Amerindian mtDNA Haplogroups and Celiac Disease Risk HLA Haplotypes in Mixed-blood Latin American Patients

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ABSTRACT

Background and Objective: Risk haplotypes have been described in celiac disease (CD), but the influence of native genes on CD in Hispanic Americans is unknown. The aim of the study was to measure the frequency of Amerindian mitochondrial DNA (mtDNA) haplogroups (inherited by the maternal line) in mixed-blood patients with CD from Chile, Argentina, and Uruguay, and to assess the relation between these and human leukocyte antigen (HLA) alleles and haplotypes and clinical presentations.

Patients and Methods: Clinical history, histological data, and genetic studies were conducted following 2 protocols: a case-control study of 72 Chilean patients with CD and controls, and an assessment of 43 (additional) samples of celiac patients from Chile, 96 from Argentina, and 57 from Uruguay, compared with the mtDNA frequency in the corresponding country. HLA typing was performed by a commercial kit, and mtDNA was determined by means of polymerase chain reaction and restriction fragment length polymorphisms analysis.

Results: A total of 73.6% of cases had typical presentations. The most frequent HLA alleles were HLA-DQB∗201 and 202. No-DQ2/DQ8 HLA haplotypes were found in 7% of cases. mtDNA frequencies for typical Amerindian haplogroups were found in 71% of cases and 64% of controls ($P\ \chi^2 = 0.016$); in the comparative analysis, mtDNA distribution was not different from the figures reported for the respective general country population. No relation was found between haplotypes or haplogroups and clinical presentations.

Conclusions: mtDNA haplogroups A/B/C/D were frequently found in celiac patients and controls, but no relations appeared between haplogroups, haplotypes, and clinical presentations.

Key Words: celiac disease, ethnicity, HLA haplotypes, mtDNA haplogrups

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Celiac disease (CD) is an autoimmune disorder occurring in genetically susceptible individuals, triggered by gluten and related prolamins. This induces innate and adaptive immune responses that lead to variable damage to the small intestine. Gluten is partially degraded and modified in the duodenum-jejunum, resulting in gluten peptides that bind to human leukocyte antigens (HLA)-DQ2 or HLA-DQ8 with high affinity, triggering inflammatory T-cell responses. These MHC genes represent the main known genetic predisposition to CD, especially DQ2 and DQ8 variants, which are found in up to 95% of patients with CD (1).

CD prevalence in Europe is close to 1% in the population, being higher in northern areas (2). In the Americas, the situation has not been systematically assessed. A recent study in the United States reported that an active search of the disease yielded 2.25% positive cases (3). In Latin America, serologic studies of prevalence have described figures of 2.6% among ''mestizo'' (mixed blood) Mexican adults (4); 1:681 individuals (5) in samples from a blood bank in Brasilia, Brazil; and 1.6% in children (6) living in Montevideo, Uruguay. In Argentina, a prevalence of 1:167 (7) was reported, depending on the type of sample studied. In Chile, the frequency of typical clinical presentations based on patients who consulted with classical digestive symptoms and were diagnosed by small-intestinal biopsy was reported at 1:1800 subjects (8). The recently published National Health Survey conducted in a country representative sample yielded 1% of cases positive as per high transglutaminase levels (9).

The distribution of HLA genes in celiac patients living in the Americas varies among the regions. In a study in Cuba, 86.3% of patients carried DQA1∗0501 and DQB1∗02 (10) alleles; in Buenos Aires, Argentina, DQ2 frequency was 95% (11), whereas in Brazil, HLA-DRB1∗03, HLA-DRB1∗07, and HLA-DQB1∗02 were the most frequent findings among patients with CD (12). In Chile, DQ8 conformation was reported to predominate over DQ2, a feature thought to be concurrent with the high frequency of DR4 found in the local native Mapuche population (DR4 is in linkage disequilibrium with DQB1∗0302) (13). These data suggest that the frequency of Amerindians genes is high in Latin America, but the relation between these genes and the prevalence of CD is unknown.

Latin American history shows that in the 16th Century, Spanish conquerors were mainly men who colonized the land and mixed with native women. One of the most used markers to assess the ethnic origin of populations and migrations following the maternal ancestry is mitochondrial DNA (mtDNA), a small molecule with a low rate of mutation that has been used to classify the human population following mtDNA haplogroups (14). These mtDNA haplogroups have been used to trace some diseases and

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follow migrations. In Europe, 10 haplogroups describe 95% of the total population: H/I/J/K/M/T/U/V/W/X (15). In Sweden, Finland, and the Tuscany region of Italy, H haplogroup is carried by 40% of the population, whereas haplogroups I/J/K/M/T/U/V/W/X show lower frequencies, representing the remaining approximately 60% (15). In contrast, in the Americas, 4 different haplogroups (16) are described: A/B/C/D; they represent approximately 90% of haplogroups described in this region, but their frequencies show marked differences among the countries: 20.4% in Montevideo, Uruguay (17); 36% in Rio do Janeiro, Brazil (18); 25.9% in Buenos Aires, Argentina (19); 90.4% in northern Peru (20); and 84% in Santiago, Chile (21). The haplogroups formerly described in European populations are most infrequently found in South America, except for mixed-blood individuals whose ancestors originated from a mixture of native and migrating groups.

Given this background, in the present study, we assessed the potential influence that maternal native ancestry has on the appearance of CD, setting 2 objectives: to assess the frequency of HLA haplotypes and mtDNA haplogroups in celiac and nonceliac Chilean individuals (cases and controls), including the evaluation of their relation with clinical presentations, and to expand the assessment to mtDNA haplogroups to samples available from patients diagnosed in Chile, Uruguay, and Argentina in the last 10 years, comparing the results to mtDNA haplogroups and frequencies reported in each country, in the general population.

PATIENTS AND METHODS

Design and Study Groups

The first objective was addressed by assessing cases diagnosed following ESPGHAN 1999 (22) criteria, which included a positive serological test (anti-endomysial antibodies [EMA] and/or transglutaminase antibodies [tTG]) plus a small-intestinal biopsy showing different degrees of histological damage. Patients with silent presentations were not included. Cases were diagnosed at San Juan de Dios and Excequiel González Cortés hospitals and the Catholic University of Chile, between 1999 and 2008. Controls were chosen from asymptomatic individuals who responded to a call for volunteers at the same hospitals, with no history suggestive of CD (see below). The protocol was approved by the institutional review board of INTA, University of Chile. Candidates received full information about the study and those who accepted signed informed consent forms. In the case of children younger than 12 years of age, the consent was signed by their parents/legal guardians.

Data were obtained from clinical charts and the patients, including clinical presentation (typical/atypical), positive/negative EMA and/or tTG antibodies, and findings on the jejunal biopsy at the time of diagnosis. Histological findings were classified following the Marsh criteria (23). Typical presentation was defined as the presence of diarrhea, abdominal distention, and weight loss and/or poor growth as symptoms leading to diagnosis. Atypical CD was defined by constipation, anemia, and/or short stature (<-3 SD weight to height) of unknown cause, in the absence of the symptoms that defined the first group. Controls were apparently healthy individuals with no history suggestive of CD (explicitly including constipation, abdominal pain, and anemia), negative EMA and tTG tests, and normal immunoglobulin A (IgA), matched by sex.

The second objective was approached by studying mtDNA haplogroups in samples obtained in the last 10 years in 43 Chilean, 96 Argentinean, and 57 Uruguayan cases.

Measures and Procedures

At the end of the interview that collected clinical data, 5-mL anticubital venous blood samples were collected, between 8 and 9 AM. IgA-EMA was determined by indirect immunofluorescence using slides with monkey esophagus sections as substrate (IMMCO Diagnostics, Amherst, NY). Each patient's serum was diluted 1:10 in phosphate buffer at pH 7.4, and the presence of an immunofluorescent (brilliant green) pattern was considered positive. IgA-tTG was measured using a commercial ELISA kit (IMMCO Diagnostics), expressing the results to be negative $\leq$20 U, borderline = 20 to 25 U, and positive $\geq$25 U.

Genomic DNA Extraction and mtDNA Analysis

DNA was extracted from peripheral blood lymphocytes using standard techniques (24). Frequencies of haplogroups were determined amplifying the 4 main polymorphic regions (A/B/C/D), using a previously described set of primers (21); the final products were analyzed by means of restriction fragment length polymorphisms (RFLP) analysis (25).

To fulfill the second objective, available DNA samples from patients with CD diagnosed in Resistencia (Argentina), in Montevideo (Uruguay), and in Santiago (Chile) during the last decade were assessed (9). Published data from each country were used for comparison of frequencies of the mtDNA haplogroups found (17,19,21).

HLA-DQ alleles encoding HLA-DQ2 and HLA-DQ8 were determined using the Dynal RELI^{TM} SSO HLA-DQB1 Typing Kit (Invitrogen/DYNAL, Carlsbad, CA). Results were expressed following the current the World Health Organization nomenclature following the manufacturer's indications.

Statistical Analysis

For the case-control protocol, the sample size was calculated using previously published data that showed that the frequency of Amerindian genes in an apparently healthy population in Santiago was 84% (21,25). Using α error at 0.05 and power at 80%, the number of individuals per group was 54. Based on previous experiences showing that 25% to 30% of patients do not provide complete data for analysis, we set the final number at 72. For the second objective, we measured all of the available samples (universe).

In the case-control protocol, haplotype frequencies were compared by odds ratio (OR), using STATA 9.0 (StataCorp, College Station, TX). In other analyses, data are presented as mean $\pm$ SD and 95% confidence interval (CI). Pearson χ^2 was used for comparison of Chilean, Argentinean, and Brazilian patients. $P < 0.05$ was considered significant.

RESULTS

A total of 72 cases of CD and their sex-matched controls were analyzed in the first protocol; the general characteristics of celiac cases are shown in Table 1. As expected, patients diagnosed earlier in life had more prominent features: digestive symptoms, abdominal pain, and diarrhea. The mean age at diagnosis was 8 years for the whole study group, 4 years for typical cases, and 18 years for the atypical presentations. Although all of the subjects declared that they followed a gluten-free diet, 31 showed positive EMA and/or tTG at the time of assessment, and in 10 of them, both antibodies were positive. Among atypical patients with CD, 2 cases with normal histology had positive IgA-EMA and a clear positive clinical response to the gluten-free diet (Tables 1 and 2). Autoimmune conditions were detected in only 4 cases (5.5% of total sample), all of them having typical clinical presentations (Table 1). Intense histological lesions were the most frequent finding in both typical and atypical cases (Table 2). Less intense lesions were found

TABLE 1. Clinical characteristics of patients with CD at the time of diagnosis

		CD cases (n = 72)			
		Typical clinical presentation		Atypical clinical presentation	
	Total, n	n	Frequency, %	n	Frequency, %
No. patients	72	53	73.6	19	26.4
Sex, female/male	51/21	38/15	74.5/71.4	13/6	25.5/28.6
Symptoms					
Diarrhea	53	53	100	—	—
Abdominal distension	53	53	100	—	—
Constipation	6	—	—	6	100
Abdominal pain	35	28	80	7	20
Malnutrition	24	24	100	—	—
Weight loss	38	35	92.1	3	7.9
Autoimmune disease					
DM type 1	1	1	100	—	—
Down syndrome	2	2	100	—	—
Dermatitis herpetiformis	1	1	100	—	—
Other diseases					
Anemia	21	13	61.9	8	38.1
Osteoporosis	4	3	75	1	25
Short stature	4	3	75	1	25
Positive antibodies					
EMA	31	26	83.9	5	16.1
tTG	31	25	80.6	6	19.4

CD = celiac disease; DM = diabetes mellitus; EMA = endomysial antibodies; tTG = transglutaminase antibodies.

only in atypical presentations, 6% were classified as Marsh 2, and 3% had normal histology.

HLA typification revealed that the most frequent alleles among patients with CD were HLA-DQB1∗201 and 202 (55%), whereas among controls the highest frequency was HLA-DQB1∗301 and 302 (36%). The predominant conformation was DQ2. Alleles conforming heterodimers DQ7 were found in 16% and 28% among patients with typical and atypical presentations ($P > 0.05$). Distribution of risk by HLA-DQB1∗ genotypes and mtDNA haplogroups in cases and controls is shown in Tables 3 and 4, respectively. For the analysis of haplotypes, we defined the homozygote condition as the high-risk category and no DQ2/DQ8 as the low-risk category; comparison of these 2 groups yielded an OR of 44 (CI 9–267); as expected, the medium risk category yielded a lower value of OR, 18 (CI 5.22–104.6) (Table 3). With regard to haplogroups, C showed the highest frequency and was more frequent among patients ($\chi^2 = 0.01$, $P < 0.05$) (Table 4). Comparison of cases with A/B/C or D haplogroup against controls resulted in a $P < 0.02$ (group χ^2), OR 5.81 (CI 1.1–399.1) (Table 4).

Intensity of histological damage showed no relation to haplotypes (expressed as alleles or genotypes) or to haplogroups. No differences were found by haplotypes or by mtDNA

TABLE 2. Case-control study: histological damage in patients with CD

		CD cases			
		Typical clinical presentation, n = 53		Atypical clinical presentation, n = 19	
Histological damage	Total, n = 72	n	%	n	%
Normal	2	—	—	2	100
Marsh 1	—	—	—	—	—
Marsh 2	4	—	—	4	100
Marsh 3	66	53	80.3	13	19.7

CD = celiac disease.

Marsh criteria: type 0: normal mucosa; type 1: lesion infiltration: characterized by increased intraepithelial lymphocytes; type 2: hyperplastic lesion: type 1 + elongation of crypts; type 3: injury destructive: type 2 + villous atrophy: 3a (partial villous atrophy), 3b (subtotal villous atrophy), 3c (total villous atrophy); type 4: hypoplastic lesion: total atrophy + crypt hypoplasia.

TABLE 3. Case-control study: distribution of risk by HLA-DQB1* genotypes in patients and controls

	CD cases, n = 72						
	Total	Typical presentation, n = 53		Atypical presentation, n = 19		Controls, n = 72	
HLA haplotype	n (%)	n	%	n	%	Total n (%)	OR (CI)
DQ2/DQ2 (high risk)	25 (34.7)	20	80	5	20	7 (9.7)	44 (9–267)
DQ2 or DQ8/other (medium risk)	44 (61.1)	32	72.7	12	27.3	28 (38.9)	18 (5.22–104.6)
Not DQ2/DQ8 (low risk)	3 (4.2)	1	33.3	2	66.7	37 (51.4)	

CD = celiac disease; CI = confidence interval; HLA = human leukocyte antigen; OR = odds ratio.

haplogroups when analyzed by clinical presentation at the time of diagnosis (typical or atypical) (Tables 3 and 4).

Analyses of patients with CD diagnosed in Chile, Argentina, and Uruguay (second protocol) are shown in Table 5. The frequency distribution of haplogroups differed among Uruguayan, Chilean, and Argentinean patients; comparison of the 3 study groups by Pearson χ^2 was 85.00, Pr = 0.001; the difference was mainly caused by the high frequency of non-A/B/C/D haplogroups among Uruguayan patients. In Chilean patients, haplogroup B showed the highest frequency. Because we obtained results from a previous study in the same area of the country, we contrasted our results with the previous ones. These comparisons showed that in 1999, haplotypes DQ8 and DQ2 were 43% and 25%, respectively (8), whereas in the present study, these figures were 10% and 55%, respectively. In 1999, haplogroup B (32.5%) showed the highest frequency, whereas in the present study, haplogroup C was the most prevalent (43%).

DISCUSSION

The risk of CD associated with HLA haplotypes in the group assessed was similar to descriptions that originated in European

TABLE 4. Case-control study: distribution of mtDNA haplogroups in patients and controls

	CD cases (n = 72)					
	Total	Typical presentation, n = 53		Atypical presentation, n = 19		Controls (n = 72) Total
mtDNA haplogroup	n (%)	n	%	n	%	n (%)
Haplogroup A	5 (6.9)	4	7.6	1	5.3	8 (11.1)
Haplogroup B	20 (27.8)	13	24.5	7	36.8	11 (15.3)
Haplogroup C*	31 (43.1)	23	43.4	8	42.1	27 (37.5)
Haplogroup D	15 (20.8)	12	22.6	3	15.8	18 (25)
Other haplogroups†	1 (1.4)	1	1.9	0	0.0	8 (11.1)
Total A/B/C/D haplogroup	71 (98.6)	52	98.1	19	100	64 (88.9)

CD = celiac disease; CI = confidence interval; OR = odds ratio.
* Cases against controls, $P = 0.01$ (χ^2).
† White or Asian haplogroups. Group χ^2 (cases with A or B or C or D haplogroup) against controls, $P = 0.016$ (χ^2, OR 5.81, CI 1.1–399.1).

TABLE 5. Distribution of mtDNA haplogroups in patients with CD from Chile, Argentina, and Uruguay

	Chile (n = 43)		Argentina (n = 96)		Uruguay (n = 57)	
	n	%	n	%	n	%
Haplogroup A	4	9.3	21	21.9	0	0.0
Haplogroup B	14	32.6	22	22.9	2	3.5
Haplogroup C	9	20.9	29	30.2	8	14.0
Haplogroup D	6	13.9	10	10.4	0	0.0
Other haplogroups*	10	23.3	14	14.6	47	82.5
Total A/B/C/D haplogroup	33	76.7	82	85.4	10	17.5

* White or Asian haplogroups, $P = 85.0026$, Pr = 0.000 (Pearson χ^2 of the 3 study groups).

population groups (26). It is worth commenting that in our assessed patients, DQB1∗201 was not associated with severe histological damage, which contrasts with some previous reports (27) and agrees with others (28). The lack of association found could be related to the high proportion of cases with severe mucosal damage (66/72) among our patients, their higher heterogeneity in comparison to the Jores et al (27), study or both.

Although A/B/C/D mtDNA haplogroups were found more frequently among cases (patients with CD) than controls, the fact that no mtDNA haplogroups were detected in only 1 case does not allow us to reach conclusions; however, it is interesting that the presence of native mtDNA haplogroups could modify the risk of CD and the finding deserves further investigation. Should this be the case, relevant implications would lead to ways to approach this condition in Latin America, and health policies promoting the active search for CD should be implemented. Independent of whether mtDNA haplogroups add risk of CD, our results suggest that efforts to actively search for CD in South America are worth promoting.

Discussing the influence of genetic characteristics of the disease in the countries assessed, one must keep in mind that exposure to gluten is a strong confounder in the analysis; it is well known that the Amerindian diet was, and in many South American areas still is, based on corn, potatoes, and quínoa. Consumption of wheat (and rye and barley) started approximately 500 years ago, and variations in dietary staples consumed in different regions are considerable. Being unable to incorporate gluten exposure in the analysis was a limitation of this study. Understanding the roles of genetic characteristics and gluten exposure in the current picture of CD in Hispanic America represents an attractive challenge for future research.

Haplogroups B/C and D were the most frequent findings among Chilean individuals, in both cases and controls. It is interesting to compare the results obtained in the present study with those of approximately 10 years ago, when the mtDNA haplogroup B predominated. Haplogroup distribution in Chile varies from north to south, depending on the native groups that created the current populations (21). Because haplogroup C predominates among Mapuche groups and they live in the south of Chile, we can speculate that our present results reflect the rural-to-urban migration from southern areas to Santiago (capital city). Data from national censuses (9) seem to support this idea because migration in this direction has occurred in the last decade and Mapuche groups have participated in this phenomenon. Another difference detected between the 2 studies is that in 1999 patients presented only classical clinical presentations with early digestive symptoms, whereas the group presently assessed was more heterogeneous in age and clinical presentation, including both typical and atypical forms of the disease. This may reflect that active search for the disease has been incorporated in clinical practice in the last 10 years.

mtDNA haplogroup frequencies coincided with the figures previously reported in the general populations in both the Chilean and Uruguayan groups, but not in Argentinean patients. The present results disagree with previously published data perhaps because of the different areas from which the samples were obtained. There is a strong contribution of Italian migrating groups in Buenos Aires, but not in Argentinean areas such as Resistencia (in the north of the country), where European migrations were few and therefore the presence of native genes is stronger (29). Thus, we interpret our results as suggesting genetic differences in the composition of the Argentinean population.

In summary, these results, obtained in the southern cone of South America, show that mtDNA haplogroups A/B/C/D are frequent, both in celiac cases and in nonceliac controls. Whether they modify the risk for CD deserves further study in a larger group of patients. Interestingly, in Chile, we detected in 2009 a change in the predominant HLA haplotypes among patients with CD, from DQ8 (in 1999) to DQ2.

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