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Genetic Differences in HLA-DQA1* and DQB1* Allelic Distributions Between Celiac and Control Children in Santiago, Chile

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ABSTRACT: Celiac disease is a permanent gluten intolerance strongly associated with HLA class II antigens. The over presentation of particular HLA alleles and haplotypes has been described in several populations. Different lines of evidence obtained during the last years suggest that a particular HLA-DQ heterodimer, encoded by the DQA1*0501 and DQB1*0201 genes in cis or trans conformation, confers the primary disease susceptibility. We report the HLA class II allelic distribution and DQA1/DQB1 genotypes in 62 Chilean celiac patients compared with 124 control subjects in Santiago, Chile. We found a pronounced increase of the "susceptible" alleles :DQA1*0501 (0.480 vs 0.169, $P_c < 0.0005$), DQB1*0302 (0.430 vs 0.242, $P_c = 0.002$) and DQB1*0201 (0.250 vs 0.125, $P_c = 0.037$) in celiac patients in comparison with control children. As for "protective" alleles, we detected a high frequency of DQA1*0101 (0.310 vs 0.160, $P_c = 0.01$), DQA1*0201 (0.105 vs 0.010, $P_c < 0.0075$) and DQB1*0301 (0.250 vs 0.100, $P_c = 0.010$) in controls. In relation to risk haplotypes, the main combination observed was the conformation DQ8 (DQB1*0302/DQA1*0301) over DQ2 (DQB1*0201/DQA1*0501). In conclusion, results show that celiac disease in Chilean patients is primarily associated with DQ8 conformation. This is concordant with the high frequency of DR4 alleles (in linkage disequilibrium with DQB1*0302) detected in Amerind groups in Chile, where DQB1*0302 is more frequent than DQB1*0201. *Human Immunology 60, 262–267 (1999).* © American Society for Histocompatibility and Immunogenetics, 1999. Published by Elsevier Science Inc.

KEYWORDS: HLA-DQ genes; celiac disease; genetic markers; genetic admixture; Amerindians

ABBREVIATIONS

CD	celiac disease
MHC	major histocompatibility complex
PCR	polymerase chain reaction
SSO	sequence-specific oligonucleotides
IC	confiance interval
OR	odds ratio

INTRODUCTION

Celiac disease (CD) or gluten sensitive enteropathy is associated with small bowel lesions consisting of severe degrees of crypt cell hyperplasia and villous atrophy induced by toxic gliadin components contained in the diet [1–9]. Although its pathogenesis remains unclear it is agreed that the presence of genetic susceptibility and environmental variables, mainly exposure to dietary gluten are key factors in determinig its clinical appearance. There is evidence which suggests that breast milk may protect against celiac disease but this hypothesis remains to be demonstrated [5, 7]. The genetic component is clearly evidenced by an increased prevalence among first-degree relatives of affected individuals, a high concordance rate in monozygotic twins (approximately 70%) and among HLA-identical twins (30%) [10, 11]. The genetic control of this illness is related to the major histocompatibility complex (MHC) region of chromo-

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some 6, which encodes the HLA class l and class II molecules [12, 13]. Predisposition to the disease is closely associated with the inheritance of specific DQA1* and DQB1* alleles. In Northern Europe, CD is associated with HLA-B8, DR3, DR7 and DQ2 [14–16]. In contrast, in Southern Europe, where HLA-DR3 is less common, the disease is also associated with HLA-DR5. To reconcile the various HLA-DR associations observed in different ethnic groups, it has been accepted that celiac disease is associated with specific HLA-DQ2 alpha/beta heterodimers, encoded by the alleles DQB1*0201 and DQA1*0501, which indeed are the alleles found in over 90% of celiac patients [17–19].

There is little information about CD in Latin America. Its incidence in Chile has been calculated to be 1:1846 live births (unpublished data). The present study was carried out a) to describe the HLA-DQA1* and DQB1* allele distribution in Chilean patienrs with CD and b) to compare these with both the distribution observed in healthy Chilean individuals and the alleles described in celiac patients from other areas of the world.

PATIENTS AND METHODS

Study Population

The 95 celiac patients over 10 years of age who were registered in Hospital Exequiel González, Hospital Felix Bulnes and Hospital San Juan de Dios, Santiago, Chile, were contacted by mail and invited to particpate in this study. 62 were located at the addresses registered at the hospital and they attended the interview in which the study was explained. All families accepted to participate and provided written consent. The study was approved by the Committee on Ethics and Human Research, INTA, University of Chile. In all cases diagnosis was established following the criteria set by ESPGHAN [20], (i.e., three small intestinal biopsies, showing the characterisitc severely abnormal mucosa on biopsy I, histological recovery on biopsy II and reappearance of morphological damage on biopsy III). There were 23 males and 39 females, their mean age at diagnosis was 3.9 years (3.1 and at the time of this study 17.9 years (5.1. The control population consisted of 124 non-celiac subjects, with no family history of CD or other autoimmune diseases, who were randomly selected from the same geographical area as the celiac patients. Their mean age was 13.8 years (5.8; 53 were males and 71 females. Celiac and control subjects were ethnically similar, belonging to the strata III of sociogenetic classification in Chile, whose definition takes into consideration several factors such as blood system (A, B, 0 and Rh), socioeconomic status and surnames analysis; This estimation was based on high frequency of A and B alleles observed in Spaniards and other migrant European populations while these alleles are practically nonexistent in present Chilean native groups [21, 22]

Methods

Genomic DNA was extracted from peripheral blood lymphocytes using standard techniques [23,24]. The DNA was subjected to PCR with specific primers according to standard protocols. For the oligonucleotide dot-blot analysis we used the combination of the DQA1 and DQB1 sequence specific oligonucleotides (SSO) probes, allowed for the determination of known alleles in both homozygous and heterozygous individuals. The official nomenclature of the World Health Organization committee for factors of the HLA system was used for each allele [25].

Statistical Analysis

Allele and genotype frequencies were computed as sample proportions. The comparison of such frequencies in cases and controls was statistically assessed by the chi-square test corrected by multiple comparisons [26]. OR and exact 95% IC were computed from data [27]. The reference groups for OR calculations were *0101/*0101 for DQA1 gene and *0501/*0501 in DQB1 gene.

RESULTS

HLA-DQB1 Alleles

The allele distribution for DQB1 locus is shown in Table 1. In summary, the most common allele among controls was DQB1*0301 (25.0%) as compared with celiac subjects (10%), p-value = 0.010. DQB1*0302 allele frequency was higher in celiac patients than in controls (43% vs 24.2%, $P_c < 0.002$). Studies in Caucasians have revealed that DQB1*0602 and DQB1*0301 are more frequent among healthy subjects and therefore are thought to be protective alleles for CD. In the present study they were decreased among patients, but the difference was significant only for DQB1*0301. Among celiac patients other relevant allele in this locus was DQB1*0201 (25% vs 12.5%, $P_c < 0.037$).

HLA-DQA1 Alleles

As shown in Table 1, in the control subjects the most frequent alleles were DQA1*0101 (31% vs 16%, $P_c < 0.01$) and DQA1*0201 (10.5% vs 1% , $P_c < 0.0075$). DQA1*0401 was more frequent among the controls (15.7% vs 2%, $P_c < 0.0005$). The frequency of DQA1*0301 was comparable between the two groups (33% vs 25.8% among celiacs and controls, respectively). The allele DQA1*0501 was significantly increased in celiac subjects (48% vs 16.9%, $P_c < 0.0005$).

TABLE 1 DQA1 and DQB1 allelic distributions in both groups

	Celiac Children (n = 62)		Control Children (n = 124)			
Allele	n	Freq.	n	Freq.	p-value	Corrected p-value
DQA1*						
0101	20	0.160	77	0.310	0.002	0.01
0201	1	0.010	26	0.105	<0.0015	<0.0075
0301	41	0.330	64	0.258	0.14	0.7
0401	3	0.020	39	0.157	0.0001	0.0005
0501	59	0.480	42	0.169	<0.0001	<0.0005
DQB1*						
0201	31	0.250	31	0.125	0.0037	0.037
0301	13	0.100	62	0.250	0.0010	0.010
0302	53	0.430	60	0.242	0.0002	0.002
0303	5	0.040	5	0.020	0.2600	NS
0402	1	0.010	7	0.028	0.2300	NS
0501	14	0.110	39	0.157	0.1900	NS
0502	0	0.000	10	0.040	0.0240	NS
0503	0	0.000	2	0.008	0.2600	NS
0601	0	0.000	4	0.016	0.1100	NS
0602	7	0.060	28	0.113	0.1200	NS

HLA-DQA1/DQA1 Combinations

The analysis of DQA1/DQA1 combinations in celiacs and controls is shown in Table 2; the most common combinations in DQA1 locus among celiacs were DQA1*0501/0501 (32.3% vs 2.4%, OR = 44.4) and DQA1*0301/0501 (24.2% vs 8.1%, OR = 10).

HLA-DQB1/DQB1 Combinations

The most common combinations on the DQB1 locus among celiac patients were DQB1*0302/0302 (27.4% vs 9.7%), DQB1*0201/0201 (11.3% vs 2.4%), DQB1*0201/0501 (9.7% vs. 3.2%), DQB1*0201/0302 (8.1% vs. 3.2%) and DQB1*0301/0302 (11.3% vs 10.5%) (Table 3).

TABLE 2 HLA-DQA1 genotypes estimation between celiac and control children

DQA1 Genotype	Control Children (n = 124)	Celiac Children (n = 62)	O.R. IC 95%
0101-0101	0.162	0.048	1
0101-0201	0.048	0.000	—
0101-0301	0.089	0.145	5.5 (1.0–36.4)
0101-0401	0.040	0.016	1.3 (0.1–15.7)
0101-0501	0.121	0.065	1.8 (0.3–9.2)
0201-0201	0.016	0.000	—
0201-0301	0.056	0.016	0.9 (0.1–10.7)
0201-0401	0.040	0.000	—
0201-0501	0.032	0.000	—
0301-0301	0.105	0.113	3.6 (0.8–16.5)
0301-0401	0.081	0.032	1.3 (0.2–9.3)
0301-0501	0.081	0.242	10 (2.3–42.8)
0401-0401	0.048	0.000	—
0401-0501	0.057	0.000	—
0501-0501	0.024	0.323	44.4 (8.0–247.3)

HLA-DQB1/DQA1 Heterodimers

On the basis of 128 and 248 possible haplotypes between celiac patients and controls we found the next DQB1/DQA1 combinations: the most prevalent distribution among celiac patients was DQB1*0302/DQA1*0301-DQB1*0302/DQA1*0301 (25.8% vs 12.9%, patients and controls respectively, $P_c < 0.002$,). Also frequent haplotypes were: DQB1*0201/DQA1*0501-DQB1*0201/DQA1*0501 (11.3% vs 2.5%, patients and controls respectively). The conformation DQB1*0501/DQA1*0101, considered "neutral" in Northern Europe and "susceptible" in Southern Europe, was comparable in both groups. Another frequent haplotype distribution observed was DQB1*0301/DQA1*0501-DQB1*0301/DQA1*0501 (10.4% vs 3.1%, $P_c < 0.01$) that was more frequent between the control subjects compared with the celiac patients.

DISCUSSION

Several genetic systems has been studied in the Chilean Indian tribes. Information concerning the HLA-A and B loci observed in this investigations probably reflect patterns due to major ancient migrations. The variations observed in the Amerindians populations could be the product of selective forces associated with climatic differences or artificial gradients produced by non-Indian admixture [28, 29]. This is the first report of HLA-DQA1/DQB1 alleles distribution and haplotype combi-

TABLE 3 HLA-DQB1 genotype estimation between celiac and control children

DQB1 Genotype	Control Children (n = 124)	Celiac Children (n = 62)	O.R. (IC 95%)
0201-0201	0.024	0.113	5.8 (0.5–86.3)
0201-0301	0.105	0.065	0.8 (0.1–11.2)
0201-0302	0.032	0.081	1.9 (0.3–46.9)
0201-0402	0.008	0.000	—
0201-0501	0.032	0.097	2.1 (0.3–54.3)
0201-0502	0.008	0.000	—
0201-0601	0.008	0.000	—
0201-0602	0.000	0.032	—
0201-0603	0.008	0.000	—
0301-0301	0.081	0.000	—
0301-0302	0.105	0.113	1.4 (0.2–17.5)
0301-0402	0.008	0.000	—
0301-0501	0.032	0.000	
0301-0502	0.024	0.000	—
0301-0601	0.008	0.000	—
0301-0602	0.065	0.032	0.7 (0.04–11.6)
0302-0302	0.097	0.274	3.5 (0.5–41.6)
0302-0303	0.000	0.065	—
0302-0402	0.032	0.000	—
0302-0501	0.073	0.048	0.8 (0.1–13.3)
0302-0602	0.040	0.016	0.5 (0.01–13.4)
0303-0303	0.008	0.000	—
0303-0501	0.016	0.000	—
0303-0502	0.008	0.000	—
0402-0502	0.008	0.000	—
0402-0602	0.000	0.016	—
0501-0501	0.040	0.032	1
0501-0502	0.024	0.000	—
0501-0601	0.008	0.000	—
0501-0602	0.048	0.016	0.4 (0.01–11.0)
0502-0602	0.008	0.000	—
0503-0602	0.016	0.000	—
0601-0602	0.024	0.000	—

nations in Chilean celiac patients. According to the estimations made from data of the ABO blood system, nearly 40% of Aborigine population is mixed with Caucasian genes in the middle-low socio-economic stratum in the city of Santiago [21]. Results of this study show that DQ8 haplotypes predominate among our celiac patients and strongly suggest that the amerind traits would protect from CD. In general, the Ameriandians groups in Chile show a high frequency of DQB1*0301 and DQB1*0302 alleles; this special distribution could to favour the conformation DQ8 in our actual mixed population.

As expected, significant differences in some allele frequencies were found between celiac patients and controls. In Northern Europe a close association has been described between CD and DQ2 (DQA1*0501-DQB1*0201) molecules, which were found in 95% of the patients [30–32]. In Southern Europe instead, where DR3 frequency is lower, HLA class II associations have been linked to DR7 alleles, which seem to be in linkage disequilibrium with other DQ alleles such as DQA1*0201 and DQB1*0201 [32]. A second combination of HLA class II alleles is thought to influence susceptibility to CD in Eastern mediterranean populations [33]; this has been described as an alternative DQ molecule, termed DQ8. Although the HLA-DQ2 alleles remain the predominant susceptibility genes in these regions, up to 20% of celiac patients possess the DQ8 conformation. The genes encoding DQ8 (DQA1*0301-DQB1*0302) are found on DR4 haplotypes. It is interesting that in the patients anlayzed in this study although DQA1*0501 was the allele with the highest frequency, as combination other alleles were more predominant.

DR4 alleles have been described in Chilean population. Our previous data on Type 1 diabetes mellitus showed a high frequency of DR4 alleles both in diabetic patients and control subjects, (more than 40% of DR alleles were DR4) [34, 35]. In this previously analyzed group DQB1*0302 and DQB1*0301 alleles were most frequent. Therefore the predominance of DQB1*0302 over DQB1*0201 in the present study is consistent with previous findings.

As mentioned before the Mapuche genetic influence is the second most important genetic background in Chilean population. It is therefore important to evaluate the influence that amerind traits may have on CD. Unfortunately data on genetic characteristics of this native groups is limited. In the only study found reported in the literature DR4-DQ7 showed more than a two-fold increase in chilean Mapuches as compared with caucasic Chileans [36]. This suggests that the association in DR4 is favoured towards DQB1*0301 over DQB1*0302. DQ7 haplotype (DQA1*0501/DQB1*0301) has been defined as "protective" in relation to Type 1 diabetes and is more prevalent among Mapuches; interestingly, both this type of diabetes and CD are not reported among them.

Finally, with regard to DQ2 distribution, its frequency was low in comparison with descriptions from others countries [11–17]. The special ethnic composition of the group studied may be responsible for this finding [36, 37].

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