mellitus Type I, autoimmune thyroid diseases, autoimmune liver diseases or by chromosomal disease such as Down syndrome, Turner syndrome, Williams syndrome, (1). Diabetes Mellitus Type I (T1D) and CD are both multifactorial disease sand co-occur in families and even in single patients, more often than expected in the general population. Approximately 4-9% of patients with T1D also have CD while patients with CD are at increased risk of developing T1D.

Genetic testing is also an important tool for the screening of celiac patients' first degree-relatives who present uncertain serology or symptoms suggestive of malabsorption, in order to identify those who will need to undergo serological follow-up. In fact, it has been demonstrated that 20% of siblings and 6% of parents who had a positive genetic test, resulted to be affected with celiac disease. The risk is very high for DQ2 and/or DQ8 alleles carriers, but interestingly a high risk seems to be associated even to the presence of just DQB1*02 allele, carried in double dose, with the absence of DQA1*05 allele (18, 19).

Targeted screening for CD is recommended both for a benefit in management of other autoimmune diseases and for the risk, in untreated patients, of iron deficiency anemia, growth retardation, osteoporosis, fertility problems, neurologic disease and gastrointestinal malignancies such as intestinal lymphoma.

Besides, HLA typing is not recommended as an initial only screening test in people at average risk for celiac disease due to its poor positive predictive value.

Discussion

Over the last years, great importance has been given to HLA genotyping in the prediction and prognosis of many autoimmune diseases including celiac disease. Almost 95% of patients with CD express HLA-DQ2 and the rest of them usually carry the HLA-DQ8 heterodimer, encoded by DQA1*0301-DQB1*0201 alleles. However about 30%-40% of the general population carry HLA-DQ2 and/or DQ8 without developing CD. HLA typing is considered an excellent tool to be used in association to more specific tests like endoscopy, when serology is ambiguous in subjects being investigated for CD (4-8). Additionally, in individuals considered at high risk for CD as first-degree relatives, patients with immunoglobulin A deficiency, other autoimmune diseases or with Down, Turner or Williams syndromes, the HLA typing is used to identify those people in which these alleles are absent and exclude them from further investigations. The absence of predisposing alleles spares unnecessary serial serological testing while confirmation of their presence reflects an increased likelihood of developing CD. Thereby, in individuals disclosing HLA predisposing alleles, periodic screening for auto-antibodies against IgA tTG and IgA EMA should be considered to avoid a misdiagnosis of subclinical or silent forms of the disease.

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