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=0000044; 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 progression.**

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

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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.[1] Recent epidemiological studies suggest that PBC is significantly more common than has previously been estimated, with a point prevalence of 334/10^6 adults in the north east of England.[2]

PBC is phenotypically a very heterogeneous condition.[3] Some patients remain asymptomatic with only mild histological changes for many years[4 5] while others progress rapidly to cirrhosis and end stage disease.[5 6] Twin and family studies (which have reported a sibling relative risk [λ_s] 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.[3 7–10] The genetic factors contributing to PBC susceptibility remain poorly understood although sex, specific human leucocyte antigen (HLA) alleles,[11 12] and other immunoregulatory genes[13–15] 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.[16] 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.[17] 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,[18] recent studies identifying the CTLA-4 gene (also located on chromosome 2q) as a major susceptibility locus for PBC,[15] and an early, but unpublished, report of an association between PBC and *IL-1B* gene polymorphism.[19]

IL-1 is a proinflammatory cytokine which interacts with both tumour necrosis factor and

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[20] 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.[21] Four nucleotide substitutions of the IL-1β gene (*IL-1B*) have been described[22] and the *IL-1RN* gene contains a variable number of tandem repeat sequences.[23] These genetic polymorphisms have been linked with both IL-1β production in vitro[24–26] and with susceptibility to several diseases, including inflammatory bowel disease,[20] periodontal disease,[27] and most recently gastric cancer.[28]

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.[29 30] 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.[12]

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.[3] 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.[31] 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 Caucasoid 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 $MgCl_2$, 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

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'
IL-1β	IL-1β (1)	5'-gTTgTCATCAgACTTTgACC-3'
	IL-1β (2)	5'-TTCAgTTCATATggACCAgA-3'
IL-1RN	IL-1RA (1)	5'-CTCAgCAACACTCCTAT-3'
	IL-1RA (2)	5'-TCCTggTCTgCAggTAA-3'

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, the DRB1, DQB1, and DQA1 PCR amplicons were denatured and 2 µl of each PCR amplicon was dot blotted onto a series of positively charged nylon membranes (20 membranes for DRB1, 16 for DQB1, and 10 for DQA1). Each membrane was hybridised with one of a series of digoxygenin labelled allele and sequence specific oligonucleotide probes. Alleles were detected by chemiluminescence and assigned by two trained individuals according to probe specificity tables supplied by the British Society of Histocompatibility and Immunogenetics adapted from the Eleventh International Histocompatability Workshop and Conference.[32]

DETERMINATION OF IL-1 GENE POLYMORPHISMS

Genomic DNA (100 ng) was amplified in reaction mixtures containing 200 µM each of dATP, dCTP, dGTP, and dTTP (Amersham Pharmacia-Biotech), 1.5 mM $MgCl_2$, 10 mM Tris HCl, pH 8.3, 50 mM KCl, 0.01% gelatin, 0.5 µM of each primer (table 1), and 2–2.5 U *Taq polymerase* (Perkin-Elmer) on a Perkin-Elmer GeneAmp 9600 according to the following protocols.

Polymorphisms of interleukin 1B (IL-1B).

A 249 bp fragment of the fifth exon encoding *IL-1B* (positions +3816 to +4066) was amplified in a 25 µl reaction mixture according to a modification of the method of Bioque and colleagues.[33] Conditions for amplification were as follows: 96°C for 5 minutes; three cycles of 96°C for 90 seconds, 53°C for 90 seconds, 72°C for 90 seconds; 35 cycles of 96°C for 60 seconds, 53°C for 60 seconds, 72°C for 60 seconds, and a single final extension at 72°C for 10 minutes. Following amplification, 15 µl of the amplicon were digested with 6–8 units of *Taq 1* restriction endonuclease (Amersham Pharmacia-Biotech) at 65°C. Digested restriction fragments were visualised on a 3% (w/v) agarose gel with appropriate commercially available size markers (Amersham Pharmacia-Biotech). The presence of a base exchange substitution at position +3953 creates the *Taq1* restriction site in allele 1 but not allele 2, therefore *Taq 1* digestion of the 249bp *IL-1B* amplicon resulted in fragments of 114 and 135 bp (allele 1) and/or intact amplicon (allele 2).

Polymorphisms of the 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.[23] 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 60 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 χ^2 test with Yates' correction, or Fisher's exact tests on the EPISTAT statistical analysis programme (CDC-Atlanta Georgia, USA) as appropriate. Probability (p) values were corrected for multiple testing (pc) as recommended by Svejgaard and Ryder.[34] 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[11 12]). For the *IL-1* data a correction factor of 18 was used (that is, the number of different genotypes assessed (6) multiplied by the number of comparisons made (3)).

In addition, we used the method of counting suggested by Svejgaard and Ryder,[34] whereby the number of individuals positive for each allele were counted rather than the number of chromosomes (that is, copies of each gene) and odds ratios (OR) are quoted in place of relative risk.

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)

		Frequency (No (%))			
			Primary biliary cirrhosis		
Genotype	Controls (n=102)	All (n=164)	Early stage (n=76)	Late stage (n=88)	Non-progressors (n=35)
*DRB1*0801*	3	25 (15)	4 (5)	21 (24)	1
*DQA1*0401*	3	23 (14)	3 (4)	20 (23)	0
*DQB1*0402*	2	25 (15)	4 (5)	21 (24)	1
*DRB1*1501*	25	39 (24)	22 (29)	17 (19)	11 (31)
*DQA1*0102*	27	48 (29)	27 (36)	21 (24)	15 (43)
*DQB1*0602*	24	36 (22)	20 (26)	16 (18)	10 (29)
*DRB1*13*	21	16 (10)	8 (11)	8 (9)	4 (11)

Table 3 Interleukin (IL)-1 genotype distributions

		Frequency (No (%))			
			Primary biliary cirrhosis		
Genotype	Controls (n=101)	All (n=164)	Early stage (n=76)	Late stage (n=88)	Non-progressors (n=35)
IL-1B					
1,1	44 (43)	106 (65)	59 (77)	47 (53)	25 (71)
1,2	51 (50)	44 (27)	16 (21)	28 (32)	10 (29)
2,2	6 (6)	8 (5)	0	8 (9)	0
Fail	0	0	1	5	0
IL-1RN					
1,1	42 (40)	99 (60)	44 (58)	55 (63)	21 (60)
1,2	48 (46)	31 (19)	15 (20)	16 (18)	6 (17)
2,2	10 (9.5)	19 (12)	10 (13)	9 (10)	7 (20)
Other	5 (4.5)	15 (9)	7 (9)	8 (5)	1

trols (p=0.0014; OR 5.9, 95% CI 1.64–25.4). Of these 25 patients with *DRB1*0801*, 23 were also *DQA1*0401* and *DQB1*0402* positive compared with 2/3 *DRB1*0801* controls (p=0.001; OR 8.16, 95% CI 1.88–35.4). In contrast, there was no difference in the frequency of the *DQA1*0102* allele which was found in 29% of patients and 27% of controls. Nor was there any difference in the frequency of *DRB1*1501* (24% of patients and 25% of controls) and although *DRB1*13* was found at a slightly lower frequency in patients (10% *v* 21% of controls), this difference did not reach statistical significance.

When patients with early stage disease (n=76) on their last liver biopsy were compared with those with late stage disease (n=88), the genetic association with the *DRB1*0801-DQA1*0401-DQB1*0402* haplotype was found to be almost entirely attributable to an increased frequency of this haplotype in patients who had progressed to late stage disease. Thus of 25 patients with *DRB1*0801*, 21 had late stage disease and only four had early stage disease (5% *v* 24%, p=0.0009; OR 5.6, 95% CI 1.84–17.29). When the frequency of *DRB1*0801* in patients with late stage PBC was compared with controls, the overall risk associated with *DRB1*0801* increased to 10.3 (p=0.000015; OR 10.3, 95% CI 2.97–36.07) and 15.5 for the haplotype *DRB1*0801-DQA1*0401-DQB1*0402* (p=0.0000044; OR 15.5, 95% CI 3.52–68.4). Among the "long term clinical non-progressor" subgroup (n=35), *DRB1*0801* was present in only one (3% of patients; p=1.0 *v* controls, p=0.007 (OR 10.7) *v* established late stage PBC patients).

DISTRIBUTION OF IL-1 GENOTYPES (TABLE 3).
There was a marked difference in the distribution of both *IL-1B* and *IL-1RN* genotypes between patients and controls. Patients had a significantly higher frequency of the *IL-1B*1,1* genotype (65% of patients *v* 43% of controls) and a lower frequency of *IL-1B*1,2* (27% *v* 50%) (p=0.00068, pc=0.012), and a significantly higher frequency of the *IL-1RN*1,1* genotype (60% *v* 40%) and lower frequency of *IL-1RN*1,2* compared with controls (19% *v* 46%) (p=0.0002, pc=0.0036). The risk (based on OR value) of PBC associated with *IL-1B*1,1* was 2.4 (95% CI 1.38–4.06) and with *IL-1RN*1,1* was 2.3 (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%) and non-progressors (71%). Thus comparing early and late stage disease (*IL-1B*1,1*=53%), p=0.0012, pc=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=0.000003, pc=0.000054, OR=4.8, and 95% CI=2.31–10. Comparing non-progressors only, pc values for controls reached statistical significance (p=0.0045, pc=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.[4 5] 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,[4] 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.[11–14 35–38] Apart from early studies based on HLA typing by serology,[39–41] the only consistently reported HLA association in PBC is with *DRB1*0801* (or *DRB1*0803* in Japan[42–45]). Our study is no exception, finding only DRB1*0801 and failing to confirm the recent observations of Begovitch and colleagues[38] suggesting a protective role for *DQA1*0102*, which is carried on both *DRB1*1501* and *DRB1*13* haplotypes.[38] 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*[15] 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[22 24–26] 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.[26] 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.[46] 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,[20 33 47 48] and also in periodontal disease where recent studies suggest the *IL-1* genotype influences severity of disease but only in non-smokers.[27]

The real importance of these data is not in improving our understanding of the aetiopathogenesis 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[49]). 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.[12 48] 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[50] but who will decide which patients are well characterised and control the selection criteria?

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