Risk of Pediatric Celiac Disease According to HLA Haplotype and Country


**Section Editor:** Abhijeet Saha

**Summary**
This multi-centric cohort study [1] from four countries followed a group of infants with the HLA haplotype DR3–DQ2 or DR4–DQ8, from birth through the first few years of life; seeking the appearance of antibodies to tissue transglutaminase (tTG) (labeled as celiac disease autoimmunity), and development of celiac disease. This was part of a larger study evaluating the development of type 1 diabetes in a cohort of infants with genetic susceptibility (based on carrying the HLA haplotype DR3-DQ2 or DR4-DQ8) [2]. Over a median follow-up duration of nearly five years, the investigators reported 12% prevalence of celiac disease autoimmunity and 3% prevalence of celiac disease. They also identified that the respective risks of these two outcomes varied by the HLA genotype: 26% and 11% with homozygosity for DR3-DQ2 haplotype; 11% and 3% with DR3-DQ2/DR4-DQ8 haplotype; 8% and 3% with DR4-DQ8 homozygosity; and 3% and <1% among those with DR4-DQ8/DR8-DQ4 haplotype. There was statistically significant higher risk of celiac disease autoimmunity and celiac disease in infants from Europe (highest risk in Sweden), female gender, and those with family history of celiac disease.

**Commentaries**

**Evidence-based-medicine Viewpoint**

*Relevance:* Data from developed countries suggest celiac disease prevalence of 1% [3] in the general pediatric population. A similar prevalence has been reported in India [4]. There is significantly higher prevalence among those with type I diabetes (3-10%) and family members of those with celiac disease (5-20%) [5-8]. HLA DQ2 and DQ8 are considered the most important genetic risk factor for celiac disease; these haplotypes are present in nearly all patients with celiac disease [9,10]. The absence of these haplotypes has very high negative predictive value for celiac disease [11,12]. However, HLA-DQ2 is present in about one-fourth to one-third of the unaffected Caucasian population; hence it alone has low positive predictive value [13], although combination with anti tTG IgA and endomysial antibody (triple test) improves it to nearly 100% [14]. Against this background, the cohort study [1] can be regarded as highly relevant. In the Indian context, there is limited information that celiac disease is associated with multiple DR3-DQ2 haplotypes [15,16] although population-based studies are unavailable.

**Critical appraisal:** The Critical Appraisal Skills Programme (CASP tool) [17] was used to evaluate this study (Table I).

**Extendibility:** Some authors have sought to identify whether the HLA loci associated with celiac disease in Caucasian (European) population is similar in other ethnic groups. In a cohort of North Indian people, three of the several loci seen in Europeans, were observed to show strong association [18]. However other authors have suggested that there are three sets of HLA-DR3 haplotypes associated with development of type 1 diabetes [19]. Although the B8-DR3 haplotype confers the highest risk, there is considerable diversity of this haplotype in Indians unlike the single fixed haplotype observed in Caucasians [20]. These data suggest that while the results of the multi-centric cohort study may be relevant to India, the data cannot be directly extrapolated.

**Conclusions:** This cohort study suggests that infants with certain HLA haplotypes (especially DR3-DQ2 homozygosity) have higher risk for development of celiac disease autoimmunity and biopsy proven disease (compared to other haplotypes also associated with celiac disease). There are ethnic and gender variations, and family history confers increased risk.

**Pediatric Gastroenterologist's Viewpoint**

Celiac disease (CD) results from a dysregulated immune
### TABLE I  CRITICAL APPRAISAL OF THE STUDY USING THE CASP TOOL

**A. Are the results of the study valid?**

<table>
<thead>
<tr>
<th>Did the study address a clearly focused issue?</th>
<th>This cohort study has focused on a well-described population and risk factor (infants with HLA haplotype DR3-DQ2 or DR4-DQ8), and clearly defined outcomes (development of celiac disease autoimmunity and disease).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Did the authors use an appropriate method to answer their question?</td>
<td>A cohort study with a reference group for comparison (infants without the risk factor haplotype) would be appropriate to address the issue of prevalence of autoimmunity and disease, compared to the unexposed population. However, this study has focused only on those with the haplotype (and its variants).</td>
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<tr>
<td>Was the cohort recruited in an acceptable way?</td>
<td>The cohort was part of an ongoing study to determine the factors affecting type I diabetes prevalence. From a potential cohort of over 21,500 eligible infants, the authors included over 8600. Among these 6403 infants carried one of the four haplotypes of interest to this study and all were recruited. Thus there appears to be no selection bias.</td>
</tr>
<tr>
<td>Was the exposure accurately measured to minimize bias?</td>
<td>Identification of the relevant haplotypes and categorization into groups was done objectively, although the methods are not described in detail in this paper. However, it is expected that there is limited risk of bias since no subjective methods were used to identify or classify the haplotypes of interest.</td>
</tr>
<tr>
<td>Was the outcome accurately measured to minimize bias?</td>
<td>The two outcomes of interest (autoimmunity and disease) were described appropriately. The former was defined as the presence of anti tTG antibodies on two consecutive occasions when measures serially at 3 month intervals. Disease was defined by intestinal biopsy or anti tTG antibody level &gt;100 Units on two occasions. These definitions are acceptable in routine clinical practice as well as research settings. Laboratory measurements were performed at two centres (one in US and the other in UK), using internationally accepted methods for testing. In addition, the UK laboratory served as the reference lab; where all samples with anti tTG level higher than a certain value were re-tested. Intestinal biopsy specimens were subject to histopathologic grading using the standard Marsh score. These quality control measures suggest a limited risk of measurement bias. However there is no description of blinding of the laboratory personnel.</td>
</tr>
<tr>
<td>Have the authors identified all important confounding factors? List the ones you think might be important, that the authors missed.</td>
<td>The authors have not described potential confounding factors. However, previous data have shown that nearly 100% patients with celiac disease have the haplotypes under consideration; although it is present on 25-30% of the unaffected population also. Therefore, it would have been very helpful to study the pattern of development of autoimmunity and disease in a cohort without the haplotypes of interest, although this would require a very large and expensive study.</td>
</tr>
<tr>
<td>Was the follow up of complete enough and long enough?</td>
<td>The authors reported a median follow-up duration of five year. Although, they did not mention attrition, only 350 of the 786 eligible children underwent biopsy to confirm the diagnosis of celiac disease. This was at the discretion of the primary physicians and not related to the study per se.</td>
</tr>
</tbody>
</table>

**B. What are the results?**

| What are the results of this study? | Celiac disease autoimmunity and disease varied by haplotype. DR3-DQ2/ DR3-DQ2: 26% and 11%; DR3-DQ2/DR4-DQ8: 11% and 3%; DR4-DQ8/ DR4-DQ8 8% and 3%; DR4-DQ8/DR8-DQ4: 3% and <1%. Respective relative risk: in Sweden (vs USA): 1.90 (1.61, 2.25) and 1.86 (1.43, 2.41); female (vs male): 1.64 (1.42, 1.89) and 2.16 (1.71, 2.72); and with family history of celiac disease (vs no history): 1.81 (1.31, 2.50) and 2.95 (1.95, 4.46). However, this study was not designed to calculate the risk among those not exposed to the four haplotypes. |
| How precise are the results? How precise is the estimate of the risk? | In comparison to people with lowest risk category (i.e DR4-DQ8 heterozygosity), DR3-DQ2 homozygosity is associated with relative risk of 5.70 (95% CI 4.66, 6.97) for autoimmunity and 6.08 (95% CI 4.43, 8.36) for disease. The respective risk ratios for DR3-DQ2 heterozygosity are 2.09 (95%CI 1.70, 2.56) and 1.66 (1.18, 2.33). |
| Do you believe the results? | These results are in line with other reports that suggest association between celiac disease and specific haplotypes. The added information is the rate of development |
response to dietary wheat and related cereal proteins. This disease has gained importance from its HLA-linked genetic basis as evident by ethnic and racial clustering, familial aggregation, twin concordance, strong association with certain syndromes, and autoimmunity. Many environmental factors, non-HLA linked genetics (innate, adaptive and mucosal barrier) and recently early childhood intestinal microbiota dysbiosis have also been implicated in the development of this disease [21]. However HLA-linked genetics and its “gene-dose effect” continue to be the stronghold of the etiopathogenesis and thus its utility as an aid in screening and diagnosis of CD [22].

Virtually all CD patients express HLA-DQ2 or DQ8 class II molecules that bind and present gluten peptides derived from exogenous protein antigens. These antigens are endocytosed by HLA class II positive antigen-presenting cells and degraded in an intracellular endosomal/lysosomal compartment forming HLA class II-peptide complexes that are recognized by peptide-specific T cells [23]. What follows thereafter is a cascade of inflammation, villous atrophy and triggering of autoimmunity that makes the disease clinically evident.

Over the years, management of CD focused on withdrawal of gluten in diagnosed patients. However recently there has been a paradigm shift in expanding the scope of diagnosing CD early by screening high-risk groups. These high risk groups are: a) non-autoimmune first-degree relatives, selective IgA deficiency, Down syndrome, William’s syndrome and Turner syndrome); and b) autoimmune (type 1 diabetes mellitus, autoimmune thyroiditis and Sjögren syndrome). The scope has been further enhanced by screening subjects with non-gastrointestinal conditions like dermatitis herpetiformis, delayed puberty, short stature, iron deficiency anemia, osteoporosis, arthritis, ataxia, polyneuropathy, dental enamel hypoplasia and recurrent abortions.

The study being analyzed here is a prospective longitudinal study that sheds light on relationship of HLA typing (both homozygous and heterozygous) and celiac autoimmunity (development of serum tissue transglutaminase antibodies twice at least 3 months apart) and CD (serology plus small bowel biopsy features) [1]. Of 786 subjects with CD autoimmunity, small bowel biopsy was possible in 350 and CD was confirmed in 83% (n=291) children. The major caveat in this robust study is that biopsy could not be performed in 436 subjects with tTG positivity. This may have resulted in underestimation of CD or overestimation as 21 out of 436 subjects were clubbed and presumed to be CD based only on high levels (>100 U) of tTG. It was additionally found that 1% of CD autoimmunity and 2% of diseased individuals developed Type 1 diabetes mellitus on follow-up where the community prevalence of diabetes mellitus is 0.3% [1].

The researchers also found that amongst the 4 countries, Sweden had the highest risk of CD or CD autoimmunity, almost double that of USA. The authors have attributed this phenomenon to non-genetic, dietary factors. The interplay between breastfeeding and early introduction of wheat during supplementation has been projected as crucial and complex. In an unpublished study the authors cited, the Swedish children were exclusively breastfed for a longer duration (median 4 weeks) as compared to those in USA (median 1 week). However, Swedish children were given gluten-containing cereals at the earlier age as compared to USA [1]. Possibly we could interpret that breastfeeding may be protective. It is prudent to breastfeed children longer and gluten-containing diet may be introduced later during infancy (our view point). Thus the study highlights role of environmental factors besides genetic susceptibility in causation of CD.

How is this study relevant for India? In North India, a population prevalence of 0.32% (1:310) of symptomatic CD in school-going children and an overall community prevalence of 1% is reported. The prevalence of biopsy-proven CD including tTG positivity and autoimmunity
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In high-risk groups as stated above, screening with HLA may be considered. Those who are HLA predisposed (especially HLA DQ2 homozygous) must be followed up with tTG serology screening as HLA-positivity does not mean CD. Negative tTG must be coupled with serum IgA to rule out IgA deficiency (level <5 mg/dL). Those who are tTG positive or IgA deficient must undergo small bowel biopsy. In Indian situations, cost is a major consideration for HLA typing as a first line screening among high risk groups. Therefore the option of either HLA first followed by serial tTG strategy or only serial serology testing on follow-up are the alternatives available [24]. Small bowel biopsy even today for diagnosing CD seems to be mandatory in India (authors’ viewpoint). Breastfeeding needs to be re-emphasized and awareness further strengthened by various campaigns.

Nevertheless, this study by Liu, et al. has knocked the door that opens into a whole new frontier of CD. It is time we take lessons, ponder upon and surge ahead!

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Immunogeneticist’s Viewpoint

Majority of patients with Celiac disease (CD) worldwide possess HLA-DR3-DQ2 haplotype while a few carry DR4-DQ8 or others. This paper by Liu, et al. [1], describes a well-planned prospective cohort study on children CD that has re-established the importance of HLA-DQ2 as a crucial risk factor both for development of CD autoimmunity as well as phenotypic manifestation of disease at an early age. This study has also affirmed that presence of double copies of HLA-DQ2 offers a greater risk for both the above outcomes than a single copy of DQ2 and is also associated with the earliest onset.

HLA-DQ2 association has a high negative predictive value and its absence makes the possibility of CD highly unlikely. Among various HLA-DQ2 heterodimers, DQ2.5 (DQA1*05:01+DQB1*02:01) confers the greatest risk, particularly in homozygosity; followed by DQ2.2 (DQA1*02:01 + DQB1*02:02) whether in heterozygous condition with DQ2.5 or in homozygosity. The authors studied presence of such HLA class II haplotypes amongst children of various origins (USA, Finland, Germany and Sweden) and shown that cumulative risk of CDA and CD was highest among children with DR3-DQ2/DR3-DQ2 homozygotes followed by DR3-DQ2/DR4-DQ8 and DR4-DQ8/DR8-DQ4 haplotypes in descending order. The risk of CD autoimmunity was higher among children who had first degree relations with CD than with type 1 diabetes. The female predominance of CD autoimmunity was also evident in this study. Further, it is reported that risk of CD autoimmunity in Sweden was almost twice that of USA and that it developed at an earlier age despite haplotype matching. The authors attribute this difference to multiple environmental factors including interplay between breastfeeding and gluten exposure, age at which gluten was introduced in child’s diet and the probable type of infections, all of which are important in development of CD. Based on the above, the authors recommend initiation of screening for CD in at-risk children for an early diagnosis and the need to further investigate the intricate relationships between genetic ad environmental factors that may modulate development of CD in early childhood.

The DR3-DQ2 genomic segment of MHC is most commonly found as an integral component of the ancestral MHC haplotype AH8.1 i.e. the classical HLA-A1-B8-DR3 haplotype amongst the Caucasians. The extended DR3-DQ2 haplotypes in the Indian population are in contrast multiple and very different from the classical Caucasian AH8.1. The Indian DR3-DQ2 haplotypes are unique at numerous intermittent loci, are associated with CD and other autoimmune diseases. The significance of evolutionary divergence of such autoimmunogenic DR3-DQ2 haplotypes in the Indian population is not well understood. The possibility of association of different forms of DR3-DQ2 haplotypes with different disease variants of CD or T1D also remains to be determined. It is still an open question whether such haplotypes could be differently correlated with spectral phenotypes of CD i.e. GI symptomatic vs atypical symptoms vs no symptoms (Silent CD) or with no duodenal pathology (Latent CD) forms. Incidentally, HLA-DQ2 is the same genetic marker that is considered to be an important marker of unresponsiveness to HBV vaccine among the Caucasians but there is paucity of data on Indian population and it is not known if HBV response is compromised among Indian
CD patients. Hence, MHC haplotypic associations need further elucidation particularly in conjunction with different disease forms in the Indian population and in comparison with analogous counterparts in other populations.

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REFERENCES


