HLA Class II Alleles, Genotypes, Haplotypes, and Amino Acids in Primary Biliary Cirrhosis: A Large-Scale Study

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Twin and family studies suggest there is a significant genetic component to primary biliary cirrhosis (PBC). However, the inability to replicate reported associations has been a recurring problem, with the only consistently reported genetic association that between PBC and HLA-*DRB1*0801*. However, recently even this has been questioned, and a number of novel associations have also been reported. We reinvestigated HLA class II *DRB1, DQA1,* and *DQB1* alleles and haplotypes in a total of 492 well-characterized PBC patients, 412 from the United Kingdom and an additional 80 patients from northern Italy. There was a clear and significant association with HLA-*DRB1*0801* in both groups of patients compared to population-specific healthy controls (12% versus 4% in the UK patients, $P = .00087$, OR = 3.05; and 18% versus 6% in the Italian patients, $P = .021$, OR = 3.15). There were also significant protective associations with *DRB1*11* in the Italian patients (28% versus 47%, $P = .0071$, OR = 0.42), but not in the UK patients (8% versus 8%) and a protective association with *DRB1*13* in both series (14% versus 20%, $P = .042$, OR = 0.65 in the UK patients; and 10% versus 31%, $P = .00092$, OR = 0.25 in the Italian patients). In conclusion, a complex relationship exists between HLA and PBC, and some genetic associations may be population specific. (HEPATOLOGY 2006;44:667-674.)

Primary biliary cirrhosis (PBC) is an autoimmune liver disease characterized by immune-mediated damage to the biliary epithelial cells lining the small intrahepatic bile ducts.[1] Clustering of disease in geographic regions[2] and within families,[3-6] together with the recent reports of 75% concordance in monozygotic twins[7] and a sibling relative risk (λ_s) of 10.5 for PBC,[3] all suggest there may be a significant heritable component to the disease.[8-10]

The role of host genes in the pathogenesis of PBC remains poorly understood, though female sex, specific HLA alleles,[11-23] and other immunoregulatory genes[8,12,24-27] are all thought to be important. However, as with many non-Mendelian "complex" diseases, unraveling the genetic basis of PBC has proved very difficult, with failure to replicate reported associations a recurring problem. The most consistent reports refer to the associations with HLA, yet even these are controversial.[11-23]

The most frequently described HLA association in PBC is that with the *DRB1*08* family of alleles; *DRB1*0801* in Europeans[12-14,22,23] and North Americans of European descent,[11,15,20] and *DRB1*0803* in Japanese.[16-19] In Europeans the association, though strong in relative terms, accounts for a minority of patients.[11-15,20,22,23] More recent studies have described novel associations in PBC, and one report suggested the *DRB1*0801* association, reported in North America (United States and Canada),[15,20] England,[13,14,23] and Germany,[12] was not present in northern Italy.[21] Among the novel findings reported recently are a strong protective association with *DRB1*11*[21,28] and *DRB1*13*.[28] These latter findings have yet to be replicated in PBC patients from northern Europe.

A second controversy surrounds our own observations that the HLA-*DRB1*08* allele is more common in patients with late-stage disease than in patients with early-

Abbreviation: PBC, primary biliary cirrhosis.

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stage disease.[23] PBC is a phenotypically heterogeneous disease[29]; some patients remain asymptomatic, with only mild histological changes,[29,30] whereas others progress rapidly to cirrhosis and end-stage disease.[29] We previously hypothesized that case mix could be a factor in the variation in reported strength of this association,[23] further compounded by the effects of treatment on disease progression and the small sample sizes in subgroup analyses.

In the present study we present data for a total of 492 PBC patients, 412 from the United Kingdom (UK) and a smaller group from northern Italy. The aims of the study were to investigate the recent reports of associations with *DRB1*11* and *DRB1*13* from Italy in UK patients and to confirm or refute the reported absence of a *DRB1*0801* association in Italian patients. Further, we hoped to both identify population-specific differences in the distribution of HLA *DRB1*, *DQA1* and *DQB1* alleles in PBC and reexamine the relationship between specific HLA alleles, genotypes, and haplotypes and disease progression in PBC. The data presented illustrate the value of looking at different populations to identify and confirm genetic associations, the importance of large series in such analyses, and the implications for understanding investigations of HLA in other genetically complex liver diseases.

Patients and Methods

Subjects in UK Series. Four hundred and twelve well-characterized PBC patients from 2 major UK centers and 236 geographically and racially matched controls were studied. All patients and controls were of northern European ancestry. Thirty-three were male (8%) and 379 female (92%).

Subjects in Italian Series. For comparison, 80 well-characterized PBC patients and 95 geographically and racially matched controls from a single center in northern Italy were also studied. All were of European ancestry and resided in the Padova area of northern Italy. Eight patients were male (10%) and 72 female (90%).

All patients had definite disease defined as all of the 3 standard criteria: (1) liver histology diagnostic of or compatible with PBC, (2) cholestatic liver function tests, and (3) positive serum antimitochondrial antibody titer $\geq$ 1:40 detected by immunofluorescence. Subjects were excluded from the study if their biopsy (or any other clinical data) suggested additional, potentially confounding causes of liver pathology.

The liver biopsies of 246 patients (173 UK patients and 73 Italian patients) were reviewed to confirm diagnosis in order to determine stage of disease. Patients were classified as having advanced-stage (late) disease, that is, Scheuer stage III or IV, or early-stage disease, that is, Scheuer stage I or II.[31] The most recent liver biopsies of 163 patients (67%) showed histologically advanced disease (Scheuer stage III or IV).

Table 1. Sequences of HLA Class II Primers

Locus	Primers	Sequences
HLA	DRB (1)	5′-CCC CAC AgC ACg TTT CTT g-3′
	DRB (2)	5′-CCg CTg CAC TgT gAA gCT CT-3′
HLA	DQB (1)	5′-CAT gTg CTA CTT CAC CAA Cgg-3′
	DQB (2)	5′-CTg gTA gTT gTg TCT gCA CAC-3′
HLA	DQA (1)	5′-ATg gTg TAA ACT TgT ACC AgT-3′
	DQA (2)	5′-TTg gTA gCA gCg gTA gAg TTg-3′

All subjects and controls gave informed consent, and the study was cleared by the relevant local hospital ethics committees. DNA samples were labeled and stored by code only and analyzed without prior knowledge of individual identities.

Determination of HLA DRB1, DQA1, and DQB1 Genotypes. HLA genotyping of all 492 PBC patients and 331 controls was performed by standard polymerase chain reaction protocol for a total of 32 HLA DRB and *DQB* alleles or groups of alleles. In addition, HLA genotyping of 255 PBC patients (175 from the United Kingdom) and all 331 controls was performed for 10 *DQA1* alleles or groups of alleles. For each locus a pair of *DRB-*, *DQA-,* or *DQB*-specific primers corresponding to the second exon sequence was used (Table 1) to amplify approximately 100 ng of genomic DNA in a 50-μL reaction mix comprising 200 μmol/L each of dATP, dCTP, dGTP, and dTTP (Amersham Pharmacia-Biotech, St. Albans, UK); 1.5 mmol/L $MgCl_2$; 10 mmol/L Tris-HCl (pH 8.3); 50 mmol/L KCl; 0.01% gelatin; 1 μmol/L of each primer; and 2-2.5 U Taq polymerase (Perkin Elmer, Norwalk, CT) on a Perkin-Elmer GeneAmp 9600. PCR cycling parameters were as follows: 94°C for 120 seconds, 94°C for 10 seconds, 56°C for 60 seconds (10 cycles), 94°C for 30 seconds, 52°C for 30 seconds, 72°C for 45 seconds (23 cycles), and a final extension at 72°C for 300 seconds.

Following amplification, PCR amplicons were denatured and dot-blotted on a series of positively charged nylon membranes (20 membranes for *DRB1*, 16 for *DQB1,* and 10 for *DQA1*). Each membrane was hybridized with one of a series of digoxigenin-labeled allele- and sequence-specific oligonucleotide probes (SSO). Alleles were detected by chemiluminescence and assigned by 2 trained individuals according to probe specificity tables supplied by the British Society of Histocompatibility and Immunogenetics adapted from the 11th International Histocompatibility Workshop and Conference.[32]

Statistical Analysis. Allele and genotype distributions were compared with the χ^2 test using the EPISTAT

Table 2. Distribution of *DRB1, DQA1*, and *DQB1* Genotypes in Those with PBC Versus Controls for Both UK and Italy

Allele Family and Population	Patients N (%) UK (N = 412) Italian (N = 80)	Controls N (%) UK (N = 236) Italian (N = 95)	Probability and Odds Ratio (95% Confidence Interval)
*DRB1*08*-UK	50 (12)	10 (4)	$P = .00087$ OR = 3.05 (1.48-6.46)
*DRB1*08*–Italy	14 (18)	6 (6)	$P = 0.021$ OR = 3.15 (1.06-9.76)
*DRB1*11*–UK	34 (8)	20 (8)	ns
*DRB1*11*–Italy	22 (28)	45 (47)	$P = 0.0071$ OR = 0.42 (0.21-.83)
*DRB1*13*–UK	57 (14)	47 (20)	$P = 0.042$ OR = 0.65 (0.41-1.01)
*DRB1*13*–Italy	8 (10)	29 (31)	$P = 0.00092$; OR = 0.25 (0.1-0.63)
*DRB1*15*–UK	73 (18)	53 (13)	ns
*DRB1*15*–Italy	13 (16)	16 (17)	ns
*DQA1*0102*-UK[a]	53 (30)	69 (29)	ns
*DQA1*0102*–Italy	23 (29)	31 (33)	ns
*DQA1*0401*–UK[a]	24 (14)	10 (4.2)	$P = 0.00056$ OR = 3.59 (1.59-8.3)
*DQA1*0401*–Italy	14 (18)	7 (7)	$P = 0.04$ OR = 2.67 (0.94-7.79)
*DQB1*0301*–UK	109 (26)	80 (34)	$P = 0.045$ OR = 0.7 (0.49-1.01)
*DQB1*0301*–Italy	26 (33)	47 (49)	$P = 0.023$ OR = 0.49 (0.25-0.95)
*DQB1*0402*–UK	44 (11)	9 (4)	$P = 0.002$ OR = 3.02 (1.39-6.77)
*DQB1*0402*-Italy	15 (21)	6 (6)	$P = 0.012$ OR = 3.42 (1.16-10.52)

[a]NOTE. Total for *DQA1* in UK only is 175 patients.

statistical analysis program (Centers for Disease Control, Atlanta, GA) as appropriate. No correction factor was necessary as *only a priori*-identified alleles/haplotypes were found to be significant. Associations with alleles were tested by counting individuals positive for each allele as suggested by Svejgaard and Ryder,[33] and associations with haplotypes by counting the number of chromosomes. Odds ratios (ORs) are given instead of relative risk as the accepted standard.

Results

Distribution of HLA*-DRB1 *Alleles. There were 3 significant differences in *DRB1* allele distribution (Table 2). First, both patient populations had significantly higher *DRB1*0801* frequencies than their population-specific healthy controls: 12% of UK patients versus 4% of controls ($P = .00087$, OR = 3.05); 18% of Italian patients versus 6% of controls ($P = .021$, OR = 3.15). Second, both patients populations had significantly lower frequencies of *DRB1*13* alleles compared to controls, though the difference in UK patients was only of borderline significance: 14% of UK patients versus 20% of controls ($P = .042$, OR = 0.65); 10% of Italian patients versus 31% of controls ($P = .00092$, OR = 0.25). Third, and in contrast to the observations above, a strong protective association with *DRB1*11* was noted but *only* in the Italian patients (28% versus 47%, $P = .0071$, OR = 0.42).

Distribution of HLA* DQA1 *and* DQB1 *Alleles. Overall, there was only one novel association at either the *DQA1* or *DQB1* locus. In both the UK and Italian populations the *DQB1*0301* allele appeared to encode a reduced risk of PBC (OR = 0.7 and 0.49, respectively, Table 2). This allele is very commonly but not exclusively found in *DRB1*11* haplotypes.

The only other significant associations at *DQA1* or *DQB1* were with *DQA1*0401* and *DQB1*0402,* both of which preferentially cosegregate with *DRB1*0801*. These were significant in both patient series (Table 2, *DQA1*0401*: OR = 3.59 in UK patients, 2.67 in Italian patients; *DQB1*0402*: OR = 3.02 in UK patients, 3.42 in Italian patients).

Interestingly there were no associations with *DQA1*0102* or any other *DQA* or *DQB* alleles that preferentially cosegregate with *DRB1*13* .

Distribution of HLA* DRB1, DQA1, *and* DQB1 *Haplotypes in Patients and Controls. In the UK series

Table 3. Haplotype Distribution in PBC Patients and Controls

Allele at Each Locus			Patients N	Controls N	
DRB1	*DQA1*	*DQB1*	UK (n = 824) Italian (n = 160)	UK (n = 472) Italian (n = 190)	Probability and Odds Ratio
*0801	*0401	*0402	44 (5.3)	9 (1.9)	$P = .0027$, OR = 2.9
			16 (10)	6 (3)	$P = .0086$, OR = 3.41
*1101	*0501	*0301	35 (4.2)	20 (4.2)	ns
			21 (13)	46 (24)	$P = .0086$, OR = 0.47
*1301	*0103	*0603	27 (3.3)	17 (3.6)	ns
			3 (2)	12 (6)	$P = .0041$, OR = 0.28

there was only one significant haplotype (Table 3): *DRB1*0801-DQA1*0401-DQB1*0402* [44 of 824 (5.3%) patient chromosomes versus 9 of 472 (1.9%) control chromosomes, $P = .0027$, OR = 2.9]. Though there was a weak protective effect of *DRB1*13* alleles in UK patients, no particular *DRB1*13* haplotype could account for this. In contrast, in the smaller Italian series there were 3 significant haplotypes (Table 3): *DRB1*0801-DQA1*0401-DQB1*0402* [16 of 160 (10%) patient chromosomes versus 6 of 190 (3%) control chromosomes, $P = .0086$, OR = 3.41], *DRB1*13-DQA1*0103-DQB1*0603* [3 of 160 (2%) versus 12 of 190 (6%), $P = .0041$, OR = 0.28], and *DRB1*11-DQA1*0501-DQB1*0301* [21 of 160 (13%) versus 46 of 190 (24%), $P = .0086$, OR = 0.47]. The first and last haplotypes are highly conserved in European populations, and these findings therefore are not surprising. However, the second haplotype is only one of several possible *DRB1*13* haplotypes.

Distribution of* DRB1 *Amino Acid Residues. Comparing the amino acid sequences of the *DRB1*08* family of alleles with those of the *DRB1*11* and *DRB1*13* families showed few amino acid substitutions that could be invoked to create a model of susceptibility and resistance at the molecular level. These are: glycine for serine at position 13, tyrosine for histidine at position 16, tyrosine for phenylalanine at position 47, and leucine for alanine at position 74. Considering each possibility in turn: (1) Glycine-13 is found with all members of the *DRB1*08* family, whereas serine-13 is present in most, but not all *DRB1*11* and *DRB1*13* family members. Other *DRB1* alleles encode mostly histidine, arginine, and phenylalanine at position 13, though a few of the less common alleles do encode glycine (including *DRB1*1105, DRB1*1201, DRB1*1202, DRB1*1404,* and *DRB1*1411*). Glycine and serine are both small amino acids. However, glycine is nonpolar/hydrophobic, and serine is polar/hydrophilic. (2) At position 16 *DRB1*08* alleles encode tyrosine, whereas members of the *DRB1*11* and *DRB1*13* families and most other DRβ-polypeptides encode histidine. Tyrosine is an aromatic relatively hydrophobic amino acid, but histidine (with its imidazole group), carries a positive charge, a feature associated with the most hydrophilic amino acids. (3) In common with most *DRB1* alleles, *DRB1*08* alleles encode tyrosine at position 47. The exceptions to this rule are the *DRB1*11* and *DRB1*13* alleles, most of which encode another aromatic hydrophobic amino acid, namely, phenylalanine. Both phenylalanine and tyrosine are aromatic amino acids and relatively nonpolar, but tyrosine is significantly more polar than phenylalanine. (4) At position 74 most *DRB1*08* alleles encode leucine, whereas most *DRB1*11* and *DRB1*13* and most other *DRB1* alleles encode alanine (exceptions to this rule are the less common alleles *DRB1*0805* and *DRB1*0818,* which both have alanine-74, and *DRB1*1313*, *DRB1*1318*, *DRB1*1123* and *DRB1*1125,* which all have leucine-74). Leucine and alanine are both aliphatic hydrophobic amino acids; however, leucine is a significantly bigger molecule, with 6 carbon atoms compared with alanine which has only 3. Taken together, these data suggest that amino acid residues glycine-13, tyrosine-16, and leucine-74 may all contribute to *DRB1*08*-encoded susceptibility to PBC, whereas the amino acid residues serine-13 and phenyalanine-47 may both contribute to *DRB1*11*- and *DRB1*13*-induced resistance to PBC.

Relationship With Disease Progression. In the UK patient series there is a modest relationship between possession of *DRB1*0801* and histological stage of disease. Thus, of a total of 175 patients for whom data were available, 4 of 51 (8%) patients with early-stage disease had *DRB1*0801* compared with 23 of 122 (19%) patients with late-stage disease (OR = 2.73, $P = .069$). However, using the same parameters to measure progression, namely, histological score, no such relationship was apparent in the Italian patients. Thus, 7 of 35 (20%) patients with early-stage disease had *DRB1*0801* compared with 7 of 41 (17%) patients with late-stage disease. A comparison of the distribution of *DRB1*11* and *DRB1*13* alleles in the Italian series in those with early-

stage disease (stages I and II) versus those with late-stage disease (stages III and IV) also showed no significant differences (*DRB1*11*, 23% versus 34%; *DRB1*13,* 14% versus 7%). Overall, this suggests that *DRB1*0801, DRB1*1301,* and *DRB1*1101* are not predictive of disease progression in PBC, and such reported relationships may be the result of other factors such as case ascertainment bias.

Discussion

We made several significant and important observations in the present study. First, there was an association with the HLA *DRB1*0801-DQA1*0401-DQB1*0402* haplotype in both the UK and the Italian PBC patients. This observation contrasts with a recent report suggesting this genetic association does not exist in Italy.[21] Our data showed similarity in the distribution of most but not all HLA alleles in patients and controls from the United Kingdom and Italy, contradicting the idea Italians are "genetically different" from northern Europeans. Clearly, there are some differences, but these may not be as marked as implied by the authors of the earlier study.[21] The observation of a very strong genetic association with *DRB1*08*, albeit a different allele (*DRB1*0803*), in Japanese patients with PBC is also strong evidence against the former suggestion regarding PBC.[16-19]

Second, our data did agree with the observation suggesting *DRB1*11* may protect from the development of PBC.[21] We found a significant reduction in the frequency of *DRB1*11* in Italian patients but this was not found in the UK series. This failure is not attributable to small sample size or inadequate statistical power. The size of our study population (412) was sufficient to detect relatively small effects on disease risk with a high level of statistical confidence. Interestingly, *DRB1*11* is much more common in southern Europe (Table 4), suggesting population differences may account for some of the reported differences in the incidence and prevalence of PBC in different geographic locations.[2] Historically the possibility of a *DRB1*11* association in PBC was first suggested by Gores et al.,[11] who found reduced frequency of the DR5 serotype (9.6% of patients versus 25.2% of controls) in their 1987 study. *DRB1*11* is the major subfamily of DR5. However, until recently this observation had not been replicated. Thus, *DRB1*11* was reported in 7% of 159 UK patients compared to 12% of 162 controls,[13] studies in California revealed 16% of patients with *DRB1*11* versus 17% of controls,[15] and a recent large series from Toronto[20] reported 12.3% versus 14.4% of controls. However, with three independent reports of the same protective association—Gores et al.,[11] Invernizzi et al.,[21] and the present study—we need to reconsider the relationship between *DRB1*11* and PBC. There are two possible explanations for the *DRB1*11* findings. Either *DRB1*1101* is simply a marker for a true MHC-encoded protective allele in PBC, or there are genuine population-specific differences in these genetic associations with PBC. Table 4 shows the frequencies of several PBC-related HLA haplotypes and alleles in several populations where PBC occurs. Note the major differences between northern and southern Europe relate only to haplotypes 1 and 3 (*DRB1*4* and *DRB1*11*), whereas haplotypes 2 and 4 (*DRB1*08* and *DRB1*13*) are relatively evenly distributed between these populations.

The possibility of genuine population-specific differences in the genetic basis of PBC is not without precedent. There are reproducible population-specific differences in the HLA class II haplotypes associated with autoimmune hepatitis,[9,34,35] primary sclerosing cholangitis,[36] and responses to viral liver diseases, especially the hepatitis B

Table 4. Distribution of PBC-Related Haplotypes in Northern Versus Southern Europeans (U.S. and Japanese Data Shown for Comparison)

Allele/Haplotype	Population	Genotype Frequency	Number of chromosomes
*DRB1*04*	USA—reference 15	17%	478
	UK—present study	22%	472
	Italy—present study	7.3%	190
	Italy—reference 21	3.1%	1,116
	Japan—reference 46	25.3%	2,748
*DRB1*0801*	USA—reference 15	1.9%	478
	UK—present study	1.9%	472
	Italy—present study	3%	190
	Italy—reference 21	5.3%	1,116
	Japan—reference 46	13.6%	2,748
*DRB1*11*	USA—reference 15	8.3%	478
	UK—present study	4.2%	472
	Italy—present study	24%	190
	Italy—reference 21	17.4%	1,116
	Japan—reference 46	5.75%	2,748
*DRB1*13*	USA—reference 15	6.9%	478
	UK—present study	3.6%	472
	Italy—present study	6%	190
	Italy—reference 21	<5.2%	1,116
	Japan—reference 46	6.2%	2,748

NOTE. Data for all *DRB1*04* haplotypes not available. *DRB1*0801* haplotypes mostly carry *DQA1*0401-DQB1*0402*, *DRB1*11* haplotypes carry *DQA1*03-DQB1*0301*, *DRB1*13* cosegregates with a variety of different *DQA1* and *DQB1* alleles, and haplotype data are not generally available. Data for Japan[46] are included for reference only. However, note that at the level of resolution applied in the current study the only major difference between northern Europe and Japan is in the much higher frequency of *DRB1*08* alleles in Japan; all other allele families appear to be at similar frequencies. However, this similarity may be misleading because higher-resolution genotyping reveals more significant differences between Europeans and Japanese than is apparent at low resolution. In addition, comparisons show that although *DRB1-DQA1-DQB1* haplotypes are mostly conserved (and therefore predictable) within European subpopulations, they are most frequently different between Europeans and Japanese. Data for United States are based predominantly (though not exclusively) on northern Europeans.

and C viruses.[9] It is possible these differences are related to geographic differences in exposure to potential environmental triggers, especially but not exclusively infectious agents, several of which have been implicated in the pathogenesis of PBC.[37-40]

Alternatively, if the *DRB1* alleles discussed here are simply markers for nearby "true" susceptibility alleles in PBC, there are 2 immediate candidates to consider: the *DQB1* locus and the MHC-encoded C4 loci. It is difficult to unravel the relative contribution of *DQB1* to the increased risk of PBC because the *DRB1*0801* haplotype almost always carries *DQB1*0402*. However, when considering HLA haplotypes associated with a reduced risk of PBC, the weak association with *DQB1*0301* reported here is of considerable interest. This allele is almost always found in combination with the *DRB1*11* (DR11) family and is occasionally found on *DRB1*13* haplotypes. In addition, *DQB1*0301* is very commonly found in combination with the *DRB1*04* (DR4) family. In European populations specific differences in the distribution of the predominant *DQB1*0301* haplotypes may lead to different genetic associations at *DRB1,* all of which arise as a result of linkage disequilibrium with *DQB1*0301*. Interestingly, in hepatitis C virus infection the dominant protective HLA haplotype appears to be *DRB1*04-DQB1*0301* in some UK studies and *DRB1*11-DQB1*0301* in studies from France and Italy, with *DQB1*0301* the common element in the two haplotypes.

The second series of alternative candidates for MHC-encoded susceptibility and resistance to PBC that must be considered is MHC-encoded complement C4 genes. To date, very few studies have investigated C4 alleles in PBC. However, both studies that have looked at C4 reported significant associations, first with *C4B*2,*[24] and later with both *C4B*2* and *C4A*Q0.*[12] It is possible that different complement alleles carried on the *DRB1*0801, DB1*11,* and *DRB1*13* haplotypes determine subtle but significant differences in complement activity that are sufficient to explain the relative difference in disease risk associated with these haplotypes. If this hypothesis were true, then the population-specific differences discussed here would be coincidental.

The third observation, that of a significant association with *DRB1*1301,* has not been universally reported in PBC. In the present study this association, though relatively strong in Italian patients, was at best weak in the UK series, just reaching significance in the 412 patients studied. Additional, even bigger investigations are needed in both populations to determine whether this association applies to both populations or is only relevant to Italians. Interestingly, Begovitch et al.,[15] in a study of 51 patients, reported a lower than expected frequency of the HLA *DRB1*1302-DQA1*0102-DQB1*0604* haplotype in PBC. This association was not replicated here, nor was the hypothesis that the association may have been a result of the protective effect of the *DQA1*0102* allele. This latter allele is also carried on the most common *DRB1*1501* haplotype, but neither *DQA1*0102* nor *DRB1*1501* was found to be associated with PBC in the UK or the Italian patients (Table 2). The *DRB1*1301-DQA1*0103-DQB1*0603* haplotype has been identified as a genetic determinant of susceptibility to PSC,[36] and DR13 is associated with susceptibility to persistent hepatitis B virus infection,[41] fibrosis due to schistosoma infection,[42] type 1 AIH in children,[43] and, in South America, persistent HAV infection.[44]

The fourth observation made in the present study relates to the commonly used explanation for genetic associations between disease and specific HLA alleles, that is, the associated alleles have specific antigen presentation profiles. Antigen presentation is the key event in initiation of the immune response, and it is a reasonable hypothesis that genetic associations with HLA occur because HLA alleles determine the relative dynamics of an immune response. Analysis of the amino acid residues encoded by the different associated alleles discussed here revealed 4 major differences, all of which have potential and are worthy of further investigation. These are glycine-13, tyrosine-16, and leucine-74 (encoded by *DRB1*0801*) versus serine-13 and phenylalanine-47 (encoded by most *DRB1*11* and *DRB1*13* alleles, respectively). Three of these 4 positions are of potential functional importance. The amino acid residue at position 13 affects the binding of antigen side chains associated with both the fourth and seventh pockets of the expressed DR molecule (Fig. 1).[45] The amino acid residues at positions 47 and 74 influence

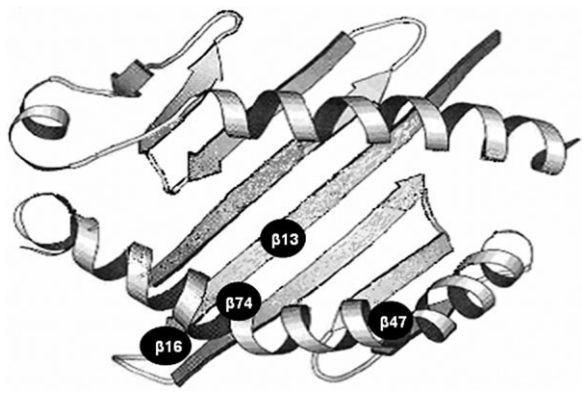

Fig. 1. A generic ribbon diagram of an HLA *DRB1* molecule (adapted from Stern et al., 1994[45]) looking into the peptide-binding groove from above. The molecule is composed of 2 opposing α-helices and a series of supporting β-pleated sheets. The relative positions of the 4 amino acids discussed are indicated by black dots. Of these 4 positions, 3 are on the DRβ β-pleated sheet and 1 is on the DRβ α-helix.

Table 5. Summary of Major Biochemical Differences Between HLA DRB1 Alleles Associated With an Increased Risk of PBC and Those Associated With a Reduced Risk

	Allele			Significant Difference		
Position	*DRB1*08*	*DRB1*11/13*	Other	*DRB1*08*	*DRB1*11/13*	Binding Pocket
13	Glycine	Serine	Histidine Alanine Arginine	Hydrophobic	Hydrophilic	4th and 6th
16	Tyrosine	Histidine	Histidine	Relatively hydrophobic	Hydrophilic	–
47	Tyrosine	Phenylalanine	Tyrosine	Relatively hydrophobic	Extremely hydrophobic	7th
74	Leucine	Alanine	Alanine	Size–large (Mr = 131) (Hydrophobic)	Size–small(Mr = 89) (Hydrophobic)	4th

NOTE. Tyrosine and phenylalanine, though essentially aromatic and hydrophilic, do have regions that are both nonpolar and polar and as such may be classified as amphiphatic. However, as neither amino acid carries a charge, they are most often classified as hydrophobic, especially when compared with highly charged amino acids such as lysine, arginine, or histidine.

the binding of antigenic side chains associated with the sixth and fourth binding pockets, respectively. Changes in hydrophobicity and hydrophilicity at positions 13, 16, and 47 and a major change in the size (molecular weight) of the residue at position 74 may influence the binding characteristics of the MHC molecule and promote or restrain the genesis of PBC (Table 5).

The last observation of the present study is that the proposed relationship between HLA class II alleles and disease progression/severity as measured by the histological index[23] has not been upheld 5 years on. The current observation regarding the UK patients is in keeping with published data from Toronto[20] and also our own observations of the Italian patients in this study.

The present study has confirmed the association with HLA-*DRB1*0801* in PBC in patients from the United Kingdom and Italy and has stimulated new interest in the role of non-*DRB1*08* haplotypes in PBC. *DRB1*0801* is relatively rare in our population, and therefore identifying other "protective" haplotypes may be particularly useful in the process of mapping MHC-encoded susceptibility and resistance to PBC and thus contributing to the debate on PBC pathogenesis.

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