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Distinctive HLA-II association with primary biliary cholangitis on the Island of Sardinia

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Abstract

Background: The *HLA DRB1**08 allele associated with primary biliary cholangitis (PBC) among Caucasians is of low frequency in the Sardinian population.

Objective: The aim of our study was to type a cohort of PBC patients from the island of Sardinia for HLA class II antigens.

Methods: Twenty Sardinian patients affected by PBC, 14 with autoimmune hepatitis (AIH) and 89 healthy controls (HCs) were typed for HLA class II alleles by dot-blot analysis.

Results: The PBC-associated *HLA DRB1**08 allele was detected in none of the studied individuals. The *DRB1**0301–*DQB1**0201 was the prevalent HLA haplotype, detected in 19 (47.5%) out of 40 PBC haplotypes (OR = 3.0; 95% CI 1.5–6.2) and in 11 (39.3%) out of 28 AIH haplotypes (OR = 2.2; 95% CI 0.94–5.0), but in only 41 (23%) out of 178 HC haplotypes. Moreover, PBC patients showed an increased frequency of homozygosity for the *DQB1**0201 allele (35% compared with 6.7% of the HCs; OR = 7.5; 95% CI 2.2–25.7). The frequency of the DRB1*11 allele in the PBC group was about half of that seen in the Sardinian HCs (7.5% vs 15.7%) (p = ns).

Conclusions: Our study confirmed the low frequency of the *HLA DRB1**08 allele among Sardinians, either in the general population or PBC patients. The high prevalence of the *HLA DRB1**0301–*DQB1**0201 haplotype is a distinctive genetic feature of PBC among Sardinians. Our study strengthens the hypothesis that still unknown genetic, epigenetic, and environmental factors must be involved in the pathogenesis of different HLA-associated liver diseases, and it represents a pathfinder that warrants exploration in a future extensive study.

Keywords

Immune-mediated liver diseases, autoimmune liver diseases, autoimmune hepatitis

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Introduction

Primary biliary cholangitis[1] is the recently proposed name for primary biliary cirrhosis (PBC), a chronic, non-suppurative cholangitis of still unknown etiology, characterized by the progressive destruction of the small intrahepatic bile ducts, which might eventually lead to end-stage liver failure.[2] PBC is likely a multifactorial disease resulting from the interaction of genetic and environmental factors, including a possible infectious agent[3] and epigenetic mechanisms,[4] as suggested by its predominance in females, its pattern of occurrence in twins, its prevalence in specific geographical areas, and its frequent clustering with autoimmune diseases within the same family.[1–6] PBC presents with clinical and histological features highly suggestive of an

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immune-mediated pathogenesis, such as T-lymphocyte and plasma cell infiltration of biliary ducts, highly specific circulating autoantibodies and association with autoimmune diseases.[2] Serum antimitochondrial antibodies (AMA) are the hallmark of PBC,[7] and one of the major autoantigens is the E2-subunit of the pyruvate dehydrogenase complex (PDC-E2).

Unlike in autoimmune hepatitis (AIH) and other immune-mediated diseases, the human leukocyte antigen (HLA) *DRB1**08 allele has been recognized as the major HLA antigen predisposing to PBC in Caucasian and Asian populations.[8–10] However, in sporadic cases with PBC[11] and in the Danish population,[12] *HLA-DRB1**0301–*DQB1**0201 is the prevalent haplotype associated with PBC. Studies on the distribution of HLA class II haplotypes have revealed that Sardinians have a highly homogeneous genetic background that is distinct from that of other Caucasian populations.[13] In particular, the *HLA DRB1**08 allele has an extremely low frequency in Sardinia,[13] but no study has investigated the frequency of this allele in Sardinian PBC patients. The aim of this study was to evaluate the prevalence of HLA class II genes in a group of Sardinian patients affected by AMA-E2-positive PBC.

Materials and methods

Patients

Twenty patients (mean age, 57.7 ± 15.3 years, female/male (F/M) ratio of 18/2) affected by PBC were typed for HLA class II genes along with 14 patients (mean age, 29.8 ± 19.2 years with an F/M ratio of 5/9) affected by AIH. As an independent Sardinian cohort, we analyzed 89 ethnically matched healthy controls (HCs) with no family history of autoimmune disorders from the Blood Transfusion Service of the Azienda Ospedaliera Brotzu, Cagliari, Italy. Moreover, data previously published on HLA class II haplotypes in a large cohort of 631 randomly selected newborns were also included as controls for the Sardinian general population.[13] The diagnosis of PBC was in line with the guidelines published by the American Association for the Study of Liver Diseases.[14] All our patients had biochemical evidence of cholestasis with elevation of alkaline phosphatase activity, and all were positive for AMA reacting with AMA-E2. None of the 20 PBC patients showed evidence of overlapping AIH.[14] According to the AIH classification,[15] among the 14 AIH patients analyzed, eight were Type 1 AIH and six Type 2 AIH. All the patients and controls enrolled in this study were of Sardinian descent. All provided informed consent.

HLA class II genes

DNA was extracted using the method previously reported.[13] Amplification of the polymorphic second exon of 23 *DRB1*, *DQB1* genes and dot-blot analysis of amplified DNA with sequence-specific oligonucleotide probes were performed using the previously described oligonucleotide probes and procedures.[13]

Statistical evaluation

The association with distinct HLA variants was evaluated between groups with χ^2 test. Fisher's exact test was used when appropriate. Odds ratios (ORs) were calculated considering as controls the HCs and the previously published Sardinian newborns.[13] Ninety-five percent confidence intervals (95% CI) on ORs were calculated according to the Woolf method.[16] All p values are two sided, and p values ≤ 0.05 are considered to be statistically significant. Statistical analysis was carried out using the statistical software Stata 11.0 (StataCorp LP, College Station, TX, USA).

Results

Table 1 shows the *DRB1*, *DQB1* genotypes of the 20 PBC patients, 14 AIH patients and 89 HCs. The most frequent genotype detected in Sardinian PBC patients was the *DRB1**0301–*DQB1**0201/*DRB1**0301–*DQB1**0201 homozygosity (20% compared with 5.6% in HCs). It is interesting that the second most frequent genotype detected in PBC patients was *DRB1**0301–*DQB1**0201/*DRB1**0701–*DQB1**0201 (15% compared with 1.1% in HCs), which share the same homozygosity at the *DQB1* locus. Therefore, in Sardinia, PBC patients showed an increased frequency of homozygosity for the *DQB1**0201 allele (35% compared with 6.7% of the HCs; OR = 7.5; 95% CI 2.2–25.7).

In Table 2 the frequencies of the six major *DRB1*, *DQB1* haplotypes detected in our PBC and AIH patients are shown in comparison to HCs and those previously published in Sardinian newborns, respectively. The *DRB1**0301–*DQB1**0201 was the prevalent HLA haplotype, detected in 19 (47.5%) out of 40 PBC haplotypes (OR = 3.0; 95% CI 1.5–6.2 and 3.2, 95% CI 1.7–6.1, respectively) and in 11 (39.3%) out of 28 AIH haplotypes (OR = 2.2; 95% CI 0.94–5.0 and 2.3, 95% CI 1.1–5.0, respectively), but only in 41 (23.0%) out of 178 HC haplotypes and in 277 (21.9%) out of 1262 Sardinian newborns. No difference in the frequency of this HLA haplotype was found between PBC and AIH groups (Table 2, 47.5% vs 39.3%; p = ns).

Table 3 shows the distribution of PBC-associated HLA DRB1 alleles in Italian[8] vs Sardinian individuals, both in the general population and those with PBC. The frequency of the *HLA DRB1**0301 allele was two-fold higher in Sardinian PBC patients compared both to the Sardinian HCs and Sardinian newborns, while among continental Italians no differences in *HLA DRB1**0301 frequency were observed among groups.[8] As regards to *HLA DRB1**08, our results confirmed the very low frequency of this allele among Sardinians, either in the general population or in PBC patients. Conversely, the frequency of this allele was about three-fold higher in Italian PBC patients compared to the Italian general population.[8]

The *HLA DRB1**08 allele was not detected in any of the participants tested (Tables 1–3). Two HLA class II alleles (Table 3), namely *HLA DRB1**11 and *DRB1**13, have been recently described as conferring protection against PBC among Caucasians.[8] While in our series the *DRB1**13 allele showed similar frequency between groups, the *DRB1**11 allele frequency in the PBC group was about half of that seen in the Sardinian HCs or newborns (7.5% vs 15.7% and 13.5%, respectively), a finding that lacked statistical significance ($p = \text{ns}$).

Table 1. Distribution of *DRB1*, *DQB1* genotypes in patients with PBC or AIH and HCs.

No.	DRB1-DQB1 genotype	PBC (20)	AIH (14)	HCs (89)
1	0301-0201/0301-0201	4 (20%)	1 (7%)	5 (5.6%)
2	0301-0201/0701-0201	3 (15%)	–	1 (1.1%)
3	0301-0201/0101-0501	2 (10%)	–	4 (4.5%)
4	0301-0201/1601-0502	2 (10%)	2 (14.2%)	5 (5.6%)
5	0301-0201/110-0301	2 (10%)	2 (14.2%)	10 (11%)
6	0301-0201/1401-0503	1 (5%)	1 (7%)	2 (2.2%)
7	0301-0201/040-0302	1 (5%)	–	1 (1.1%)
8	0301-0201/1001-0501	–	2 (14.2%)	2 (2.2%)
9	0301-0201/130-060	–	1 (7%)	–
10	0301-0201/040-0302	–	1 (7%)	1 (1.1%)
11	1601-0502/1601-0502	1 (5%)	–	5 (5.6%)
12	1601-0502/040-0302	1 (5%)	–	–
13	1601-0502/110-0301	1 (5%)	–	–
14	1601-0502/0101-0501	1 (5%)	–	–
15	0701-0201/130-060	1 (5%)	–	–
16	0701-0201/1601-0502	–	1 (7%)	–
17	040-0302/040-0302	–	1 (7%)	–
18	040-0302/0101-0501	–	2 (14.2%)	–

PBC: primary biliary cholangitis; AIH: autoimmune hepatitis; HCs: healthy controls;

Discussion

The Sardinian population has a high degree of genetic homogeneity.[13] HLA class II genes have a unique and very homogeneous distribution in all the districts of the island.[13] In spite of its geographical location, when compared to other Caucasians, Sardinians show a very high frequency of the *HLA DRB1**0301–*DQB1**0201 haplotype, which is responsible for the high genetic susceptibility to clinically overt autoimmune diseases, including type 1 diabetes, Hashimoto's thyroiditis, celiac disease and multiple sclerosis.[17–19] In particular, multiple sclerosis has been associated with the *HLA DRB1**0301–*DQB1**0201 haplotype only in Sardinia but not in other Caucasian populations where the association involves a different *HLA DRB1* allele.[20] Similarly, we found that Sardinian PBC patients lack expression of *HLA DRB1**08, which is the major HLA allele conferring predisposition to PBC in other Caucasian and Asian populations, while carrying the same HLA haplotype that predisposes to other, both hepatic and extra-hepatic, immune-mediated diseases.[15] Thus, along with type 1 diabetes,

Table 2. Frequency of the six major *DRB1*, *DQB1* haplotypes in Sardinian PBC (a) and AIH (b) patients in comparison to healthy controls (HCs) (c) and Sardinian newborns (d).

DRB1	DQB1	Sardinian PBC (a) ($n = 40$)	Sardinian AIH (b) ($n = 28$)	Sardinian HCs (c) ($n = 178$)	Sardinian newborns (d) ($n = 1262$)	(a) vs (c)	(a) vs (d)	(b) vs (c)	(b) vs (d)
0301	0201	**19 (47.5%)**	**11 (39.3%)**	**41 (23.0%)**	**277 (21.9%)**	$\mathbf{p < 0.05}$	$\mathbf{p < 0.001}$	$p = \text{ns}$	$\mathbf{p < 0.05}$
0402-5	0302	2 (5.0%)	4 (14.3%)	3 (1.7%)	94 (7.5%)	$p = \text{ns}$	$p = \text{ns}$	$p = \text{ns}$	$p = \text{ns}$
0701	0201	4 (10.0%)	1 (3.6%)	6 (3.4%)	69 (5.5%)	$p = \text{ns}$	$p = \text{ns}$	$p = \text{ns}$	$p = \text{ns}$
1101-4	0301	3 (7.5%)	2 (7.1%)	28 (15.7%)	171 (13.5%)	$p = \text{ns}$	$p = \text{ns}$	$p = \text{ns}$	$p = \text{ns}$
1301-3	060	1 (2.5%)	1 (3.6%)	2 (1.1%)	35 (2.8%)	$p = \text{ns}$	$p = \text{ns}$	$p = \text{ns}$	$p = \text{ns}$
1601	0502	6 (15.0%)	3 (10.7%)	37 (20.8%)	241 (19.0%)	$p = \text{ns}$	$p = \text{ns}$	$p = \text{ns}$	$p = \text{ns}$

PBC: primary biliary cholangitis; AIH: autoimmune hepatitis.

Table 3. Frequency of PBC-related HLA DRB1 alleles in the general population and in PBC patients–both Italian (8) and Sardinian.

	n. alleles	*DRB1**03	*DRB1**08	*DRB1**11	*DRB1**13
Italian population	3984	7.8%	2.3%	30%	11.2%
Italian PBC	1328	10.9%	7.2%	13.6%	8.6%
Sardinian newborns	1262	21.9%	1%	13.5%	2.8%
Sardinian healthy controls	178	23.0%	0%	15.7.%	1.1%
Sardinian PBC	40	47.5%	0%	7.5%	2.5%

PBC: primary biliary cholangitis; HLA: human leukocyte antigen.

Hashimoto's thyroiditis, celiac disease and multiple sclerosis, at least in the Sardinian population, *DRB1**0301–*DQB1**0201 appears to be associated also with PBC and AIH.

Moreover, Sardinian PBC patients showed an increased homozygosity for the DQB1*0201 allele. In celiac disease, the HLA DQB1*0201 homozygosity influences the severity of the intestinal mucosa lesions in a dose-dependent manner.[18] Future studies could therefore investigate whether a dose-dependent effect of HLA DQB1*0201 homozygosity may affect the natural course and outcome of PBC in Sardinia. In parallel, two HLA alleles (DRB1*11 and DRB1*13) recently reported to confer protection against PBC in Caucasians[8] were not significantly different between PBC and controls in our study, even if the lack of statistical difference was likely due to the low power of the study, at least for the DRB1*11 allele.

Considering the high genetic risk of autoimmune diseases in Sardinia, it was important to include in our study an independent cohort of Sardinian adult healthy controls with no family history for autoimmune diseases, as some of the newborns considered as Sardinian "general population" are expected to develop autoimmune diseases later in life. The high frequency of the *DRB1**0301–*DQB1**0201 genes in the Sardinian population has been explained by the selective advantage of an increased immune protection toward epidemic infectious diseases, like malaria and plague, which have caused substantial mortality in the history of the island.[21,22] Therefore, the selection of specific genetic traits among the surviving Sardinians could be responsible, at least in part, for the high frequency of autoimmune diseases observed on the island today.[21,22]

The reason why the *DRB1**08 allele is underrepresented in Sardinia, and especially in Sardinian PBC patients, is not clear. Besides the role played by selective forces, the peculiar distribution of HLA genes found in all the various regions of the island is suggestive of an ancestral origin likely due to a founder effect on a population that has remained genetically unaffected by external immigration.[13,22] Moreover, recent genome-wide association studies (GWAS) showed that genetic predisposition to PBC mainly lies within the HLA region;[23] the non-HLA risk loci so far identified are similar in hepatic and non-hepatic immune-mediated diseases,[24] indicating that each disease phenotype must be attributable to the involvement of other factors.

These findings strongly suggest that multiple factors play a role in the pathogenesis of autoimmune diseases, which likely arise from a complex interaction between genes and acquired factors. The results of our study provide further evidence that the Sardinian population is characterized by a different genetic background compared to other populations. Several immune-mediated disorders may be linked to peculiar HLA haplotypes, in support of the concept that HLA associations with diseases might vary depending on the geographical areas.[6,14] Moreover, besides HLA associations, other unknown genetic, epigenetic, and environmental factors, including an infectious agent/s, might be implicated in the pathogenesis and clinical evolution of PBC or other autoimmune diseases.

Conclusions, strength and weakness of the study

To the best of our knowledge, this is the first study on HLA class II alleles in a cohort of Sardinian PBC patients, where for the first time significant differences emerged compared with PBC patients in continental Italian or other Caucasian populations. These results may highlight that Sardinians differ from other Caucasian populations not only in regards to the genetic background of the general population but also for specific genetic risks associated with immune-mediated disorders. This study represents a pathfinder on this topic, which warrants exploration in future, more extensive, studies.

Declaration of conflicting interests

None declared.

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