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# HLA-DQ and risk gradient for celiac disease

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ABSTRACT

Celiac disease (CD) is a rare example of multifactorial disorder in which a genetic test is of great clinical relevance, as the disease rarely develops in the absence of specific HLA alleles. We typed *DR-DQ* genes in 437 Italian children with celiac disease, 834 first-degree relatives, and 551 controls. Of patients, 91% carried DQ2 and/or DQ8 heterodimers, 6% only had $\beta$2 chain, 2% was $\alpha$5 positive, and four were DQ2/DQ8/$\beta$2/$\alpha$5 negative. Only the presence of $\alpha$5 resulted negatively associated to disease ($p = 2 \times 10^{-4}$), whereas we confirmed the effect of the $\beta$ half of DQ2 dimer on CD predisposition ($p = 4 \times 10^{-12}$). Considering 1:100 disease prevalence, we obtained a risk gradient ranging from 1:7 for DQ2 and DQ8 individuals down to 1:2518 for subjects lacking all predisposing factors. The *DQB1*02* and *DQB1*0302* concurrence ($p = 9 \times 10^{-4}$), besides the *DQB1*02/*02* homozygosity, had an additional role in disease genetic determination. The CD prevalence rose to 17.6% in sisters, 10.8% in brothers, and 3.4% in parents. In the three groups, the subjects carrying high-risk HLA molecules were 57%, 71%, and 58%; among them, 29%, 15%, and 6% respectively had CD. Those siblings and parents with no susceptible factors were not affected. These findings indicate the impact of the HLA test for CD in clinical practice.



## 1. Introduction

Celiac disease (CD, MIM 212750) is a chronic intestinal inflammation resulting in villous atrophy and flattening of the mucosa. The disease occurs in genetically predisposed individuals in response to the dietary ingestion of wheat gluten and similar proteins in barley and rye. The treatment consists of lifelong gluten exclusion from the diet that leads to histologic and clinical remission and prevents the development of refractory CD and long-term complications such as malignancy, osteoporosis, infertility, and autoimmunity.

Originally considered a rare malabsorption syndrome in childhood, CD is now recognized as a common disorder that may arise at any age, with a growing proportion of new cases diagnosed in adults and in patients with extraintestinal manifestations. Recent accurate epidemiologic studies have revealed that CD affects approximately 1% of the general population, both in Europe and in North America [1]. The prevalence of the disease increases among patients with anemia or autoimmune diseases, with short stature, or with Down, Turner, or Williams syndrome [2]. Moreover, CD clusters in families with a prevalence among first-degree relatives ranging from 2.8% to 17.2% in different series [3].

Although CD is one of the most common lifelong diseases in western countries, most affected individuals remain undiagnosed [1]. This is apparently because many patients have atypical symptoms or none at all. The disease is characterized by the production of anti-tissue transglutaminase (anti-tTG) and anti-endomysial (EmA) antibodies. Serologic screening for the presence of these autoantibodies in individuals with characteristic symptoms of CD or with associated conditions is usually the initial step in detecting new cases. Although anti-tTG and EmA appear to be good markers of the active phase of the disease, the definitive diagnosis requires a small-bowel biopsy showing the typical histologic abnormalities (villous atrophy, crypt hyperplasia, and leukocyte infiltration).

CD mostly develops in HLA-DQ2 (*DQA1*05* and *DQB1*02*)–positive individuals, whereas most of the remaining cases are HLA-DQ8 (*DQA1*03* and *DQB1*0302*) positive. The close association can be explained by the fact that the DQ2 and DQ8 $\alpha/\beta$ heterodimers mediate the activation of gluten-reactive $CD4^+$ T cells in the gut. In particular, the disease-associated HLA-DQ molecules expressed on antigen-presenting cells specifically bind gluten-derived peptides, modified by the enzyme tTG, and present them to intestinal T cells. The resulting T response leads to the production of the disease-specific antibodies and to the secretion of pro-inflammatory cytokines with consequent mucosa atrophy and clinical manifestations.

The European Genetic Cluster on Celiac Disease has demonstrated that practically all CD patients carry HLA-DQ2 and/or HLA-DQ8 molecules or one chain of the DQ2 heterodimer, coded by *DQA1*05* ($\alpha$5 chain) or *DQB1*02* ($\beta$2 chain) alleles, and that CD occurs only exceptionally in the absence of at-risk DQ factors [4]. Moreover, a gene dosage effect for the *DQB1*02* allele has been

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described in several studies [5,6]. Given the strong association, the HLA typing is routinely used as a genetic test for CD; the presence of susceptible DQ variants does not predict certain developments of the disease but strongly modifies the risk, whereas their absence makes CD very unlikely with a negative predictive value close to 100% [7]. We have recently reported evidence of gender differences in this association, with a different negative predictive value for the HLA test in female and male subjects, indicating the need to consider the gender in the disease-risk calculation [8].

Despite the fact that the diagnostic significance of the HLA test for CD is not absolutely certain, it is generally considered that it may be of help in the definition of uncertain cases. The analysis is also recommended in at-risk groups, such as first-degree relatives of patients, to decide the follow-up [2]. Indeed, the HLA genes are lifelong stable markers and their typing may discern subjects genetically susceptible or nonsusceptible to the disease long before the possible appearance of clinical or serologic signs.

The HLA-DQ association with the risk of CD has been extensively discussed, but knowledge of the practical usage of the HLA typing as a genetic test for the disease remains limited and the clinical implications of the results are still not clearly defined. We present here our experience with the HLA testing in a cohort of Italian pediatric celiac patients and first-degree relatives collected over the last 20 years, with the aim to contribute to the development of clinical practice guidelines for the use of the HLA typing as a predictive test for CD.

## 2. Subjects and methods

### 2.1. Patients and relatives

All subjects ($N = 1271$) except two siblings were described in a previous report (8). Briefly, they included 145 CD patients and 292 nuclear families (292 index cases, 34 affected and 216 unaffected siblings, and 20 affected and 564 unaffected parents).

The 437 index cases had a median age of 5 years 8 months at sample collection. Age in siblings ranged from 1 to 20 years with a median of 10 years. No differences were observed between affected and unaffected cohorts.

All relatives were tested for anti-tTG and EmA antibodies, and selected individuals underwent a small-intestinal biopsy, as previously described [9].

Informed consent was obtained from each participant.

### 2.2. Controls

The control sample ($N = 551$) included 292 healthy Italian individuals and 259 affected family based controls, as previously reported [8].

### 2.3. HLA typing

All individuals were typed for *DRB1*, *DQA1,* and *DQB1* genes by sequence-specific primer–polymerase chain reaction (SSP-PCR) using commercial kits (Dynal Biotech, Bromborough, UK).

### 2.4. Nomenclature

The term DQ2 belongs to the serologic HLA nomenclature, and it specifies an epitope on the $\beta$2 chain. However with time, in CD, the term has usually referred to a particular $\alpha$5$\beta$2 DQ2 dimer encoded by *DQA1*05* and *DQB1*02* alleles. Therefore, we only use DQ2 to indicate subjects carrying both the alleles, whereas individuals *DQA1*05* negative/*DQB1*02* positive, in which the $\beta$2 chain forms dimers with a different $\alpha$ chain, are simply named $\beta$2. A single or double dose of *DQB1*02* is indicated as *B1*02/X* or *B1*02/*02,* respectively. The phenotype coded by *DQA1*05* allele in absence of *DQB1*02* is designated as $\alpha$5.

To specify a particular haplotype, we adopted a code in which the first two digits represent *DRB1*, the third *DQA1,* and the fourth *DQB1* alleles. The fifth digit, when present, denotes the *DQB1*03* variants. For example the code 0352 stands for *DRB1*03-DQA1*05-DQB1*02*; 0432 stands for *DRB1*04-DQA1*03-DQB1*02*; 04332 stands for *DRB1*04-DQA1*03-DQB1*0302*; and 11531 stands for *DRB1*11-DQA1*05-DQB1*0301*.

### 2.5. Statistical analysis

Statistical significance was calculated by Fisher's exact test using $2 \times 2$ contingency tables. Values of $p < 0.05$ were considered significant.

Disease risks are expressed as 1:N, where N is the number of individuals among which one patient is present. Considering a disease prevalence of 1:100 in the general population, for each HLA-DQ category, N is calculated as a percentage of controls with that particular HLA-DQ status multiplied by 100 and divided by percentage of patients with the same DQ typing.

The risks in female and male subjects were calculated using the DQ frequencies previously reported in the two genders and considering the 1.8:1 female to male ratio [8].

## 3. Results

The case-control study of the 437 celiac children and 551 controls was previously described [8]. Briefly, 91.1% patients and 29.0% controls carried DQ2 and/or DQ8 heterodimers. Among the DQ2/DQ8-negative individuals, the frequencies of cases carrying *DQB1*02* ($\beta$2), *DQA1*05* ($\alpha$5), or neither of the two alleles were 66.7%, 23.1%, and 10.2%, respectively versus 14.1%, 53.4%, and 32.5% of the controls, showing a positive association with CD of the $\beta$2 phenotype ($p = 4.3 \times 10^{-12}$) but a negative association of the $\alpha$5 phenotype ($p = 2.2 \times 10^{-4}$).

Two alleles coding for at-risk DQ beta chains (**02/*02*, **02/*0302,* or **0302/*0302*) were found in 30.4% cases and 3.8% controls, leading to a very high $p$ value ($p = 5.5 \times 10^{-32}$). The *DQB1*02/*02* combination was found in 107 of 437 patients and 15 of 551 controls ($1.8 \times 10^{-26}$), and *DQB1*02/*0302* was observed in 24 of 437 and 5 of 551 ($1.8 \times 10^{-5}$). Two case patients and one control were *DQB1*0302* homozygous. Of the total 56 DQ8 cases, only one lacked the *DQA1*03* allele carrying the *DRB1*03-DQA1*05-DQB1*02* haplotype and an unusual *DRB1*11-DQA1*05-DQB1*0302* combination.

Based on the HLA-DQ typing, we obtained a gradient of disease risk ranging between 1:7 and 1:2518. Figure 1 reports the values estimated from the whole sample and in the two genders separately. Looking at the total data set, the highest risk value was for DQ2- and DQ8-positive subjects (1:7), followed by the other three categories coding for two $\beta$ susceptibility chains: DQ2, *B1*02/*02* (1:10), DQ8, *B1*02* positive (1:24), and $\beta$2, *B1*02/*02* (1:26). DQ2 subjects carrying a second *DQB1* allele different from **02 or *0302* (*B1*02/X*) resulted in a risk of 1:35. In this category, cis/trans arrangements were considered together because of the lack of statistical significance though the trend was similar to that described in other studies [10,11]. The presence of DQ8, when *DQB1*02* negative, led to a disease likelihood of 1:89; individuals *DQB1*0302* homozygous were considered together with the heterozygous ones because of their small number (two patients and one control). In the remaining three categories, the disease probability was lower than in the general population (1:100), resulting in 1:210 for $\beta$2 (*B1*02/X)* subjects, 1:1842 for the $\alpha$5 ones, and 1:2518 in the absence of all the susceptible HLA-DQ factors. In this gradient, a single or double dose of the *DQA1*05* allele was not specified because a significant different contribution to CD predisposition was not revealed. The F:M proportion in the whole cohort of patients was 1.8; however, when the patients were stratified by HLA-DQ typing, the risks in the presence of $\beta$2 phenotype (*B1*02/*02* or *B1*02/X*) gave very similar values in the two genders, and the $\alpha$5 status, even if rarely found in patients, was mostly present in male subjects [8].