DISTRIBUTION OF HLA-DPB1, -DQB1 – DQA1 ALLELES AMONG SARDINIAN CELIAC PATIENTS

M.F. BOY, G. LA NASA*, A. BALESTRIERI, M.V. CHERCHI, P. USAI
Istituto di Medicina Interna, University of Cagliari, Italy
*Istituto di Clinica Medica, University of Cagliari, Italy

SUMMARY

The Sardinian population in many aspects differs from other Caucasoid populations, particularly for its degree of homogeneity.

For this reason we have studied 50 adult Sardinian patients with celiac disease (CD) and 50 control healthy Sardinian individuals by RFLP analysis and by extensive oligotyping for 17 HLA-DPB1, 8-DQB1 and 9-DQA1 alleles, and established their -DPB1 alleles and -DQB1 -DQA1 genotypes. The heterodimer HLA-DQB1*0201/DQA1*0501, present in 96% of our patients, is strongly associated with CD susceptibility, confirming published reports.

On the other hand we found in 11 of 50 probands (22%) the presence of the allele -DQB1*0502/DQA1*0102. This genotype is extremely rare in other Caucasian populations and appears to confer limited protection in CD Sardinian patients.

KEY WORDS Celiac disease -DPB1, -DQB1, -DQA1 alleles HLA class II genes

INTRODUCTION

Human leukocyte antigens (HLA) are associated with genetic susceptibility to a large number of diseases. Some of the associations are related to specific aminoacids or epitopes of HLA class II molecules. The major histocompatibility complex (MHC), class II molecules, are cell surface heterodimers found on antigen presenting cells and B lymphocytes.

The HLA-D region of the short arm of chromosome 6, contains 12–15 non allelic class II genes and has been subdivided into three subregions, HLA DR, DQ and DP, each containing at least one functional A and B locus.

Celiac disease (CD) is an autoimmune disorder characterized by small intestinal mucosal injury and malabsorption, caused by abnormal sensitivity to gliadins, components of wheat gluten and related proteins of others grains. The condition has been reported to be MHC, class II, associated in a number of studies (Bugawan et al., 1989). CD was initially associated with HLA class I antigens A1 and B8 (Falchuck et al., 1972), later with the HLA class II, DR3 and DR7 (De Marchi et al., 1983) antigens, later still with HLA DQW2 (Tosi et al., 1983); finally a significant association with DPB1 alleles emerged. Moreover, different reports indicate significant associations of CD, with HLA DQB1 - DQA1 (Roep et al., 1988). and DPB1 alleles defined by restriction fragment length polymorphism (RFLP) analysis (Howell et al., 1988; Niven et al., 1987) or by probing polymerase chain reaction amplified genomic DNA, with sequence specific oligonucleotides (Bugawan et al., 1989; Roep et al., 1988; Howell et al., 1988; Niven et al., 1987).
al., 1987; Rosenberg et al., 1989). Data on the DP associations are nevertheless questionable not only among different ethnic groups, but also within various regions populated by the same ethnic group. On the other hand, several studies confirm that the heterodimer-alpha/beta, encoded by the DQA1*0501/DQB1*0201 genes, is the HLA genetic determinant most closely associated with CD (Sollid et al., 1989, Sollid and Throsby, 1990; Spurkland et al., 1990).

We have studied a group of Sardinian patients, with the late onset form of CD and have focused on these genes, hoping to establish more clearly whether alleles of the DQ and DP regions are always implicated in CD susceptibility.

MATERIAL AND METHODS

Patients and normal controls

The patient group comprised 50 unrelated adults (13 males, 37 females), with the late onset form of celiac disease. Family studies have been performed and complete HLA haplotypes were determined. Fifty ethnically matched controls, with no family history of autoimmune disorders, were typed.

Serological HLA typing

HLA class I and II antigens were determined using the standard microlymphocytotoxicity assay.

RFLP analysis (28 patients and 22 controls with DQw2-DR3 genotype were investigated using traditional methods)

High molecular weight DNA was extracted and then digested with the following restriction enzymes: Bam HI, Eco RI, Hind III, Taq I, Rsa I, Pvu I, Msp I, Pst I, Xba I, Bgl II. It was subjected to electrophoresis in 0.8% agarose gel and transferred onto nylon membranes (Z probe Biorad), by Southern’s method. Hybridization was performed with the following cDNA probes: betaDR, betaDQ and alphaDQ, betaDP and alphaDP.

Oligonucleotides

Oligonucleotides were synthesized using a DNA synthesizer (Beckman system I plus) and purified by the applied biosystem OPC (oligonucleotide purification cartridge) method.

The sequence of the ASO probes and primers used to amplify the second exon of the HLA-DQB1, -DQA1 and -BPB1 genes have already been reported (Bugawan et al., 1989; Spurkland et al., 1990; Khalil et al., 1990).

PCR amplification

Amplification by PCR was performed on approximately 1 µg human genomic DNA, using 50 pmol of each primer. Two units of Taq polymerase (Perkin-Elmer-Cetus) were added to a 100µl vol. of polymerase buffer containing 50 mM KCL, 10 mM TRIS pH 8.4, 2.5 mM MgCl, and 0.001% gelatin.

Each of the four deoxynucleotide triphosphates was present at 2 mM (8 mM total dNTP). The reactions were amplified for 30 cycles using a DNA thermal cycler (Perkin-Elmer-Cetus) with a two step temperature cycle (denaturation: 96° for 30"; annealing and extension: 65° for 45").
**Dot spot**
A 10 µl aliquot of each amplified DNA was mixed with 8 µl of 10 N NaOH and 10 µl of 0.50 mM EDTA.

Samples were kept at room temperature for 10 min., then applied to a nylon membrane (Biorad Z probe) using a DOT SPOT apparatus (Schleicher and Schuell, Keene, NH).

**Hybridization**
The oligonucleotide probes were 5' end labeled with gamma 32P dATP; filters were hybridized for 3 hours at 60° C in Church buffer (EDTA 1 mM pH 7.5; Na₂HPO₄ 0.5 m pH 7.2, SDS 7%) and 2x10⁶ cpm/ml probe. After hybridization the filters were washed in 1 mM EDTA pH 7.5, 40 mM Na₂HPO₄ pH 7.4, 1% SDS, for 10 minutes at T. Melting.

The filters were exposed to Kodak X-AR 5, at -80°C with intensifying screen for 2 hours and/or overnight.

**Statistical evaluation**
The Chi-Square test was used as appropriate.
Applying the Bonferroni criterium, P Values were corrected for the number of comparisons made.

The relative risk (according to Wolf’s test with Haldane’s correction) and confidence limits were calculated.

**RESULTS**
The frequencies of polymorphic restriction fragments observed in our patients and controls, versus those described by other authors, are shown in Table 1.

No significant differences between patients and controls DQw2, DR3 were found. In particular the alphaDP 3.5 Kb, BgI II fragment associated with CD, was found in 50% of our patients versus 56% of matched controls.

Results concerning HLA-DQB1 and -DQA1 typing obtained by DOT BLOT analysis are given in Table 2 and 3.

Almost all patients carried HLA-DQB1*0201/-DQA1*0501 alleles (96%). In 22% of patients the HLA-DQB1*0502/-DQA1*0102 genotype was found although this has been uncommon in Italian CD already described. Among the latter group two were only celiac individuals lacking the DQB1*0201 allele.

The DQB1 alleles showed the most significant association with CD. Homozygotes for the DQB1*0201 allele comprised 38% of patients (P= >0.001; RR= 6.3; IC 95% =16–48). We noticed also the DQA1*0201 allele occurred in 26% of celiacs compared with 4% of controls (p= 0.005; RR= 6.5; IC 95% =8–36).

Results of HLA-DPB1 genes analysis revealed a slight increase in the frequency of DPB1*0301 (31%) and DPB1*0402 (21%) alleles.

**DISCUSSION**
The findings in Sardinian CD patients differ somewhat from analysis of DQ and DP polymorphism as described for other Caucasian populations.
Table 1. Frequency of DQ and DP RFLPs in CD patients and controls from Sardinian and Caucasian populations (NS difference in Sardinian population, Chi squared test).

| RFLP Probe | 4KB RSA I α-DP | 16KB Xba I α-DP | 3.5KB Bgl II α-DP | 4Kb Bgl II α-DQ |
|------------|----------------|-----------------|-------------------|----------------|
|            | No. | %   | No. | %   | No. | %   | No. | %   |
| Sardinian  |      |     |      |     |      |     |      |     |
| Celiacs    | 21/28 | 75   | 18/28 | 64   | 14/28 | 50   | 18/28 | 100   |
| Controls   | 14/22 | 64   | 12/21 | 57   | 10/18 | 56   | 16/20 | 80    |
| Other Caucasian populations |      |     |      |     |      |     |      |     |
| Celiacs    | 18/19 | 95   | 16/19 | 84   | 51/65 | 78   | 29/30 | 97    |
| Controls   | 3/11  | 27   | 4/11  | 36   | 29/83 | 35   | 33/48 | 68    |

Table 2. HLA-DQA1 locus subtyping in 50 Celiac patients and 50 ethnically matched Sardinian controls.

| HLA-DQA1* | Celiacs (50) | Controls (50) | RR | 95%IC |
|-----------|--------------|---------------|----|-------|
|           | No. | %   | No. | %   |      |      |
| DQA1*0501/DQA1*0501 | 18  | 36  | *6  | 12  | 3    | 7–41 |
| DQA1*0501/DQA1*0302 | 4   | 8   | 2   | 4   | 2    | 0–13 |
| DQA1*0501/DQA1*0201 | 13  | 26  | 2   | 4   | 6.5  | 8–36 |
| DQA1*0501/DQA1*0102 | 12  | 24  | 4   | 8   | 3    | 2–30 |
| DQA1*0501/DQA1*0101 | 3   | 6   | 2   | 4   | 1.5  | 0–10 |

*p=0.01, ^p=0.005 (Chi squared test): NS according to Bonferroni’s criterium.
RR: relative risk. IC: interval of confidence.

Table 3. HLA-DQB1 locus subtyping in 50 Celiac patients and 50 ethnically matched Sardinian controls.

| HLA-DQB1* | Celiacs (50) | Controls (50) | RR | 95%IC |
|-----------|--------------|---------------|----|-------|
|           | No. | %   | No. | %   |      |      |
| DQB1*0201/DQB1*0201 | 19  | 38  | *3  | 6   | 6.3  | 16–48 |
| DQB1*0201/DQB1*0502 | 9   | 18  | 3   | 6   | 3    | 0–25  |
| DQB1*0201/DQB1*0302 | 4   | 8   | 3   | 6   | 1.3  | 0–12  |
| DQB1*0201/DQB1*0301 | 12  | 24  | 5   | 10  | 2.4  | 0–29  |
| DQB1*0201/DQB1*0501 | 3   | 6   | 2   | 4   | 1.5  | 0–10  |
| DQB1*0201/DQB1*0602 | 1   | 2   | 0   | 0   | /    | 0–6   |
| DQB1*0201/DQB1*0301 | 2   | 4   | 1   | 2   | 2    | 0–13  |

*p=<0.001 (Chi squared test).
RR: relative risk. IC: interval of confidence.
CD patients in the USA and England showed a very high incidence of certain alphaDP and betaDP chain alleles (Howell et al., 1988; Niven et al., 1987); the most common allele in the USA was DPB1*0301 followed by DPB1*0402 and by DPB1*0101 (Kagnoff et al., 1989), while in a Caucasian English population an increase of DPB1*0101 was observed (Rosemberg et al., 1989). The same RFLPs did not reveal any significant DP association in an Argentinian population (Herrera et al., 1989). A low frequency of the alphaDP 3.5 Kb, Bgl II fragment present in 50% of our patients was noted, and the other RFLPs examined showed a moderate increase when compared with controls carrying the DQw2-DR3 haplotype.

The hypothesis that can be invoked to explain the above findings is that, in the Sardinian population, these alleles are preferentially associated with the DQw2 genotype independently from the disease. Previous research studies of the DPB1 alleles have been undertaken in different Italian regions: a group of CD pediatric patients from the Naples area in southern Italy, showed a significant association with DPB1*0402 and DPB1*0301 (Bugawan et al., 1989), while in the Bologna area in northern Italy, only the DPB1*0301 showed an association with CD (Colonna et al., 1990). Our results, regarding DPB1 alleles, do not confirm those found in other Italian regions.

Moreover, results determined by oligotyping for HLA-DPB1 alleles revealed in pediatric patients from the Naples area that the DPB1*0402 allele (52%) prevails. Whilst in our patients the frequencies of DPB1*0301 and DPB1*0402, respectively 31% and 21%, almost overlap those of adults checked in the Bologna area.

Regarding the DQB1 alleles, we found the most significant association in CD was homozygosity for the DQB1*0201 allele, identified in 38% of patients.

An interesting finding of this study is the relatively high frequency of DQB1*0502/DQA1*0102 genotype determined in 11 of 50 probands (22%) that confirms the limited general protection provided by these alleles in the Sardinian population.

The presence of the DQB1*0502 allele in 7.8% of patients has been previously reported in a study among Sardinian IDDM (Carcassi et al., 1991), in fact this allele, extremely rare in other Caucasian populations, was found in the Sardinian general population, with a frequency of 19.6% (Carcassi et al., 1991).

The DQB1*0502 has a serine in position 57 of the HLA-DQB1 chain, the absence of aspartic acid in this position seems generally to confer susceptibility to IDDM and not resistance.

In keeping with the above we could hypothesize that the DQB1*0502/DQA1*0102 alleles are also involved in CD susceptibility in the Sardinian population.

Moreover the examination of DQA1 haplotypes showed a preponderance of the haplotype DQA1*0501/DQA1*0501 (36%), followed by the alleles DQA1*0201 (26%) and DQA1*0102 (24%) both in heterozygosis with DQA1*0501.

At present it remains uncertain whether the DQA1*0201 and DQA1*0102 alleles may have an additional role in susceptibility to CD.

Finally the heterodimer HLA DQB1*0201/DQA1*0501, present in 96% of our patients, confirms this as the genetic marker most closely associated with CD in Sardinian as in other patient groups.

It is possible to affirm therefore that while this association is constant in each population described, other associations may vary according to the particular linkage disequilibrium of each ethnic group.
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