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Small duct primary sclerosing cholangitis without inflammatory bowel disease is genetically different from large duct disease

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Abstract

Background & aims—Small duct primary sclerosing cholangitis (PSC) is phenotypically a mild version of large duct PSC, but it is unknown whether these phenotypes share aetiology. We aimed to characterize their relationship by investigating genetic associations in the HLA complex, which represent the strongest genetic risk factors in large duct PSC.

Methods—Four classical HLA loci (*HLA-A*, *HLA-B*, *HLA-C*, *HLA-DRB1*) were genotyped in 87 small duct PSC patients, 485 large duct PSC patients and 1117 controls across three geographical regions.

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Conflict of interest: None

Results—HLA-DRB1*13:01 (OR=2.0, 95% CI 1.2–3.4, $P=0.01$) and HLA-B*08 (OR=1.6, 95% CI 1.1–2.4, $P=0.02$) were significantly associated with small duct PSC compared with healthy controls. Based on the observed frequency of HLA-B*08 in small duct PSC, the strongest risk factor in large duct PSC, an estimated 32% (95% CI 4%–65%) of this population can be hypothesized to represent early stages or mild variants of large duct PSC. This subgroup may be constituted by small duct PSC patients with inflammatory bowel disease (IBD), which greatly resembled large duct PSC in its HLA association. In contrast, small duct PSC without IBD was only associated with HLA-DRB1*13:01 ($P=0.03$) and was otherwise distinctly dissimilar from large duct PSC.

Conclusions—Small duct PSC with IBD resembles large duct PSC in its HLA association and may represent early stages or mild variants of large duct disease. Different HLA associations in small duct PSC without IBD could indicate that this subgroup is a different entity. HLA-DRB1*13:01 may represent a specific risk factor for inflammatory bile duct disease.

Keywords

primary sclerosing cholangitis; large duct; small duct; human leukocyte antigens; inflammatory bowel disease

Introduction

Primary sclerosing cholangitis (PSC) is a chronic inflammatory bile duct disease of unknown aetiology frequently associated with inflammatory bowel disease (IBD), which occurs in about 60–80% of PSC patients of Northern European decent (1). An abnormal cholangiogram showing strictures and intervening dilatations of the intra- and/or the extra-hepatic biliary tree and exclusion of specific etiologies (i.e. secondary sclerosing cholangitis) is required for the diagnosis of classic PSC, hereafter referred to as large duct PSC. In some patients, clinical, biochemical and histological abnormalities may strongly suggest the presence of PSC, but without apparent corresponding cholangiographic changes. These patients have small duct PSC, and this entity is approximately ten-fold less frequent than large duct PSC (2, 3).

To date, it is not clear whether small duct PSC represents (i) an early stage of large duct PSC, (ii) a mild variant of large duct PSC, or (iii) a separate disease condition (2). Studies on the natural history of small duct PSC have shown that small duct PSC has a more favorable prognosis than large duct PSC, longer transplantation-free survival, fewer liver related deaths, and a less frequent development of cholangiocarcinoma (CCA) (4–8). Twenty-three percent of the small duct PSC patients progressed to large duct disease in the largest study presented to date, suggesting that in a quarter of the patients, small duct disease represents an early stage of large duct PSC (8). The detailed diagnostic criteria in small duct PSC are still being discussed, especially whether the presence of IBD should be a prerequisite for the diagnosis (9). Currently, IBD is not required for the diagnosis of small duct PSC, supported by the observation that not all large duct patients have co-existing IBD (8). However, this means that some patients with cholangiopathies of other aetiologies may be diagnosed with small duct PSC, due to similar histological features (9–11).

With a 9–39 times increased risk of disease in siblings of PSC patients (12), importance of genetic risk factors in PSC has been demonstrated and sixteen robust genetic risk loci have been found in large duct PSC to date (13–16). The by far strongest genetic risk factors in large duct PSC are encoded within the human leucocyte antigen (HLA) complex at chromosome 6 (13, 14, 16). Multiple HLA alleles have repeatedly been associated with PSC, including HLA-B*08 and DRB1*03:01 (serotypes HLA-B8 and DR3), initially reported more than 30 years ago (17). Positive associations have also been found for HLA-DRB1*13:01 (13, 18), B*07 and DRB1*15:01 (18–20), while negative associations have been reported for HLA-DRB1*04, *07 and *11 (18, 21–23).

No genetic studies have so far been conducted in small duct PSC, probably due to the small numbers of patients available. Characterization of the genetic similarities or differences between small duct and large duct PSC would provide important data on the pathogenesis of, and relationship between, small duct and large duct sclerosing cholangitis. Thus, in this cross-sectional study we included small and large duct PSC patients from Scandinavia, UK and US and genotyped key HLA genes, which are the strongest known genetic risk factors in large duct PSC. Due to the controversy regarding the diagnosis of small duct PSC in patients without IBD, we also aimed to investigate the subgroups with and without IBD.

Material and methods

Study population

Eighty seven small duct PSC patients from Scandinavia (n=43, i.e. Norway (n=14) and Sweden (n=29)), UK (n=28) and US (n=16) were included in this study. Disease definition was based on three consensus criteria (2); 1) chronic cholestasis of unknown etiology, excluding other causes of cholestatic liver diseases; 2) a diagnostic-quality cholangiogram (either endoscopic retrograde cholangiopancreatography or magnetic resonance cholangiopancreatography) showing a normal biliary tree; and 3) liver histology compatible with PSC. This was a cross-sectional study and patients were classified as small duct PSC based on current diagnosis, meaning that patients with an initial small duct diagnosis, later progressing to large duct, were not included in the small duct group. Diagnosis of IBD was based on commonly accepted clinical, endoscopic, radiological and histological criteria. Four hundred and eighty-five geographically matched large duct PSC patients (357 Scandinavian, 77 UK and 51 US) and 1117 healthy controls (368 Scandinavian, 600 UK and 149 US) were included for comparison. Clinical characteristics of small duct and large duct PSC patients are summarized in Table 1. Written informed consent was obtained from all study participants. Ethical approval was obtained from the research ethics committees at each participating medical center in accordance with the declaration of Helsinki.

HLA genotyping

Sequencing-based HLA-genotyping of the patients was performed at three different locations (Oslo, London, Oxford) according to protocols established at the various centers (protocols are available from authors upon request). HLA genotypes were available for all the Scandinavian large duct PSC patients from previous studies (24, 25). US control genotypes were generated using HLA genotypes from type 1 diabetic multiplex families.

The affected family based controls method was applied, using parental alleles never transmitted to the affected sib-pair to assign control alleles (26). Scandinavian control data consisted of randomly selected individuals from the Norwegian Bone Marrow Registry, previously published in (24).

HLA-A, *B* and *C* genotypes were resolved to a consistent two-digit resolution. For *HLA-A* and *HLA-B* it was necessary to resolve to serotype nomenclature level to ensure comparability with all the Scandinavian controls. Most *HLA-DRB1* alleles were resolved to four-digit resolution. To avoid too many allelic subgroups and subsequent power limitation due to small numbers, two-digit resolution is presented, except for the *DRB1*13* allele, due to the differential effects of 13:01 and 13:02 in large duct PSC (27).

Statistical methods

Single locus association analyses were performed in the individual geographical panels using Chi-square test or Fisher's exact test in PASW v.18 (SPSS, Chicago, IL, USA) and Microsoft Excel (Redmond, WA, USA). Odds ratios (ORs) were calculated according to Woolf's formula with Haldane's correction. The main association tests were performed in R v.3.0.1 as meta-analyses (<http://CRAN.R-project.org/package=meta>) across the geographical panels. Woolf's test was used to test for heterogeneity of the ORs; if significant, the random effects model rather than the fixed effect model was used for association testing. The Mantel-Haenszel method was used to calculate common ORs. The significance level was set to 0.05. Established HLA associations in large duct PSC were a priori hypothesized to be associated with small duct PSC and the subgroups with and without IBD, and uncorrected *P*-values below 0.05 were therefore considered significant for these. Associations in small duct PSC, previously not observed in large duct PSC, were adjusted for multiple testing by Bonferroni's correction according to the number of alleles analysed at each HLA locus. Estimation of *HLA-B-DRB1* haplotypes was done in Phase v.2.1.1, using 1000 iterations. Linkage disequilibrium (LD) calculations were done using Unphased v.3.0.10. Monte Carlo simulations, calculated in MATLAB, were used to estimate a percentage, *X*, of potential "contaminating" large duct PSC patients in the small duct PSC cohort. Power calculations for the Mantel-Haenszel method were done in PS: Power and Sample Size v.3.0.43 (28) assuming that effect sizes (ORs) observed in this study for associated alleles in large duct PSC were equal to ORs in small duct PSC (Supplementary Table 3).

Results

Small duct PSC is associated with *HLA-DRB1*13:01* and *B*08*

Genotyping success rate for *HLA-A*, *B*, *C* and *DRB1* was 96%. Only two HLA alleles; *HLA-DRB1*13:01* (11% vs. 6%, OR = 2.0, 95% CI 1.2–3.4, $P_{\text{uncorrected}} = 0.01$) and *B*08* (i.e. B8 serotype) (20% vs. 13%, OR = 1.6, 95% CI 1.1–2.4, $P_{\text{uncorrected}} = 0.02$) were associated with small duct PSC (Tables 2 and 3), both being established large duct PSC risk variants. Allele frequencies were similar in the three different panels (Tables 2 and 3, Supplementary Table 1). No associations were found for *HLA-A* or *HLA-C* (Supplementary Table 1).

Distinct contributions of HLA-DRB1*13:01 and HLA-B*08

In order to explore the relationship between the HLA-B*08 and DRB1*13:01 alleles, frequencies of the various haplotypes formed by *HLA-B* and *HLA-DRB1* alleles were estimated (Supplementary Table 2). There was no LD between HLA-DRB1*13:01 and the *HLA-B* alleles (r^2 range <0.01 to 0.02 in controls), and the difference in DRB1*13:01 frequency in small duct PSC vs. controls (11% vs. 6%, Table 3) was caused by an increase of several different haplotypes carrying the DRB1*13:01 allele (Supplementary Table 2). HLA-B*08-DRB1*03 was the most common HLA-B*08 haplotype, and these alleles showed considerable LD with each other ($r^2=0.50$ in controls). The estimated frequency of this haplotype was higher in small duct PSC than in controls (13% and 10% respectively), but this increase could not explain the entire HLA-B*08 association in small duct PSC, given a frequency of HLA-B*08 of 20% in small duct PSC compared with 13 % in controls (Supplementary Table 1).

The HLA associations in small duct PSC differ from the HLA associations in large duct PSC

The frequency of the HLA-DRB1*13:01 risk allele was not significantly different between large duct and small duct disease. In contrast, the HLA-B*08 allele was more prevalent in large duct PSC than small duct PSC, (33% vs. 20%, respectively, OR = 1.9, 95% CI 1.3–2.9, $P_{\text{uncorrected}} = 0.002$). Several other established large duct PSC associated variants were also significantly different; HLA-A*01 ($P_{\text{uncorrected}} = 0.01$) and DRB1*03 ($P_{\text{uncorrected}} = 0.0004$) were more prevalent in large duct than small duct PSC, while the opposite was observed for DRB1*04 ($P_{\text{uncorrected}} = 0.02$) and DRB1*07 ($P_{\text{uncorrected}} = 0.01$) (Table 4). If considering small duct PSC a compound phenotype consisting of two subgroups; (i) early large duct disease with HLA allele frequencies equal to large duct PSC and (ii) small duct disease with HLA allele frequencies equal to the healthy controls, the allele frequencies of HLA-B*08 in the current study would be consistent with a prevalence of large duct PSC of 32% (95% CI 4–65%) in our small duct PSC cohort.

The HLA associations in small duct PSC with IBD resembles large duct PSC

When comparing only the small duct PSC patients with concomitant IBD (n=53) with healthy controls, three significantly associated alleles were identified (Table 4); HLA-C*07 (OR = 1.6, 95% CI 1.1–2.4, $P_{\text{uncorrected}} = 0.02$), DRB1*03 (OR = 1.9, 95% CI 1.2–3.1, $P_{\text{uncorrected}} = 0.01$) and DRB1*04 (OR = 0.5, 95% CI 0.2–0.9, $P_{\text{uncorrected}} = 0.02$), in addition to DRB1*13:01 (OR = 2.0, 95% CI 1.03–3.9, $P_{\text{uncorrected}} = 0.04$) and B*08 (OR = 2.4, 95% CI 1.5–3.7, $P_{\text{uncorrected}} = 0.0003$). There were no significant differences between small duct PSC with IBD and large duct PSC for any of the alleles associated with large duct PSC (Table 4).

Only HLA-DRB1*13:01 confers susceptibility to small duct PSC without IBD

Thirty five percent of the small duct population had no concomitant IBD diagnosis. When comparing with controls, only DRB1*13:01 was significantly associated (frequency 13% vs. 6%, OR = 2.4, 95% CI 1.1–5.5, $P_{\text{uncorrected}} = 0.03$) in this subgroup. None of the other large duct PSC associated alleles were associated with small duct PSC without IBD, but there

were trends towards protective effects of typical large duct PSC predisposing alleles, most notable for HLA-DRB1*03 (4% in small duct PSC without IBD vs. 14% in controls, OR = 0.3, 95% CI 0.1–1.0, $P_{\text{uncorrected}} = 0.05$) (Table 4). When comparing small duct PSC without IBD with small duct PSC with IBD and large duct PSC, multiple alleles were significantly different (HLA-B*08, C*07, DRB1*03, DRB1*04, DRB1*07 and DRB1*11, the latter two only vs. large duct PSC), while there were no significant differences observed for HLA-DRB1*13:01 (Table 4). In contrast, in large duct PSC, only HLA-DRB1*04 was significantly different between patients with and without IBD (OR = 0.41, 95% CI 0.24–0.68, $P_{\text{uncorrected}} = 0.0007$, Supplementary Table 4).

Discussion

In this first genetic study of small duct PSC, the HLA-DRB1*13:01 and B*08 alleles were significantly associated with this condition. The strength of the HLA-DRB1*13:01 association was similar to that of large duct PSC, irrespective of IBD status in the small duct PSC patients, suggesting that haplotypes carrying DRB1*13:01 are a general risk factor for inflammatory bile duct disease. HLA-B*08 and several other alleles typically associated with large duct PSC were only associated with small duct PSC with concomitant IBD, while there were no trends of associations in small duct PSC without IBD. Despite phenotypical similarities between small duct PSC with and without IBD (8), the obvious difference in HLA predisposition detected in this study indicate that small duct PSC without IBD to a large extent could represent cholangiopathies of other aetiologies.

The strongest genetic association identified in small duct PSC was with the HLA-DRB1*13:01 allele, previously repeatedly associated with large duct PSC (13, 18). This was the only HLA allele associated with small duct PSC irrespective of IBD status. In contrast to many other large duct PSC associated HLA alleles, HLA-DRB1*13:01 increases the risk of only a limited number of other diseases and has been associated with protection against autoimmune diseases like narcolepsy (29), type 1 diabetes (30) and rheumatoid arthritis (31) and with clearance of hepatitis B (32). Given the association in all subgroups of sclerosing cholangitis, the HLA-DRB1*13:01 haplotype could be hypothesized to specifically increase susceptibility to inflammatory bile duct disease. This could be related to a restricted peptide-binding repertoire of the HLA-DRB1*13:01 molecule due to distinct electrostatic properties in its groove (27), or could represent a closely linked variant in a nearby gene. The HLA-DRB1*13:01 haplotype should therefore be a priority in further genetic or functional studies in PSC and related phenotypes.

The strongest genetic risk factor in large duct PSC, HLA-B*08 (14, 16), was significantly more prevalent in small duct PSC than healthy controls, but less prevalent than in large duct PSC. The intermediate position of the HLA-B*08 association in small duct PSC between healthy controls and large duct PSC is consistent with a heterogeneous small duct group that could consist of one subgroup which is HLA-B*08 associated, and one that is not. The simulations performed based on the results in the present study are compatible with a HLA-B*08 associated subgroup of 32% with a confidence interval of 4–65%. Notably, stratified analysis in our study indicate that this HLA-B*08 associated subgroup primarily consists of small duct PSC with concomitant IBD. In addition, no significant differences were found

between small duct PSC with IBD and large duct PSC when frequencies of the various HLA alleles were compared. This genetic similarity may suggest that small duct PSC with concomitant IBD represent mild or early stages of large duct disease, and future follow-up studies of small duct patients investigating the natural history in relation to HLA variants would be of great interest.

In small duct PSC patients without IBD, besides HLA-DRB1*13*01, there were no overlapping HLA associations with large duct PSC or small duct PSC with IBD. Different HLA associations (i.e. in terms of alleles and strength of association) have also been found in phenotypical distinct subgroups of several other HLA-associated diseases. Examples are rheumatoid arthritis (33) and myasthenia gravis (34), where differences have been observed with respect to autoantibody status and onset of disease, respectively. With only a 13 % frequency of the DRB1*13:01 allele in the small duct population without IBD, the majority of these patients will have genetic influences other than variants within the HLA complex. One possible contributor to the genetic predisposition is variation within the *ABCB4* gene, which has been detected in patients with unexplained cholestasis (10, 11). This study therefore provides a rationale for dedicated assessment of multiple genes with known involvement in monogenic cholestatic diseases in patients with small duct PSC but no IBD (35). The different HLA associations in small duct PSC with and without IBD stand in contrast to large duct PSC, where similar HLA associations in patients with and without IBD have been observed previously (25), and only the DRB1*04 allele had a different frequency in the present study. Thus, based on the HLA associations, the inclusion of small duct disease without IBD in the PSC disease spectrum is debatable.

A limitation of this study was the low number of small duct PSC patients and even smaller numbers included in the subgroup analyses with and without IBD. Previously reported HLA associations in large duct PSC were considered specific hypotheses, i.e. the study was specifically assessing the role of large duct PSC risk factors in small duct PSC, and a significance level of 0.05 was therefore reasonable for these (36). None of the possible novel observations were robust to the applied correction according to Bonferroni's method. False negative findings are difficult to exclude, but power calculations showed that e.g. the lack of association of the established large duct PSC risk HLA alleles, B*08 and DRB1*03, in small duct PSC patients without IBD can unlikely be explained by limited statistical power (Supplementary Table 3). Independent confirmation of these results would still be important, in particular in other population groups, e.g. Asian or African-American, which could also facilitate the identification of the true risk genes in the HLA complex.

In conclusion, the DRB1*13:01 allele contributes to the risk of all investigated PSC subgroups and may represent a specific risk factor for bile duct disease. While the HLA associations in small duct PSC with IBD and large duct PSC are highly similar, the differences observed in small duct PSC without IBD make it reasonable to discuss whether a diagnosis of IBD should be re-introduced as a prerequisite for the diagnosis of small duct PSC.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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List of abbreviations

PSC	primary sclerosing cholangitis
IBD	inflammatory bowel disease
CCA	cholangiocarcinoma
HLA	human leukocyte antigen
OR	odds ratio
LD	linkage disequilibrium

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Table 1

Clinical characteristics of patients with large duct primary sclerosing cholangitis and small duct primary sclerosing cholangitis with and without IBD

Trait		Large duct PSC (n=485)	Small duct PSC (n=87)	Small duct PSC with IBD (n=53)	Small duct PSC without IBD (n=29)
Gender	Male, n (%)	329 (68)	55 (63)	34 (64)	17 (59)
Age at diagnosis (years)	Median (range)	36 (12–81)	42 (12–79)	40 (12–79)	42 (29–64)
IBD*	IBD, n (%)	398 (83)	53 (65)	-	-
	UC, n (%)	294 (61)	37 (45)	-	-
	CD, n (%)	50 (10)	13 (16)	-	-
	IBD unclassified, n (%)	54 (11)	3 (4)	-	-
CCA	n (%)	61 (13)	0 (0)	0 (0)	0 (0)
OLT	n (%)	150 (34)	7 (8)	3 (6)	4 (14)
Death	n (%)	114 (25)	7 (8)	5 (9)	2 (7)

CCA: cholangiocarcinoma; CD: Crohn's disease; IBD: inflammatory bowel disease; OLT: orthotopic liver transplantation; PSC: primary sclerosing cholangitis; UC: ulcerative colitis.

* IBD status missing for 5 (1%) and 5 (6%) individuals in the large duct and small duct group respectively.

Table 2

Associations at *HLA-B* in small duct primary sclerosing cholangitis and controls

	Scandinavia		UK		US		Overall meta-analysis		
	Small duct PSC 2n=86		Small duct PSC 2n=51		Small duct PSC 2n=32		OR	95% CI	<i>p</i> uncorrected
	Alleles %	Alleles %	Alleles %	Alleles %	Alleles %	Alleles %			
HLA-B*									
5	10	4	4	5	3	5	1.69	0.90–3.17	0.11
7	19	16	12	15	9	12	0.97	0.62–1.52	0.91
8	19	13	16	13	28	13	1.62	1.08–2.44	0.02
12	15	15	16	19	9	15	0.84	0.54–1.32	0.45
13	3	1	0	2	6	2	2.32 ^a	0.90–5.98	0.08
14	5	3	4	4	6	5	1.37	0.64–2.93	0.41
15	6	11	8	7	9	4	0.85	0.46–1.57	0.61
16	0	2	0	1	0	1	0.50 ^a	0.10–2.57	0.41
17	0	3	12	4	0	6	0.67 ^{ab}	0.05–9.04	0.76
18	5	2	4	4	0	5	1.21 ^a	0.53–2.76	0.65
21	2	2	4	2	3	4	1.22	0.48–3.11	0.68
22	0	1	2	3	0	2	0.69 ^a	0.16–2.90	0.61
27	2	8	4	4	0	4	0.43 ^a	0.17–1.11	0.08
35	3	8	6	6	13	9	0.78	0.40–1.51	0.46
37	1	2	4	1	0	2	1.35 ^a	0.44–4.10	0.60
3901	2	0	2	1	3	1	4.23 ^a	1.40–12.78	0.01 ^c
40	6	10	4	7	6	7	0.59	0.29–1.18	0.13

Only alleles with frequency 1 % in at least one of the groups are presented. CI: confidence interval; OR: odds ratio; PSC: primary sclerosing cholangitis.

^aZero cell correction performed by adding 0.5.

^bSignificant test of heterogeneity, *p* (X^2_{2df})=0.01, random effects model used.

^c Bonferroni corrected *p*-value; 0.24.

* HLA-B presented with serotype nomenclature, i.e. B5 corresponds to HLA-B*51:XX and B*52:XX alleles, B7 to B*07:XX, B8 to B*08:XX, B12 to B*44:XX and B*45:XX, B13 to B*13:XX, B14 to B*14:XX, B15 to B*15:XX, B16 to B*38:XX and B*39:XX, B17 to B*57:XX and B*58:XX, B18 to B*18:XX, B21 to B*49:XX and B*50:XX, B22 to B*54:XX, B*55:XX and B*56:XX, B27 to B*27:XX, B35 to B*35:XX, B37 to B*37:XX, B3901 to B*39:01, B40 to B*40:XX.

Table 3

Associations at *HLA-DRB1* in small duct primary sclerosing cholangitis and controls

	Scandinavia		UK		US		Overall meta-analysis			
	Small duct PSC 2n=86	Controls 2n=734	Small duct PSC 2n=50	Controls 2n=1200	Small duct PSC 2n=31	Controls 2n=236	OR	95% CI	P _{uncorrected}	
HLA-DRB1	Alleles %	Alleles %	Alleles %	Alleles %	Alleles %	Alleles %				
01	9	13	10	13	10	15	0.69	0.40–1.18	0.17	
03	17	14	14	13	26	15	1.32	0.87–2.00	0.20	
04	14	22	18	18	13	14	0.74	0.48–1.15	0.19	
07	10	7	14	16	6	14	0.94	0.56–1.57	0.81	
08	2	4	0	2	0	2	0.56 ^a	0.17–1.82	0.33	
09	0	1	2	1	6	1	2.01 ^a	0.63–6.41	0.24	
10	2	1	2	0	0	1	3.30 ^a	0.98–11.14	0.05 ^b	
11	5	6	10	6	10	11	1.07	0.58–1.99	0.82	
12	1	2	0	1	3	0	0.96 ^a	0.26–3.57	0.96	
13:01	12	6	8	6	13	4	1.97	1.16–3.35	0.01	
13:02/03/05	3	6	4	5	3	4	0.66	0.28–1.52	0.32	
14	1	2	4	3	6	3	1.35	0.53–3.48	0.53	
15	21	15	14	15	3	12	1.08	0.69–1.67	0.74	

Only alleles with frequency 1 % in at least one of the groups are presented. CI: confidence interval; OR: odds ratio; PSC: primary sclerosing cholangitis.

^aZero cell correction performed by adding 0.5.

^bBonferroni corrected P=0.82.

Table 4
Frequencies of eight HLA alleles associated with large duct PSC and odds ratios of association analyses between subgroups

	Large duct PSC (n=485)	Small duct PSC (n=87)	Small duct PSC with IBD (n=53)	Small duct PSC without IBD (n=29)	Controls (n=1117)	Large duct PSC vs. controls	Large duct PSC vs. small duct PSC	Large duct PSC vs. small duct PSC with IBD	Large duct PSC vs. small duct PSC without IBD	Small duct PSC with IBD vs. controls	Small duct PSC without IBD vs. controls	Small duct PSC with IBD vs. small duct PSC without IBD
	Freq	Freq	Freq	Freq	Freq	OR (95 %CI)	OR (95 %CI)	OR (95 %CI)	OR (95 %CI)	OR (95 %CI)	OR (95 %CI)	OR (95 %CI)
HLA-A *												
1	30%	19%	21%	18%	17%	2.1 (1.7-2.5)	1.8 (1.2-2.7)	1.6 (1.0-2.6)	1.8 (0.9-3.7)	1.3 (0.8-2.0)	1.1 (0.5-2.2)	1.2 (0.5-3.0)
HLA-B *												
8	33%	20%	26%	5%	13%	3.3 (2.6-4.0)	1.9 (1.3-2.9)	1.4 (0.9-2.2)	6.8 (2.3-20.5)^a	2.4 (1.5-3.7)	0.4 (0.1-1.3) ^d	5.7 (1.7-19.2)^a
HLA-C												
07	50%	40%	46%	25%	34%	1.8 (1.5-2.2)	1.4 (1.0-1.9)	1.1 (0.7-1.7)	2.6 (1.4-5.0)	1.6 (1.1-2.4)	0.7 (0.4-1.2)	2.4 (1.2-5.1)
HLA-DRB1												
03	34%	18%	25%	4%	14%	2.9 (2.4-3.6)	2.2 (1.4-3.3)	1.5 (0.9-2.4)	9.6 (2.7-34.3)^a	1.9 (1.2-3.1)	0.3 (0.1-1.0) ^d	7.3 (1.8-29.2)^a
04	8%	15%	11%	22%	19%	0.4 (0.2-0.9)^b	0.6 (0.3-0.9)	0.9 (0.4-1.7)	0.4 (0.2-0.8)	0.5 (0.2-0.9)	1.2 (0.6-2.3)	0.4 (0.2-1.0)
07	5%	11%	9%	16%	13%	0.4 (0.3-0.6)	0.4 (0.3-0.8)	0.6 (0.3-1.2)	0.3 (0.1-0.6)	0.8 (0.4-1.6)	1.4 (0.7-2.9)	0.5 (0.2-1.4)
11	3%	7%	6%	11%	6%	0.5 (0.4-0.8)	0.5 (0.3-1.1)	0.6 (0.3-1.6)	0.4 (0.1-1.0)	0.9 (0.4-2.0)	1.7 (0.7-4.0)	0.6 (0.2-2.0)
13:01	15%	11%	11%	13%	6%	2.7 (2.0-3.6)	1.3 (0.8-2.3)	0.6 (0.7-2.7)	1.0 (0.4-2.4)	2.0 (1.0-3.9)	2.4 (1.1-5.5)	0.8 (0.3-2.1)

Eight significant HLA associations found in the meta-analysis of large duct PSC vs. controls in our study populations are listed. Significant ($P_{\text{uncorrected}} < 0.05$) Mantel-Haenszel tests are highlighted in bold. Freq: overall allele frequencies in the geographical panels; IBD: inflammatory bowel disease; PSC: primary sclerosing cholangitis.

^aZero cell correction performed.

^bRandom effects model used.

* HLA-A and B presented with serotype nomenclature, i.e. A1 corresponds to HLA-A*01:XX alleles and B8 to HLA-B*08:XX alleles.