Original Article

Genetic susceptibility for celiac disease is highly prevalent in the Saudi population

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Abstract

Background/Aim: To determine the frequency of celiac disease (CD)-predisposing human leukocyte antigen (*HLA)-DQ* genotypes in the Saudi population, where the prevalence of CD is 1.5% as recently reported in a mass screening study.

Patients and Methods: In a cross-sectional population-based study, a total of 192 randomly selected healthy school children (97 females, mean age 10.5 ± 2.2 years, all negative for tissue transglutaminase-IgA) were typed for *DQA1* and *DQB1* genes by polymerase chain reaction sequence–specific oligonucleotide probes.

Results: Of the 192 children, 52.7% carried the high-risk CD-associated *HLA-DQ* molecules: homozygous *DQ2.5 (*2.6%), *DQ2.5/DQ2.2 (*4.7%), heterozygous *DQ2.5 (*28.15%), homozygous *DQ8 (*4.2%), *DQ8/DQ2.2 (*3.6%), and double dose *DQ2.2 (*9.4%). Low-risk CD-associated *HLA-DQ* molecules (single dose *DQ2.2* and heterozygous *DQ8*) constituted 3.6% and 9.4%, respectively. Among the very low–risk groups, individuals lacking alleles that contribute to *DQ2/DQ8* variants (33.5%), 13.5% carried only one of the alleles of the high-risk *HLA-DQ2.5* heterodimer called "half-heterodimer" (*HLA-DQA1*05* in 12% and *HLA-DQB1**02 in 1.5%), and 20.8% lacked all the susceptible alleles (*DQX.x*). Gender distribution was not significantly different among the CD-risk groups.

Conclusion: We report one of the highest frequencies of CD-predisposing *HLA-DQ* genotypes among healthy general populations (52.7%) worldwide, which might partly explain the high prevalence of CD in the Saudi community.

Keywords: Celiac disease, genetic susceptibility, HLA typing, HLA-*DQ2.5*, *HLA-DQ8*, Saudi Arabia

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INTRODUCTION

Celiac Disease (CD) is an autoimmune enteropathy characterized by chronic inflammation of the small intestinal mucosa triggered by gluten uptake that occurs in genetically susceptible individuals.[1] CD is a worldwide health problem, and its prevalence varies among different populations; this diversity is apparently determined by genetic and environmental factors. Human leukocyte antigen *(HLA)-DQ2.5* protein (encoded by *HLA-DQA1*05* and *DQB1*02* alleles) and *HLA-DQ8* protein (encoded by HLA-DQA1*03 and DQB1*03:02 alleles) are recognized predisposing factors for CD.[2] The genes encoding for

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HLA-DQ molecules are found in a region known as class II on chromosome 6, known as -DQ. *HLA-DQA1* and *HLA-DQB1* genes encode for α- and β-heterodimers, respectively. These are cell surface receptors located on antigen presenting cells, and they form a cleft that binds gliadin peptides [Figure 1]. Following deamidation by tissue transglutaminase 2 (TTG2) and presentation to T-helper cells, T-helper cells are activated, causing secretion of inflammatory cytokines that result in villous atrophy.[2] The preferential binding of *HLA-DQ* molecules to gluten peptides is a key step in the pathogenesis of CD; hence, the development of CD in individuals who are *HLA-DQ2* and -*DQ8* negative is extremely rare.[3]

A variable distribution of CD-predisposing *DQ* alleles and environmental factors among different populations and ethnicities could explain the variable prevalence of CD that has been reported in different parts of the world. In the United States and Europe, where the prevalence of CD ranges between 0.5 and 2%, 25–30% of individuals carry *HLA-DQ2* and *DQ8* susceptibility heterodimers,[4] whereas the low prevalence of HLA predisposing *DQ2*/*DQ8* molecules in Far-East Asian countries could explain, in addition to the low wheat consumption, the anecdotal reports of CD in these countries.[5]

We recently conducted a mass screening study in Saudi Arabia and estimated the prevalence rate of CD at 1.5% among 7930 school aged-children.[6] Therefore, we propose that the high prevalence of CD among the pediatric Saudi population could be due to the common presence of CD-predisposing *HLA-haplotypes-DQ2* and -*DQ8*. We conducted this cross-sectional study to define the frequency of *DQ2* and *DQ8* that have conferred high susceptibility to the development of CD in the Saudi population.

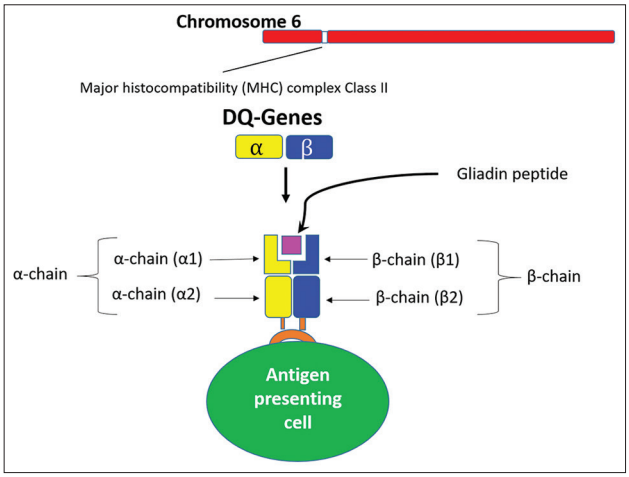

Figure 1: Class II *HLA-DQ*: The genes encoding for HLA molecules are found in the major histocompatibility (MHC) complex on the short arm of chromosome 6 (6p21.3). HLA molecules involved in celiac disease are encoded in a region known as class II by genes known as -DQ. *HLA-DQA1* gene encodes the *α* chain (*α1 and α2*), while *DQB1* encodes the *β chain (β1 and β2) of HLA-DQ* protein. Both chains are associated as heterodimers on the surface of antigen-presenting cells and form a cleft that binds antigens and presents them to T-cells

PATIENTS AND METHODS

Study design and setting

The study was a cross-sectional, population-based study to determine the frequency of CD predisposing *HLA-DQ* genotypes among Saudi students of both sexes (6–15 years of age), attending primary and intermediate schools in Riyadh, between 2014 and 2016.

Study population and recruitment of participants

The details of the methodology of the CD mass screening study (including study population, recruitment of the students, inclusion and exclusion criteria, etc.), from which the study population for the present study was selected, have been described elsewhere.[6] In brief, a total of 104 schools (61 Primary schools and 43 intermediate schools) were randomly selected using a probability proportionate sampling procedure. Parents of 7930 students (Mean age 11.22 ± 2.62 years, females 63%) have signed the informed consent forms and accepted participation in the mass screening study. The 5-milliliter blood specimens collected from each student were centrifuged at 2000 RPM for 10 minutes, and plasma was separated and stored at -20°C until analysis. Out of the 7930 serum specimens, we randomly selected 195 specimens for HLA typing. The inclusion criterion was TTG-IgA negative specimen. We excluded TTG positive specimens as our interest was principally to ascertain the frequency of *HLA-DQ* genotypes among non-Celiacs.

Study procedures

DNA was isolated from the whole blood using the Magna Pure compact instrument (Roche Diagnostics, Mannheim, Germany) and the MagNa Pure Nucleic Acid Isolation Kit (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions. HLA-Typing for celiac disease susceptibility genes was performed for the 195 randomly selected specimens using Sequence Specific Oligonucleotide (SSO) method. In summary, the SSO method is a DNA-based tissue typing technique using polymerase chain reaction to amplify the target DNA. The amplified product was hybridized with *DQA1*-specific and *DQB1*-specific nucleotide probes bound to fluorescently labeled beads that identified alleles encoded in the DNA sample. The process was carried out using Luminex technology in the Lab Type Kit (One lambda®, Canoga Park, USA). Reactivity patterns were interpreted with HLA Fusion software (One lambda®, Canoga Park, USA) to

identify the alleles present. Further details of the procedure are available at http://www.onelambda.com.

HLA-DQ genotype notation

The type and configuration of the *DQA1* and *DQB1* alleles determined the *DQ* molecule that formed as shown in Table 1. Individuals with *HLA-DQ2* encoding genotypes [*DQ2.5 (homozygous), DQ2.5 (heterozygous), DQ2.5/DQ2.2, DQ2.2*] were denoted as *DQ2* positive, while carriers of *HLA-DQ8* encoding genotypes [*DQ8 (homozygous), DQ8 (heterozygous), DQ8/DQ2.2*] were denoted as *DQ8* positive. We followed the WHO nomenclature[7] in which HLA is followed by a hyphen followed by the gene (e.g. *DQA1, DQB1,* etc.), an asterisk (separator), allele group (field 1), colon (field separator), and protein (field 2). Disease risks for each HLA genotype are based on the published data from Europe,[8-10] as shown in Table 2. The genotyping procedure at King Fahad Medical City laboratory is continuously monitored for quality assurance by the College of American Pathologists.

Ethical consideration

This study was approved by the Institutional Review Board of King Fahad Medical City (number 11-066) and Ministry of Education in Saudi Arabia. All study participants, or their legal guardians, provided informed written consent prior to study enrollment.

Statistical analysis

Descriptive statistics (SPSS for Windows, version 21.0; SPSS Inc, Chicago, IL) were used to calculate the frequencies of the most common HLA types. Pearson's chi-squared test was used to test any association between gender and *HLA-DQ* molecules. A P value < 0.05 was considered statistically significant.

RESULTS

Out of 195 randomly selected serum specimens, 3 specimens were excluded because they tested positive for TTG-IgA, and CD was confirmed on duodenal biopsies. The remaining 192 serum specimens (97 females; mean age 10.5 ± 2.2 years) tested negative for TTG-IgA and underwent HLA typing. The frequency of each *HLA-DQ* genotype is presented in Table 3. Of the 192 healthy children, 52.7% carried the CD-associated *HLA-DQ* molecules, *DQ2* or *DQ8* variants, making them highly susceptible to develop CD. The *HLA-DQ2* positive group (*DQ2.5* homo- or heterozygous, *DQ2.5/DQ2.2, DQ2.2* homo- or heterozygous) constituted 48.5%, whereas the *DQ8* positive group (*DQ8* homo- or heterozygous, *DQ8/DQ2.2*) constituted 17.2% of the total cohort. Among individuals lacking alleles that contribute to *DQ2/DQ8* variants (33.5%), 13.5% carried only one of the alleles of the risk *HLA-DQ2* heterodimer called "half-heterodimer" (*HLA-DQA1*05* in 12% and *HLA-DQB1**02 in 1.5%), and 20.8% lacked all the susceptible alleles (*DQX.x*).

Table 1: Types of celiac disease risk heterodimers that result from the variable configuration of the DQA1 and DQB1 alleles

Celiac risk heterodimer	DQA1	DQB1
DQ2.5 and DQ8		
Strand 1 →	DQA1*05	DQB1*02
Strand 2 →	DQA1*03	DQB1*03:02
DQ2.5 (Homozygous)	DQA1*05	DQB1*02
	DQA1*05	DQB1*02
DQ2.5 (heterozygous)	DQA1*05	DQB1*02┼
DQ2.5/DQ2.2	DQA1*05	DQB1*02
	DQA1*02	DQB1*02
DQ8 (homozygous)	DQA1*03	DQB1*03:02
	DQA1*03	DQB1*03:02
DQ8 (heterozygous)	DQA1*03	DQB1*03:02┼
DQ8/DQ2.2	DQA1*03	DQB1*03:02
	DQA1*02	DQB1*02
DQ2.2 (homozygous)	DQB1*02	DQB1*02
	DQB1*02	DQB1*02
DQ2.2 (heterozygous)	DQB1*02	DQB1*02
	DQA1 ≠ **0201* or **05*	DQB1≠ **02 or *0302*
DQ2.x (half heterodimer of DQB1*02)	DQA1≠*05^	DQB1*02┼
DQX.5 (half heterodimer of DQA1*05)	DQA1*05	DQB1≠*02 OR *03:02¥
DQX.x	DQA1≠*05^	DQB1≠*02 OR *03:02¥

┼In one of the DQB1-1 OR DQB1-2 alleles; ¥There is no *02 OR *03:02 in the DQB1 alleles; ^There is no *05 in the DQA1 alleles

Table 2: HLA-DQ status and risk of celiac disease

HLA status disease risk	HLA status disease risk
DQ2.5 and DQ8	Very high
DQ2.5 (with a double dose of DQB1*02)	Very high
DQ2.5/DQ2.2	Very High
DQ2.5 (with a single dose of DQB1*02)	High
DQ8 (homozygous)	High
DQ8/DQ2.2	High
DQ2.2 (with a double dose of DQB1*02)	High
DQ2.2 (with a single dose of DQB1*02)	Low
DQ8 (heterozygous)	Low
DQ2.x	Extremely low
DQX.5	Extremely low
DQX.x	Extremely low

Table 3: Frequency of HLA-DQ genotypes among 192 healthy students and the associated risk to develop celiac disease

HLA-DQ heterodimer	Number=192 (%)	
DQ2.5 and DQ8	0	Extremely high risk
DQ2.5 (homozygous)	5 (2.6%)	
DQ2.5/DQ2.2	9 (4.7%)	
DQ2.5 (heterozygous)	54 (28.15%)	High risk
DQ8 (homozygous)	8 (4.2%)	
DQ8/DQ2.2	7 (3.6%)	
DQ2.2 (double dose of DQB1*02)	18 (9.4%)	Low risk
DQ2.2 (single dose of DQB1*02)	7 (3.6%)	
DQ8 (heterozygous)	18 (9.4%)	
DQ2.x	3 (1.5%)	Extremely low risk
DQX.5	23 (12%)	
DQX.x	40 (20.8%)	

The frequency of consanguinity in our study cohort was 41%, and the family history of CD was 2.6%. The consanguinity rate was similar in the 4 CD-risk groups. In addition, gender distribution was not significantly different among the 4 CD-risk groups, but there was a tendency for extremely high-risk HLA molecules to predominate among males ($P = 0.08$).

DISCUSSION

Our data indicate that more than half of the Saudi pediatric population (52.7%) carries CD-predisposing *HLA-DQ* genotypes that confer high risk to develop CD, which might partly explain the recently reported high prevalence of CD in the Saudi community (1.5%).[6] To the best of our knowledge, we report one of the highest frequencies of CD-predisposing *HLA-DQ* genotypes among healthy general populations worldwide. Frequencies of *HLA DQ2* and *DQ8* among other populations ranged between 0 and 28% and between 1 and 9%, respectively.[11] It is generally assumed that the prevalence of CD risk alleles in Europe is between 25% and 30%.[4] However, in Sweden, where the prevalence of CD was 2.13%, the frequency of CD-predisposing *HLA-DQ* genotypes was 53%.[12] In addition to the strong genetic susceptibility, we believe that the high frequency of CD among the pediatric Saudi population could be because of the high intake of cereals (as most of the staple foods in Saudi community contain wheat, rye, or barley). The consumption of gluten-containing cereals in the Saudi population is very high according to the Food and Agriculture Organization data.[13] The yearly cereal intake per person between 2009 and 2014 was 151 kg in Saudi Arabia, compared to 107 kg/person/year in North America and 137 kg/person/year in Europe.[13]

The analysis of correlation between wheat consumption, *HLA-DQ2 and DQ8* frequency, and CD prevalence showed a significant correlation between the combination of both risk factors and the incidence of CD worldwide.[14] In Africa, there is a gradient in CD prevalence from North to South; the prevalence of CD among Northern populations (such as Saharawi, Libya, Tunisia, and Egypt) shows a high frequency of *HLA-DQ2 (*23-39%) and high level of wheat consumption, which is much higher (0.8–5.6%) than the CD prevalence in the southern populations (such as Papua New Guinea, Burkina Faso, Rwanda, Tanzania, and Cameroon) where the frequency of *HLA-DQ2* is low (0–15%) and where the staple diet is low in wheat.[11,13-16] A review of medical literature for the mass screening studies performed in Asia, including our study, indicates a gradient in CD prevalence. The highest rates were reported from the west of the continent (Saudi Arabia 1.5–2% and Iran 0.8–1%),[17,18] intermediate rates from India (0.4%),[19] and only anecdotal reports of CD from the Far East.[5,20] This observation is corroborated by a gradient distribution of *HLA-DQ* molecules (Saudi Arabia 52.7% (present study), Iran 58%,[21] North India 31.9%,[22] and less than 10% in Japan and China).[5,20] Likewise, the level of wheat consumption in West Asian countries is very high compared to the low wheat consumption in Far-Eastern countries.[13] No CD prevalence studies were conducted in Japan or China; however, the low frequency of *HLA-DQ* molecules in these countries predicts low prevalence rates for CD.[5,20]

The importance of *HLA-DQ* genotyping in clinical practice emerges from two important pieces of information. First, the absence of *HLA-DQ2 and DQ8* molecules is significant for its high negative predictive value, as it strongly argues against the diagnosis of CD.[3,8,23] Second, *HLA-DQ* molecular typing allows for the definition of a CD risk gradient associated with each particular *HLA-DQ* status.[8,23] In this regard, it is useful to consider *HLA-DQ* genotype gradient risk as a first step in selecting individuals who must undergo serologic follow-up; this "2-step CD-screening approach" is especially important in high-risk groups (such as family members of a celiac case, individuals with an autoimmune disease such as type 1 diabetes, and certain conditions associated with CD, e.g., Down and Turner syndromes).[1] Such a strategy should assist in the early diagnosis of CD in at-risk individuals who often have unclear symptoms.[24] An early diagnosis means early intervention with a gluten-free diet, which may prevent the development of significant comorbidities.[25,26] Some researchers took this "2-step approach" further and proposed using the *HLA-DQ* molecular test as a first step in mass screening of the general population for CD.[26-29] Based on our study, more than half of the Saudi population is genetically susceptible to develop CD, and consequently, the 2-step approach would be much less cost-effective than a one-step approach (i.e., screening with TTG-IgA). The cost effectiveness of the 2-step approach is dependent on the frequency of the HLA risk alleles in the studied population. The lower the prevalence of HLA risk alleles, the larger the percentage of the population that could be excluded from further testing of TTG-IgA. Based on the current knowledge and the need for cost-effectiveness, it is clear that any future screening program should be tailored to the characteristics of the specific target population.

The majority of *HLA-DQ* risk gradient studies in CD originated from Europe and the United States,[8-10,23,30] and the detailed information of *HLA-DQ* risk gradient

on CD patient cohorts from diverse ethnic groups is lacking. These studies confirmed that *DQ2.5* haplotype homozygosity, *DQ2.5/DQ8,* and *DQ2.5/DQ2.2* conferred the highest risk for CD and were associated with the earliest onset and most severe phenotype. The prevalence of *HLA-DQ2* and *-DQ8* in the general population varies geographically.[31] Our data, similar to the data from the Middle East and South America, show a higher frequency of *HLA-DQ8 (*17-20%) among the general population compared to 1–9% among Caucasians.[31] In addition, the prevalence of "high-risk" CD predisposing genotypes is higher in the Saudi population compared to the European population. These differences suggest that variable combinations of *HLA-DQ* risk alleles in Saudi CD patients might confer different risk gradients for some *HLA-DQ* molecules compared to Caucasian CD patients. Therefore, studies that explore the *HLA-DQ* genotypes of Saudi CD patients and compare them to the *HLA-DQ* data among the controls reported here are needed to enable clinicians in Saudi Arabia to categorize *HLA-DQ* molecules into risk groups. This information will help local physicians in genetic counseling of CD families to determine more precise CD occurrence risks and appropriate plans for serologic follow-ups.

Although 52.7% of the Saudi population carries high-risk *HLA-DQ* molecules, only 1.5% develop CD.[6] This underscores the role of additional genetic or environmental factors. CD is a multigenetic disorder, which means that the expression of these *HLA-DQ2* or *HLA-DQ8* molecules is necessary but not sufficient to cause disease. Genome-wide association studies have identified a large number of non-HLA genes associated with CD, such as those coding for cytokines, chemokines and their receptors, cell adhesion molecules, and T- and B-cell activators.[32-36] The high consanguineous marriage rate in the Saudi community (40–60%)[6,37] and the clustering of CD cases in certain families are important reasons to conduct genome-wide association studies in Saudi Arabia. These studies would enhance the identification of new non-HLA genetic loci, which could shed light on new genes and genetic pathways involved in disease pathogenesis, and the possibility of a monogenic-form of CD could be explored.[38]

Data on the differences in the association between *HLA-DQ* molecules in male and female subjects from the general population are scarce in the literature. Our mass screening study in Saudi Arabia,[6] like many others, confirmed CD to be more prevalent in females than in males (F:M = 2-4:1), but data from our present study showed no difference between females and males in the frequency of high-risk *HLA-DQ* molecules. These data are in agreement with a large Italian study.[38] This observation suggests that the HLA genes, *per se*, probably have no role on the CD sex bias. The causes of the CD sex bias are not yet clear, and other genetic (non-HLA) or non-genetic factors, such as exposures to environmental agents, endogenous hormones, sexual dimorphism of the immune response, X inactivation, genes on X or Y chromosomes, and epigenetic modifications, have been considered as potential causes of the sex discrepancy.[39-41]

CONCLUSION

The high genetic susceptibility of the Saudi population could partly explain the recently reported high prevalence of CD in the Saudi community. Based on this finding, *HLA-DQ* typing should not be used as a screening test to exclude CD. As the CD-risk gradient associated with *HLA-DQ* molecules among Caucasians cannot be extrapolated and applied completely to Arabs, it remains to be determined how different *HLA-DQ* molecules modify the risk of developing CD in the Saudi population. Our data offers a first step toward defining the genetic structure of *HLA-DQ* molecules in different regions of Saudi Arabia. More research is needed to define the role of non-HLA genetic and environmental factors.

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Conflicts of interest

There are no conflicts of interest.

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