HLA and interleukin 1 gene polymorphisms in primary biliary cirrhosis: associations with disease progression and disease susceptibility

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Abstract

Background and aims—Twin and family studies suggest that there is a genetic component to primary biliary cirrhosis (PBC) but the genetic associations which have been described are weak with marked variations between centres. PBC is heterogeneous and genetic associations with disease progression may be obscured when the PBC population is analysed only as a whole and not subdivided.

Methods—We have investigated two candidate gene loci in 164 well characterised patients, 88 (54%) of whom had advanced disease.

Results—There was an increased frequency of the HLA DRB1*0801-DQA1*0401-DQB1*0402 haplotype in patients who had progressed to late stage disease (23% v 2% of controls; p=0.000044; odds ratio (OR) 15.5, 95% confidence interval (CI) 3.52–68.4) but not in those with early stage disease (4% v 2%). Patients had a higher frequency of the IL-1B*1,1 genotype and lower frequencies of the IL-1B*1,2 and *2,2 genotypes (p=0.00078; OR 2.37, 95% CI 1.38–4.06), and higher frequency of the IL-1RN*1,1 genotype and lower frequency of the IL-1RN*1,2 genotype (p=0.0011; OR 2.28, 95% CI 1.34–3.89). The difference in the IL-1B*1,1 genotype distribution was most marked in patients with early stage disease (77% v 43% of controls; p=0.000003; OR 4.8, 95% CI 2.31–10) but the IL-1RN genotype distribution was similar in patients with early and late stage disease.

Conclusions—These data indicate a complex relationship between immunoregulatory genes and PBC. While the IL-1 genes are markers of both disease susceptibility and progression, HLA genes appear to be principally associated with disease progresssion.

Keywords: human leucocyte antigens; primary biliary cirrhosis; interleukin 1

Primary biliary cirrhosis (PBC) is an autoimmune liver disease characterised by immune mediated damage to the biliary epithelial cells lining the small intrahepatic bile ducts. Recent epidemiological studies suggest that PBC is significantly more common than has previously been estimated, with a point prevalence of 33/100000 adults in the north east of England.2

Primary biliary cirrhosis is phenotypically a very heterogeneous condition.2 Some patients remain asymptomatic with only mild histological changes for many years3 4 while others progress rapidly to cirrhosis and end stage disease.5 6 Twin and family studies (which have reported a sibling relative risk [λs] of 10.5 for PBC, a value similar to those reported for other autoimmune diseases) suggest that there is a genetic component to PBC susceptibility.7 8 9 The genetic factors contributing to PBC susceptibility remain poorly understood although sex, specific human leucocyte antigen (HLA) alleles,10 11 and other immunoregulatory genes12 13 are thought to play a role. Those genetic associations which have been described in PBC, particularly the associations with HLA DRB1*0801, are weak, with considerable variation between individual studies.14 One possible explanation for this variation is case mix. The large patient cohorts required for genetic studies are mostly available at tertiary referral centres where the patient population may be biased towards more severe, unusual, or less easily managed cases. Thus series from tertiary referral centres may suffer from over representation of patients with end stage disease referred for consideration of liver transplantation. Studies based on such patient groups often report strong associations which are not confirmed in patient populations recruited on a community basis.15 For this reason studies from tertiary referral centres are not always a true reflection of the “patient gene pool”. In general, studies of autoimmune disorders based on patient groups from tertiary referral centres may report differences in allele distribution suggestive of increased susceptibility to disease but which in reality reflect genetic influences on disease progression.

Among potential new candidate susceptibility genes in PBC, there is strong circumstantial evidence to support investigation of the interleukin (IL)-1 gene family on chromosome 2q13–14. Evidence includes: early reports of abnormal IL-1 production by monocytes in PBC,16 recent studies identifying the CTLA-4 gene (also located on chromosome 2q) as a major susceptibility locus for PBC,17 and an early, but unpublished, report of an association between PBC and IL-1B gene polymorphism.18

IL-1 is a proinflammatory cytokine which interacts with both tumour necrosis factor and other immunoregulatory genes and PBC. While the IL-1 genes are markers of both disease susceptibility and progression, HLA genes appear to be principally associated with disease progresssion.

Keywords: human leucocyte antigens; primary biliary cirrhosis; interleukin 1

Abbreviations used in this paper: HLA, human leucocyte antigens; PBC, primary biliary cirrhosis; IL, interleukin; IL-1RA, interleukin 1 receptor antagonist; PCR, polymerase chain reaction; OR, odds ratio.
IL-6. IL-1 is principally produced by monocytes and has wide ranging and variable functions. Among these, IL-1 may be important in both inflammation and fibrosis. The IL-1 gene family on chromosome 2q13 encodes three proteins, including IL-1α, IL-1β, and the IL-1 receptor antagonist (IL-1RA). IL-1RA competes with IL-1β (and IL-1α) for binding to the IL-1 receptors and is a potent inhibitor of IL-1 activity. Four nucleotide substitutions of the IL-1β gene (IL-1B) have been described and the IL-1RN gene contains a variable number of tandem repeat sequences. These genetic polymorphisms have been linked with both IL-1β production in vitro and with susceptibility to several diseases, including inflammatory bowel disease, periodontal disease, and most recently gastric cancer.

In the present study we have investigated two candidate regions of the human genome, HLA on chromosome 6p21.3 and the IL-1 gene cluster on chromosome 2q13–14, in a large and particularly well characterised series of population based PBC patients to identify novel genetic associations and to illuminate the role of HLA and IL-1 genes in both disease susceptibility and disease progression. The extent to which histological and clinical quantification of disease progression is possible in PBC makes this disease an ideal model in which to study genetic influences on disease progression. This is particularly important in light of recent observations of non-random clustering of autoimmune diseases in families, indicating that there may be significant overlap of susceptibility loci in autoimmune diseases and sharing of common pathways in the genesis of autoimmunity. In PBC, the diagnosis is based on biopsy evidence of disease activity with a clear, and internationally accepted, staging system. A second advantage of studying PBC is that the disease is not associated with the HLA A1-B8-DRB1*0301 haplotype which confounds the analysis of genetic associations in other autoimmune diseases.

The data presented illustrate the importance of identifying disease progression genes, and question the over simplistic approach to genetics of complex disease. This study may have important implications for future investigations of complex disease, particularly with the completion of the human genome project, and may also indicate a rethink of our approach to clinical trials analysis.

**Patients and methods**

**SUBJECTS**

We studied 164 consecutive well characterised patients with PBC. All were of northern European ancestry and resident within the Newcastle area, defined by postal code, and sought following extensive case finding methods described by our group. Sixteen were male (9.8%) and 148 female (90.2%). All subjects had definite disease using standard criteria (all three of (i) liver histology diagnostic of, or compatible with, PBC; (ii) cholestatic liver function tests; and (iii) positive serum antimitochondrial antibody titre >1:40 detected by immunofluorescence). Subjects were excluded if their biopsy (or any other clinical data) suggested additional potentially confounding causes for liver pathology. All liver biopsies were reviewed for confirmation of diagnosis and stage by two independent histopathologists. Patients were classified as “advanced (late) disease” stage (that is, stage III or IV) or “early disease” (that is, stage I or II). Eighty eight PBC patients (54%) had histologically advanced disease (Scheuer stage III or IV) on their last liver biopsy. Patients with early stage disease on their last biopsy are under regular clinical follow up. None of these patients had clinical, radiological, or biochemical features to suggest development of more advanced disease. To perform repeat liver biopsies solely for the purpose of histological staging for the present study was considered unethical. Within the group of 76 (46%) patients with histological stage I/II disease on their last liver biopsy, a subgroup (hence forth referred to as the “long term clinical non-progressors”) were identified who had been followed for a minimum of 10 years from initial diagnosis of PBC (median follow up 13 years, range 10–23 years) and who at the time of the study had normal liver synthetic function, normal bilirubin levels (median bilirubin 6.1 µmol/l (range 2.2–11.2, upper limit of normal 17 µmol/l)) and exclusion of any clinical and/or ultrasound features suggestive of progression to cirrhosis. None of these 35 “long term clinical non-progressors” received treatment in the form of corticosteroids, ursodeoxycholic acid, or other trial immunotherapies during the period of follow up.

For comparison, 102 geographically and racially matched controls were studied. All subjects were of northern European Caucasian origin and resident in the Newcastle area. All subjects and controls gave informed consent and the study was approved by the ethics committee of the Newcastle Hospitals Trust. Samples were labelled and stored by code only and analysed without prior knowledge of individual identities.

Genomic DNA was extracted from 10 ml of EDTA whole blood using a standard phenol/chloroform extraction protocol.

**DETERMINATION OF HLA DRB1, DQA1, AND DQB1 GENOTYPES**

HLA genotyping was performed using a standard polymerase chain reaction (PCR) protocol for a total of 42 different HLA DRB, DQA, and DQB alleles or groups of alleles with DRB, DQA, and DQB specific primers corresponding to the second exon sequence of each locus (table 1). Briefly, 100 ng of genomic DNA were amplified in a 50 µl reaction mix containing 200 µM each of dATP, dCTP, dGTP, and dTTP (Amersham Pharmacia-Biotech, St Albans, UK), 1.5 mM MgCl₂, 10 mM Tris HCl, pH 8.3, 50 mM KCl, 0.01% gelatin, 1 µM of each primer, and 2–1.25 U Taq polymerase (Perkin Elmer, Norwalk, Connecticut, UK) on a Perkin-Elmer GeneAmp 9600. PCR cycling parameters were as follows: 120 seconds 94°C, 94°C for 10 seconds, 56°C for 60 seconds (10
Table 1 Sequences of human leucocyte antigen (HLA) and interleukin (IL)-1 gene primers used

<table>
<thead>
<tr>
<th>Locus</th>
<th>Primers</th>
<th>Sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA</td>
<td>DRB (1)</td>
<td>5' CCC CAT CTC TTT TGT TAA ACT TGT ACC ATC 3'</td>
</tr>
<tr>
<td></td>
<td>DRB (2)</td>
<td>5' CCA GTA CAT TCT AGT GAA GCT CTA CT 3'</td>
</tr>
<tr>
<td>HLA</td>
<td>DQB (1)</td>
<td>5' CAT GTA CTA CTT CAG CAA CAG CTC 3'</td>
</tr>
<tr>
<td></td>
<td>DQB (2)</td>
<td>5' CCA GTA TGT TGG CTA CAC CAA CAG 3'</td>
</tr>
<tr>
<td>HLA</td>
<td>DQA (1)</td>
<td>5' ATG TGG TAA TAA CAC ACC AG 3'</td>
</tr>
<tr>
<td></td>
<td>DQA (2)</td>
<td>5' TTG CTA GCA GCA GTA GAT TGG TTA 3'</td>
</tr>
<tr>
<td>IL-1β</td>
<td>IL-1A (1)</td>
<td>5' CGT TCG ACC CTA CTC TGA GAG 3'</td>
</tr>
<tr>
<td></td>
<td>IL-1B (1)</td>
<td>5' CGT TCG ACC CTA CTC TGA GAG 3'</td>
</tr>
<tr>
<td>IL-1RN</td>
<td>IL-1RA (1)</td>
<td>5' CTCAG AAG CAC TCT CTA 3'</td>
</tr>
<tr>
<td></td>
<td>IL-1RA (2)</td>
<td>3' CTTCC TGTC ACC CAG TAA 3'</td>
</tr>
</tbody>
</table>

Polymorphisms of interleukin 1 receptor antagonist (IL-1RN)

A penta allelic polymorphic site containing variable numbers of an 86 bp tandem repeat sequence in intron 2 of the IL-1 receptor antagonist gene (IL-1RN) was amplified in a 25 µl reaction mixture following a modification of the method of Tarlow and colleagues. The conditions for amplification were as follows: 96°C for two minutes, 30 cycles of 96°C for 60 seconds, 58°C for 60 seconds, 72°C for 30 seconds, and a final extension at 72°C for 10 minutes. Following amplification the amplicon was visualised on a 2% (w/v) agarose gel with appropriate commercially available size markers (Amersham Pharmacia-Biotech).

Five alleles were assigned based on amplicon size: allele 1 (four repeats) 410 bp, allele 2 (two repeats) 240 bp, allele 3 (three repeats) 325 bp, allele 4 (five repeats) 500 bp, and allele 5 (six repeats) 595 bp.

Statistical analysis

Three sets of comparisons were made: firstly, the HLA allele and IL-1 genotype distribution of the total patient group was compared with that of local controls. Secondly, the HLA allele and IL-1 genotype distribution of patients with early stage disease and late stage disease were compared with local controls. Thirdly, the HLA and IL-1 genotype distributions of patients with early stage disease and late stage disease were compared. Allele and genotype distributions were compared using the χ² test with Yates’ correction, or Fisher’s exact tests on the EPISTAT statistical analysis programme (CDR-Atlanta Georgia, USA) as appropriate. Probability (p) values were corrected for multiple testing (pc) as recommended by Svejgaard and Ryder. For the HLA data we applied a correction factor of 42 (that is, the total number of DRB1, DQA1, and DQB1 alleles or groups of alleles defined) for novel (previously unreported) associations. No correction factor was applied for DRB1*0801 or the DRB1*0801-DQA1*0401-DQB1*0402 haplotype (which are well established associations in northern European PBC patients).

Results

DISTRIBUTION OF HLA DRB1*, DQA1*, AND DQB1 ALLELES.

Overall there was no significant variation in the distribution of HLA DRB1, DQA1, and DQB1 alleles, other than an expected increased frequency of the members of the DRB1*0801-DQA1*0401-DQB1*0402 haplotype (table 2). Thus 25/164 (15%) PBC patients had DRB1*0801 compared with only 3/102 con-
Table 2  Distribution of selected human leucocyte antigen (HLA) alleles based on well documented or putative associations with primary biliary cirrhosis (PBC)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Controls (n=102)</th>
<th>All (n=164)</th>
<th>Early stage (n=76)</th>
<th>Late stage (n=88)</th>
<th>Non-progressors (n=35)</th>
</tr>
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<tbody>
<tr>
<td>DRB1*0801</td>
<td>3</td>
<td>25 (15)</td>
<td>4 (5)</td>
<td>21 (24)</td>
<td>1</td>
</tr>
<tr>
<td>DQA1*0401</td>
<td>3</td>
<td>23 (15)</td>
<td>3 (4)</td>
<td>20 (23)</td>
<td>0</td>
</tr>
<tr>
<td>DQB1*0402</td>
<td>2</td>
<td>25 (15)</td>
<td>4 (5)</td>
<td>21 (24)</td>
<td>1</td>
</tr>
<tr>
<td>DRB1*1501</td>
<td>25</td>
<td>39 (24)</td>
<td>22 (29)</td>
<td>17 (19)</td>
<td>11 (31)</td>
</tr>
<tr>
<td>DQAI*0202</td>
<td>27</td>
<td>48 (20)</td>
<td>37 (26)</td>
<td>21 (24)</td>
<td>15 (43)</td>
</tr>
<tr>
<td>DQB1*0602</td>
<td>24</td>
<td>36 (22)</td>
<td>20 (26)</td>
<td>16 (18)</td>
<td>10 (29)</td>
</tr>
<tr>
<td>DRB1*13</td>
<td>21</td>
<td>16 (10)</td>
<td>8 (11)</td>
<td>8 (9)</td>
<td>4 (11)</td>
</tr>
</tbody>
</table>

Table 3  Interleukin (IL)-1 genotype distributions

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Controls (n=101)</th>
<th>All (n=164)</th>
<th>Early stage (n=76)</th>
<th>Late stage (n=88)</th>
<th>Non-progressors (n=35)</th>
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</thead>
<tbody>
<tr>
<td>IL-1B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,1</td>
<td>44 (43)</td>
<td>106 (65)</td>
<td>59 (27)</td>
<td>47 (53)</td>
<td>25 (71)</td>
</tr>
<tr>
<td>1,2</td>
<td>51 (50)</td>
<td>44 (27)</td>
<td>16 (21)</td>
<td>28 (32)</td>
<td>10 (29)</td>
</tr>
<tr>
<td>2,2</td>
<td>6 (6)</td>
<td>8 (5)</td>
<td>0</td>
<td>8 (9)</td>
<td>0</td>
</tr>
<tr>
<td>Fail</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>IL-1RN</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,1</td>
<td>42 (40)</td>
<td>99 (60)</td>
<td>44 (58)</td>
<td>55 (63)</td>
<td>21 (60)</td>
</tr>
<tr>
<td>1,2</td>
<td>48 (46)</td>
<td>31 (19)</td>
<td>15 (20)</td>
<td>16 (18)</td>
<td>6 (17)</td>
</tr>
<tr>
<td>2,2</td>
<td>10 (9.5)</td>
<td>19 (12)</td>
<td>10 (13)</td>
<td>9 (10)</td>
<td>7 (20)</td>
</tr>
<tr>
<td>Other</td>
<td>5 (4.5)</td>
<td>15 (9)</td>
<td>7 (9)</td>
<td>8 (5)</td>
<td>1</td>
</tr>
</tbody>
</table>

distributions was most marked in patients with early stage disease (77%) and non-progressors (71%). Thus comparing early and late stage disease (IL-1B*1,1=53%), p=0.0012, OR=0.022, OR=3.03, and 95% CI=1.45–6.36, and comparing early stage disease with controls (IL-1B*1,1=43%), p=0.00003, OR=0.48, and 95% CI=2.31–10. Comparing non-progressors only, OR values for controls reached statistical significance (p=0.0045, OR=0.081; OR=3.24, 95% CI=1.32–8.12).

In contrast, the IL-1RN genotype distribution was similar in patients with early (including the 35 “long-term clinical non-progressors”) and late stage disease.

Discussion

The present study indicates that there is a single HLA haplotype associated with PBC: DRB1*0801-DQA1*0401-DQB1*0402. The association is weak and attributable entirely to patients with histologically advanced disease. A second and novel genetic association with the IL-1 gene family is reported for the first time. These data indicate a complex relationship between immunoregulatory genes and PBC. In the present study, the IL-1 genes appeared to be associated with both disease susceptibility and progression while the HLA genes appeared to be associated with disease progression only.

Histological staging of liver disease is only poorly associated with prognosis in PBC and moreover, lesions may be variable and patchy leading to an under estimation of staging. This makes studies of this kind difficult. Therefore, in addition to the histological “early” disease group, we included a clinically relevant group of 35 patients identified from previous clinical studies, all of whom remain well with no progression of disease over 10 or more years of follow up. These 35 patients (referred to as “long-term clinical non-progressors”) had no clinical, histological, biochemical, or radiological evidence of disease progression over the 10 year period and their HLA and IL-1 genotypes showed marked differences in distribution compared with patients known to have progressed to advanced disease. These data are consistent with a “multi-hit” model for PBC, whereby an initial breakdown of immune tolerance results in early histological damage but where additional factors are required for disease progression. Our findings suggest that
separate genetic factors contribute to disease development (the initial “hit”) and disease progression (the second and/or subsequent “hits”).

In terms of adding to our understanding of the pathogenesis of PBC, the association with HLA DRB1*0801 in this population has been reported previously and the weak link with chromosome 6p21.3 has been discussed at length. Apart from early studies based on HLA typing by serology, the only consistently reported HLA association in PBC is with DRB1*0801 (or DRB1*0803 in Japan). Our study is no exception, finding only DRB1*0801 and failing to confirm the recent observations of Begovitch and colleagues suggesting a protective role for DQA1*0102, which is carried on both DRB1*1501 and DRB1*13 haplotypes. However, there is marked variation in the strength of the association with DRB1*0801 and PBC between centres. This may be indicative of the different referral patterns and the present study, by linking DRB1*0801 with disease progression in PBC, may finally explain the variation in the reported strength of this genetic association. Furthermore, as HLA class II associations are usually explained in terms of antigen presentation, a key event in disease initiation, the present study may indicate that DRB1*0801 is not the prime determinant of disease susceptibility or progression but may simply act as a linked marker for another, as yet unidentified, gene.

In contrast, the association with IL-1 is novel. This locus is the second novel association with PBC to be described by our group which maps to chromosome 2, albeit at different ends of the chromosome. The other newly associated gene is CTLA-4 which appears to be associated with disease susceptibility, but not with disease progression, as befits its function. At present there is no evidence of synergy between IL-1B*1, 1 and the CTLA-4*G allele. In addition, the IL-1 and CTLA-4 genes are a considerable distance apart; IL-1 maps to 2q13–14 and CTLA-4 to 2q33. These two studies based on the same series of patients identify chromosome 2q as an important location for further genetic studies in PBC. However, whether both IL-1 and CTLA-4 are simply markers for a linked “susceptibility gene or genes” elsewhere on chromosome 2q or each acts as an independent risk factor for PBC remains to be determined.

The IL-1B*1 allele is associated with high IL-1β and high IL-1 receptor antagonist (IL-1RA) production. This coordinate regulation of both the agonist and antagonist IL-1 proteins is particularly marked when the IL-1B*1 allele is found with the IL-1RN*2 allele. In the present study there was a significant deficit of the IL-1RN*2 allele in all PBC patients. High levels of IL-1RA production associated with the IL-1RN alleles may negate any functional consequence of the IL-1B*1 allele through blockade of the IL-1 receptors. As both IL-1B and IL-1RN are located on chromosome 2q13, the association between IL-1B and disease susceptibility may simply reflect linkage with IL-1RN. However, the observation of a significantly higher frequency of the IL-1B*1, 1 genotype in patients with early stage disease compared with late stage disease indicates a second effect of IL-1B which is independent of IL-1RN as the distribution of IL-1RN genotypes is equal in both early and late stage patients. Such observations are not without precedent. A similarly complex relationship between IL-1 genes and the outcome of paracetamol induced liver failure was recently reported. It is likely that this complexity is due to the multiple physiological effects of IL-1 and the inter-relationship between IL-1 receptors, agonist and antagonist, and their genes. The present data may also have important implications in inflammatory bowel disease where multiple studies have sought to assess the relationship with the IL-1 gene cluster, with mixed results, and also in periodontal disease where recent studies suggest the IL-1 genotype influences severity of disease but only in non-smokers.

The real importance of these data is not in improving our understanding of the aetopathogenesis of PBC but in drawing attention to the fact that genes influence both disease initiation (susceptibility) and disease progression. Recent therapeutic trials in PBC have failed to produce convincing evidence for beneficial clinical effects despite strong theoretical reasons for the potential benefits of the drugs concerned (most notable ursodeoxycholic acid). We may assume, based on the findings of the current study, that a proportion of patients entered into these trials will be uninformative participants, genetically less likely to progress during the time period of the trial. Understanding and identifying these genetic effects may be of great value in the design and interpretation of future trials in PBC.

The concept of a genetic basis to heterogeneity of disease progression also applies to other autoimmune diseases. It is important to be aware of case mix bias, especially when dealing with the genetics of complex disease. As the human genome project comes to a conclusion and attention turns from single gene disorders to complex disease, a clearer understanding of this issue is essential. There have already been calls for the collection of “well characterised” patient sets but who will decide which patients are well characterised and control the selection criteria?

26 Hurme M, Samanta S. IL-1 receptor antagonist (IL-1Ra) plasma levels are co-ordinately regulated by both IL-1Ra and IL-1β genes. Eur J Immunol 1998;28:2598–602.