

CLINICAL SCIENCE

Frequency distribution of HLA DQ2 and DQ8 in celiac patients and first-degree relatives in Recife, northeastern Brazil

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AIMS : The aim of this study was to evaluate the frequencies of the HLA genotypes DQ2 and DQ8 and the alleles A1*05, A1*0201, B1*0201 and B1*0302 in individuals with celiac disease in Recife, northeastern Brazil.

METHODS: HLA DQ2 and DQ8 genotyping was performed for 73 individuals with celiac disease and 126 first-degree relatives with negative transglutaminase serology. The alleles DQA1*05, DQA1*0201, DQB1*02 and DQB1*0302 were identified by sequencing using specific primers and the EU-DQ kit from the Eurospital Laboratory, Trieste, Italy and double-checked by the All Set SPP kit (Dynal).

RESULTS: Among the 73 cases, 50 (68.5%) had the genotype DQ2, 13 (17.8%) had DQ8, 5 (6.8%) had DQ2 and DQ8, and 5 did not have any of these genotypes. Among the 5 negative individuals, four had the B1*02 allele and one did not have any of the alleles studied. B1*02 was the most frequent allele in both groups (94% in the patients and 89% in the control relatives).

CONCLUSIONS: In this study, celiac disease was associated with the genotypes DQ2 and DQ8. DQ2 predominated, but the distribution of the frequencies was different from what has been found in European populations and was closer to what has been found in the Americas. The high frequencies of the HLA genotypes DQ2 and DQ8 that were found in first-degree relatives would make it difficult to use these HLA genotypes for routine diagnosis of celiac disease in this group.

KEYWORDS: Celiac disease; HLA; Relatives; Genotyping; Diagnosis.

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INTRODUCTION

The autoimmune and inflammatory processes that characterize celiac disease are triggered by gluten in genetically predisposed individuals, in the presence of environmental aggression that interferes with the integrity of the small intestine.¹ Among the known genetic markers, the HLA genes have the greatest impact on the development of the disease.² The presence of genes coding for DQ2 and DQ8 molecules of the HLA complex class II explains up to 40% of the occurrence of celiac disease in European populations.³ The association between the genotype DQ2 and celiac is explained by the high affinity of the DQ2 molecule of HLA

in cells presenting this antigen in the intestinal mucosa, towards peptides derived from gluten.⁴

In a multicenter study carried out in Europe, it was observed that the HLA genotype DQ2 is present in around 86 to 93% of celiac disease patients, while around 3 to 8% of these patients have DQ8 without DQ2.⁵ These markers are also carried by many individuals without celiac disease: 40 to 65% of first-degree relatives of celiac disease patients and 18 to 30% of the general population.⁶⁻⁸ However, in non-European populations, different frequencies are observed. In the United States, a greater proportion of HLA DQ8 and a smaller proportion of DQ2 than in Europe have been reported.⁹ HLA DQ2 and DQ8 frequencies similar to those observed in the USA have been described in Asian¹⁰, Cuban¹¹ and Chilean¹² populations.

Some authors have suggested that the HLA genotypes DQ2 and DQ8 not only are indicators of genetic vulnerability but also may be used for diagnosing celiac disease in situations that remain undefined even after biopsy.¹³⁻¹⁴

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Among first-degree relatives, just like among other groups at risk, active searching for new cases using serological tests is indicated. These groups present the highest concentration of cases with undefined diagnoses and would be the greatest candidates for genotyping.^{7,15} However, the frequency of the HLA genotypes DQ2 and DQ8 among family members is greater than among the general population and it also varies between different regions of the world. Thus, their diagnostic use also varies between populations.

The HLA system is extremely polymorphic and reflects the process of formation of each population.¹⁶ The Brazilian population is intensely miscegenated, with marked differences between the regions. In the northeastern region of Brazil, the genetic markers for this disease have not been studied yet. Recent studies have demonstrated that the frequencies of celiac disease in the Americas and in Brazil are similar to what has been described in Europe.¹⁷⁻¹⁹ This knowledge has increased the number of new diagnoses of celiac disease in Brazil, especially among high-risk groups. The diversity of celiac disease among these groups shows the need for greater knowledge about celiac disease in this population. In the present study, we aimed to evaluate the frequencies of alleles coding for the HLA molecules DQ2 and DQ8 among individuals with celiac disease and their first-degree relatives in northeastern Brazil.

METHOD

Study population and Design

This study was conducted in pediatric gastroenterology outpatient clinics in the city of Recife, State of Pernambuco, northeastern Brazil, between August 2007 and May 2008. Children and adolescents with a diagnosis of celiac disease (index cases), together with the first-degree members of their families (fathers, mothers and siblings) were invited to participate in this study.

A free and informed consent statement was obtained from all of the participants in the study. The study had previously been approved by the Ethics Committee for Research on Human Beings of the Fernando Figueira Integrated Medicine Institute (*Instituto de Medicina Integral Fernando Figueira*, IMIP), under the number 893, on November 10, 2006, and the work was performed in accordance with the principles of the 1983 Declaration of Helsinki.

The study had a cross-sectional design, in order to compare frequencies between cases and family controls. All the first-degree relatives who agreed to participate in the study underwent the human tissue transglutaminase (tTG) serological test, and those with positive findings were invited to undergo an intestinal biopsy.

Cases were taken to be celiac disease patients with a diagnosis in accordance with the criteria of ESPGHAN 1990²⁰ (index cases) and family members with positive tTG and signs of villous atrophy on intestinal biopsy. The control group was formed by first-degree relatives of patients with negative results from tTG serological tests.

One hundred and eighty-two first-degree relatives of 66 patients were identified during the study period and 161 (88.4%) first-degree relatives were included in the study. Seven first-degree relatives whose serological tests were positive and biopsies presented villous atrophy were included in the case group, while 126 first-degree relatives with non-reactive serological tests for tTG antibodies made up the control group.

Serological tests:

After blood sample collection, the blood was centrifuged and the serum thus obtained was stored in Eppendorf tubes at -18°C. The serum tTG concentration was investigated by using quantitative ELISA with a commercially available kit (ImmuLISA™, IMMCO Diagnostics, Inc, Buffalo, NY, USA). Values lower than 20 EU/ml were considered negative, as suggested by the manufacturer.

HLA DQ2 DQ8 genotyping:

Genomic DNA was extracted from 2 ml of whole blood by using the Genome Wizard DNA kit (Promega), following standard protocols. The genotyping was performed by using the EU-DQ kit from Eurospital (Trieste, Italy). PCR products were detected by means of electrophoresis, which was run on 2% agarose gel and viewed under UV light. HLA haplotypes were double-checked by using the PCR-SSP Kit AllSet (Dynal, BIOTECH A.S.A, Oslo, Norway).

The frequency of HLA DQ2 and DQ8 were based in the frequency of the alleles - A1*05, DQB1*02, A1*0201 and B1*0302 in genotyping. We consider carrier genotype DQ2 individuals who had the alleles A1*05 and DQB1*02 in genotyping and the genotype DQ8 those who had the allele B1*0302 in PCR screening.

Statistical analysis:

The frequencies of HLA genotypes and alleles were described as percentages with their respective confidence intervals. The continuous variables were described as medians and percentiles. The frequencies of the groups were compared by using the chi-squared test (χ^2). The significance level adopted was 5% ($p < 0.05$). The data were stored and analyzed using the EpiInfo 6.04 software package.

RESULTS

The characteristics of the 73 cases are described in Table 1. Among the 126 controls, 68 (54%) were women and 58 were men; 33 (26.2%) were fathers, 45 (35.7%) were mothers and

Table 1 - Sex, age and clinical characteristics of the cases at the time of diagnosis.

	Patients (n = 73)	
	n	%
Sex		
Male	37	50,7
Female	36	49,3
Age at diagnosis		
Less than 2 years	8	11,0
2 to 6 years	35	47,9
7 to 12 years	17	23,3
13 to 21 years	6	8,2
More than 21 years	7	9,6
Clinical form at diagnosis		
Classic form *	29	39,7
Mild gastrointestinal symptoms	21	28,8
Non-gastrointestinal symptoms †	8	11,0
Diagnosis by serological screening ‡	15	20,5

*Classic form: diarrhea, abdominal distension and associated malnutrition.

†Non-gastrointestinal symptoms in this group: short height, diminished bone mineral density.

‡Indication for serological screening in this group: first-degree relative, diabetes mellitus. In both groups tTG was utilized for screening.

Table 2 - Frequencies of genotypes DQ2 and DQ8 in patients and control relatives.

HLA	Patients (%)		Controls (%)		χ^2	p
	n (%)	CI	n (%)	CI		
Absent	5 (6.8)	2.5 – 15.9	27 (21.4)	14.8 – 29.8	7.28	0.006*
DQ2	50 (68.5)	56.4 – 78.5	66 (52.4)	43.3 – 61.2	4.94	0.026*
DQ8	13 (17.8)	10.1 – 28.8	20 (15.9)	10.1 – 23.6	0.13	0.72
DQ2 + DQ8	5 (6.8)	2.5 – 15.9	13 (10.3)	5.8 – 17.3	0.68	0.41
Total	73 (100)	-	126 (100)	-	-	-

*p < 0.05

48 (38.1%) were siblings. The parents' ages ranged from 23 to 56 years, with a median of 38 years (P25/75: 33/43 years), and the siblings' ages ranged from 20 months to 27 years, with a median of 13 years (P25/ P75: 8/16 years).

The HLA genotypes DQ2 or DQ8 were present in 68 of the 73 celiac individuals (93.1%; 95% CI: 84 – 97%) and 99 of the 126 controls (78.6%; 95% CI: 70 – 85%). The HLA genotypes DQ2 and DQ8 frequencies distribution in the two groups are described in Table 2.

Among the five patients who did not present HLA DQ2 or DQ8, four had the allele B1*02 without A1*05 and one did not have any of the alleles investigated with the Eu-DQ kit.

The frequencies of the alleles A1*0201, A1*05, B1*02 and B1*0302 in celiac patient and controls are described in Table 4. B1*02 was the most frequent allele in both groups and was found in 93% of the patients and 88 % of the control. No differences in the distribution of each allele alone were observed between the two groups.

DISCUSSION

The genotypes DQ2 and DQ8 were present in 93.2% of patients with Celiac Disease in the Northeast of Brazil. The HLA DQ2 was present in 75.6% and DQ8 in 17.8% of these patients and these proportions differ from those described in European populations.²¹ In celiac patients of European population the frequency of the HLA DQ2 is up of 90% e the HLA DQ8 is between five and 10%, like was described in recent study performed in 2308 cases from Dutch, UK and Irish.²²

Differences in the frequencies of the HLA genotypes DQ2 and DQ8 in non-European populations have already been described. Butterworth et al, in England, demonstrated that patients of Indian origin had lower frequency of HLA DQ2 than those of British origin.¹⁰ Lower frequencies of HLA DQ2 and higher frequency of HLA DQ8 than European have also been described among celiac disease patients in the United States (82% DQ2 and 16% DQ8 only) and in Cuba (86% DQ2)^{11,17}

In South America, Araya et al studied Chilean celiac patients of Amerindian origin and found that genotype DQ8 predominate among this group. These results can possibly be explained by the origins of the Chilean population, formed by miscegenation of the Mapuche people with Spaniards.¹² In Brazil, the miscegenation is so great that it is erroneous to identify genetic ancestry through physical characteristics.^{23,24} Thus, in our population, it is impossible to determine the composition of the group in terms of Caucasian, Amerindian or black African origin.

Another finding from this group is that 79% of the unaffected control families carried genotype DQ2 and/or DQ8, which is one of the highest frequencies so far described among first-degree relatives. Just like the genotypes DQ2 and DQ8, A1*0201, A1*05, B1*02 and B1*0302 alleles were very frequently found among the first-degree relatives of the patients. We raise the possibility that these data were overestimated because of characteristics peculiar to this group of controls. We believe that one of the biggest limitations to this study is that, among the family controls, there might be some patients. This would occur both because of the characteristic that celiac disease may be manifested at any time during life, which would put limits on evaluations made at a single time, and because of the possibility of false negative tTG findings. However, most studies on HLA among first-degree relatives used designs similar to ours, and found that no more than 59.5% of first-degree relatives in Europe presented HLA DQ2 and DQ8.^{7,15}

At present, there is no data on the distribution of HLA DQ among the general population of Recife. Nevertheless, since the frequencies of genetic markers among populations of first-degree relatives reflect and amplify those among the general population of which they form part, this allows us to speculate that in our region, a large proportion of the general population may carry these markers. One of the theories that explain the differences in HLA between different populations relates to negative selection of these molecules that has occurred during human evolution.¹⁶ This selection has been affected by the different antigen stimuli to which each population has been exposed.²⁵ In the case of celiac disease, it depends primarily on gluten exposure, and possibly also on other environmental factors.²⁶

Recife is located in northeastern Brazil, a region that was first peopled by Amerindian tribes, like the remainder of the Americas. These tribes came from places where there was no wheat-growing culture (peoples who came from Asia, arriving here via the Bering Strait).²⁷ Furthermore, differing from Europe and the Middle East (the region around the Fertile Crescent), where gluten has been consumed for more than 10,000 years, this food only came to the Americas with the European colonizers 500 years ago.^{26,28} Consequently, in our region, there has been less time for negative selection of

Table 3 - Characteristics of the patients without HLA DQ2 or DQ8, with regard to sex, age, diagnostic symptoms and alleles present in genotyping.

Patient	Sex	Age	Signs and symptoms	Alleles
1	F	3 years	Diarrhea, abdominal pain, abdominal distension and malnutrition	B1*02
2	F	8 years	Diarrhea and weight loss	B1*02
3	F	14 years	Constipation, abdominal distension, abdominal pain, ulcers and asthenia	B1*02
4	M	22 months	Diarrhea, vomiting, abdominal distension and malnutrition	B1*02 A1*0201
5	F	2 years	Diarrhea, abdominal pain, abdominal distension and malnutrition	Negative †

†For the four alleles investigated (A1*0201, A1*05, B1*02 and B1*0203)

Table 4 - Frequencies of the alleles A1*0201, A1*05, B1*02 and B1*0302 in the patient and control relative groups.

Allele	Patients (n = 73)		Controls (n = 126)		Total (n = 199)		χ^2	p
	+	%	+	%	+	%		
A1*0201	10	13,7	15	11,9	25	12,5	0.14	0.33
A1*05	55	75,3	84	66,7	139	69,8	1,65	0.19
B1*02	68	93,2	111	88,1	179	89,9	1.31	0.25
B1*0302	18	24,7	33	26,2	51	25,6	0.06	0.81

these genes, thereby leaving their frequencies relatively high in this population.

Another finding from the present study was that five out (6,8%) of the 73 celiac patients presented neither DQ2 nor DQ8, although four of them had at least the allele B1*02. Among these patients, only one did not have the classic form of celiac disease, but this patient presented considerable gastrointestinal symptoms. These findings are concordant with several previous studies in which it was demonstrated that just one of the alleles A1*05 or B1*02, coding for half of the DQ2 heterodimer molecule would confer a predisposition towards triggering celiac disease.^{2,5,21}

In our study, we found the A1*0201 allele in nine patients: one of them was negative for DQ2 and DQ8 but had the allele B1*02. These findings are concordant with European data. In a multicenter study conducted in Europe in 2002, 61 out of the 1008 celiac patients studied did not present DQ2 and DQ8 genotypes. Thirty-four out of the 61 patients presented the gene A1*0201 in association with B1*02, only four were characterized non-DQ2 and/or DQ8 haplotypes.²¹ It has been hypothesized that the genotype A1*0201-B1*0201 is capable of coding for a molecule of DQ2 with lower affinity for gliadin. The patient who did not present any of the alleles investigated was diagnosed in accordance with the criteria of ESPGHAN, with classic symptoms, positive serological tests and signs of villous atrophy from the biopsy. This patient underwent HLA genotyping repeated three times in blind with the Dynal all Set SSP kit and resulted HLA DQA1*01.

We hypothesize that in the subjects analyzed in our study the genotypes DQ2 and DQ8 conferred vulnerability towards the development of celiac disease. However, the distribution of the frequencies of the genotypes DQ2 and DQ8 were different from distributions found in European populations. The distribution was closer to what has been found in other studies conducted in the Americas. The high frequencies of the HLA genotypes DQ2 and DQ8 found in first-degree relatives would make it difficult to use these HLA genotypes for routinely diagnosing celiac disease in this specific group. In the future, it will be necessary to investigate the HLA genotypes DQ2 and DQ8 in the general population of Recife, in order to achieve better understanding of these markers in our region.

CONCLUSION

Although the genotypes DQ2 and DQ8 were presents in the main of the celiac patients in Recife, northeastern Brazil, the distribution of these genotypes was different from what had been found in European population. The quite high frequencies of the HLA genotypes DQ2 and DQ8 found in first-degree relatives would make it difficult to use these HLA genotypes for routinely diagnosing celiac disease in this group.

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