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BRIEF COMMUNICATION

HLA haplotypes in primary sclerosing cholangitis patients of admixed and non-European ancestry


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Primary sclerosing cholangitis (PSC) is strongly associated with several human leukocyte antigen (HLA) haplotypes. Due to extensive linkage disequilibrium and multiple polymorphic candidate genes in the HLA complex, identifying the alleles responsible for these associations has proven difficult. We aimed to evaluate...
Primary sclerosing cholangitis (PSC) is a rare disease characterized by chronic inflammation of intra- and extrahepatic bile ducts, ultimately leading to liver cirrhosis and liver failure. The prevalence of PSC in populations of Northern European descent is approximately 1 in 10,000, while in Southern European and Asian populations the prevalence seems to be 10 to 100-fold lower. The etiology of PSC is unknown. However, siblings of PSC patients have a 9–39 fold increased risk of disease, suggesting that genetic components are involved in PSC pathogenesis. PSC is strongly associated with the human leukocyte antigen (HLA) complex, an association that was first described in the early 1980s. The 2 most prominent risk HLA haplotypes are the HLA-A*01:01-C*07:01-B*08:01-DRB3*01:01-DRB1*03:01-DQA1*05:01-DQB1*02:01 haplotype (also known as the 8.1 ancestral haplotype [AH8.1]), and the HLA-DRB1*13:01-DQA1*03-DQB1*06:03 haplotype. The most consistently observed protective association with PSC has been with the HLA-DRB1*04-DQA1*03-DQB1*03 haplotype. Owing to the extensive linkage disequilibrium (LD) in the HLA complex, the causative alleles on these associated haplotypes have remained unknown.

Large-scale trans-ancestry studies have identified new risk loci in complex diseases such as inflammatory bowel disease (IBD) and rheumatoid arthritis, and further reported that the majority of associated risk loci were shared across ethnicities. Because allele frequencies and LD patterns differ between populations from different geographical origins, studying populations of admixed ancestry or multiple ethnicities could aid in fine mapping causative HLA alleles. This was previously demonstrated in multiple sclerosis (MS): to better localize the HLA gene responsible for the association between MS and the HLA-DRB1*15:01-DQB1*06:02 haplotype, the HLA-DRB1 and HLA-DQB1 genes of African American MS patients and controls were assessed, showing a selective association between MS and the HLA-DRB1*15:01 allele. In PSC, it is challenging to establish the necessary admixed or multi-ethnic sample size due to the low prevalence of disease in populations of non-Northern European ancestry. Nevertheless, in this descriptive study, we aimed to explore to what extent studying populations of admixed or non-European descent might aid in pinpointing the causative HLA alleles in PSC.

We included 92 PSC patients of admixed or non-European ancestry and 150 PSC patients of Scandinavian ancestry. The diagnosis of PSC was based on accepted criteria with typical findings of bile duct irregularities on cholangiography. The study was performed in accordance with the Declaration of Helsinki. Ethics committees or institutional review boards of all participating centers approved patient recruitment, and written informed consent was obtained from all patients prior to participation. Among the patients in the admixed/non-European study population, 67 had previously been characterized as “ancestry outliers” due to non-European ancestry and were therefore excluded from the final analysis of the Immunochip-based PSC study. They had originally been recruited in Finland, the United Kingdom, the Netherlands, France, Germany, Italy, the United States (USA) and Canada. The remaining patients that were included in the admixed/non-European study population comprised 21 self-reported African American PSC patients sampled in the USA and 4 PSC patients of admixed or non-European ancestry sampled in Canada (1 Iranian, 1 Pakistani/Indian, 1 admixed Canadian Caucasian/Iranian and 1 admixed Canadian Caucasian/African Canadian). The 150 Scandinavian patients were selected from a previously described Scandinavian PSC population. The 135 Norwegians and 15 Swedes were randomly selected among patients carrying HLA-DRB1 alleles found on PSC-associated haplotypes, that is HLA-DRB1*01:01, HLA-DRB1*03:01, HLA-DRB1*04:01, HLA-DRB1*07:01, HLA-DRB1*11:01, HLA-DRB1*13:01 and HLA-DRB1*15:01. Notably, we selected only a limited number of Scandinavian patients who were homozygous for both HLA-B*08 and HLA-DRB1*03:01 (n = 10). Using a...
multidimensional scaling analysis, we assessed the genetic ancestry of the patients for which Immunochip data was available, that is the 67 “ancestry outliers” and 150 Scandinavians (Figure 1).

We performed HLA typing on the admixed/non-European and Scandinavian study populations using genomic DNA and a high-throughput sequencing method. RNA baits were used for the targeted enrichment of HLA-A, HLA-B and HLA-C (HLA class I), and HLA-DPA1, HLA-DPB1, HLA-DRB3, HLA-DRB1, HLA-DQA1 and HLA-DQB1 (HLA class II). Following library preparation, sequencing was carried out on a HiSeq instrument (Illumina, San Diego, California) with 100 base pair paired-end runs, and the alleles were extracted and merged. LD-pruning (\( r^2 < 0.1 \)) and minor allele frequency (MAF) filtering (MAF > 10%) resulted in 8561 SNPs for use in the MDS analysis. Plots were generated in the statistical software environment R v.3.2.3 (https://www.r-project.org/).

**Figure 1** Multidimensional scaling (MDS) plot of the primary sclerosing cholangitis (PSC) patients with available Immunochip data, together with 1245 samples from 1000 Genomes phase3. The orange, green and blue points represent 1000 Genomes EUR (European), EAS (East Asian) and AFR (African) super-populations, respectively. The African American ASW (sub-population of AFR) are marked using purple dots. The larger red dots represent the Scandinavian patients (\( n = 150 \)), overlapping the EUR super-population, while the larger black dots show the PSC patients of admixed or non-European ancestry who were previously characterized as “ancestry outliers” (\( n = 67 \)). Using Plink 1.9, a set of 20 226 single nucleotide polymorphisms (SNPs) with rsIDs on both the Immunochip and the Illumina Omni2.5 array that was used in the 1000 Genomes Project (downloaded from ftp://ftp.1000genomes.ebi.ac.uk/vol1/ftp/release/20130502/supporting/hd_genotype_chip/ALL.chip.omni_broad_sanger_combined.20140818.snps.genotypes.vcf.gz) were extracted and merged. LD-pruning (\( r^2 < 0.1 \)) and minor allele frequency (MAF) filtering (MAF > 10%) resulted in 8561 SNPs for use in the MDS analysis. Plots were generated in the statistical software environment R v.3.2.3 (https://www.r-project.org/).

estimated using 1000 iterations in PHASE v2.1.1.21,22 We focused our further assessments on the 3 PSC-associated haplotypes carrying the risk alleles \( HLA-DRB1*13:01 \) and \( HLA-DRB1*03:01 \) and the protective \( HLA-DRB1*04 \) alleles.5 The frequencies for alleles comprising these haplotypes are listed for the admixed/non-European study population in Table S1, Supporting Information.

The \( HLA-DRB1*13:01-DQA1*01:03-DQB1*06:03 \) haplotype was observed with a frequency of 8.7% in the admixed/non-European study population. Approximately one third of all \( HLA-DRB1*13:01 \) haplotypes in the admixed/non-European study population did not carry the \( HLA-DQB1*06:03 \) allele (Figure 2A). In comparison, every \( HLA-DRB1*13:01 \) haplotype in the Scandinavian study population carried the \( HLA-DQB1*06:03 \) allele (Figure 2B). The admixed/non-European study population displayed reduced LD between \( HLA-DRB1*13:01 \) and \( HLA-DQB1*06:03 \) compared with the previously described Scandinavian PSC population (\( r^2 = 0.50 \) in the admixed/non-European study population, \( r^2 = 0.87 \) in Scandinavians). The 9 PSC patients carrying non-\( DQB1*06:03 \) alleles on the \( HLA-DRB1*13:01 \) haplotype were of African or admixed African/Caucasian ancestry (Figure S1). Of these 9 patients, 3 carried the \( HLA-DQB1*05:01 \) allele on the \( HLA-DRB1*13:01 \) haplotype (Table S2), which is not unexpected because both \( HLA-DRB1*13:01-DQB1*06:03 \) and \( HLA-DRB1*13:01-DQB1*05:01 \) are common haplotypes in African Americans.23 The \( HLA-DRB1*13:01 \) haplotype was recently hypothesized to specifically increase the susceptibility of inflammatory bile duct diseases, as it is associated with both large and small duct PSC, irrespective of IBD status.24 Association analyses to define a causative allele were inappropriate in this study due to the sample size and further the selection process of the patients in the admixed/non-European study population, which rendered it impossible to recruit an adequate healthy control population. Nevertheless, findings from this descriptive study suggest that assessing further the \( HLA-DRB1*13:01-DQA1*01:03-DQB1*06:03 \) haplotype in admixed or multi-ethnic populations of PSC patients and ethnicity matched healthy controls could aid in fine mapping the causative HLA allele, assuming the same causative allele across ethnicities.

In the admixed/non-European study population, 36% of \( HLA-DRB1*03:01 \) haplotypes carried the \( HLA-B*08:01 \) allele (Figure 3A) and 56% of \( HLA-B*08:01 \) haplotypes carried the \( HLA-DRB1*03:01 \) allele (Figure 3B), whereas in the previously described Scandinavian PSC population, 86% of \( HLA-DRB1*03:01 \) haplotypes carried the \( HLA-B*08 \) allele and 89% of \( HLA-B*08 \) haplotypes carried the \( HLA-DRB1*03:01 \) allele. The LD between \( HLA-B*08:01 \) and \( HLA-DRB1*03:01 \) was weak in the admixed/non-European study population (\( r^2 = 0.17 \)) compared to the previously described Scandinavian PSC population (\( r^2 = 0.65 \)). On the other hand, every \( HLA-DRB1*03:01 \) haplotype carried the \( HLA-DQB1*02:01 \) allele, irrespective...
of ethnic origin (Figure 3A). This observation was supported by LD measurements between HLA-DRB1*03:01 and HLA-DQB1*02:01 ($r^2 = 0.93$ in the admixed/non-European study population, $r^2 = 0.99$ in the previously described Scandinavian PSC population\textsuperscript{18}). The 10 Scandinavian PSC patients who were selected for being homozygous for both HLA-B*08:01 and HLA-DRB1*03:01 were also homozygous for the HLA-DRB3*01:01 allele and for HLA-DQA1*05:01-DQB1*02:01 (Table S3). Four of these patients were homozygous for both HLA-A*01:01 and HLA-C*07:01. Of the remaining 6 patients, 4 were heterozygous for HLA-A*01:01 and homozygous for HLA-C*07:01, and 2 were heterozygous for HLA-C*07:01 but did not carry HLA-A*01:01. AH8.1 is a common haplotype in Northern European populations, with a frequency of approximately 10\%.\textsuperscript{25,26} Alleles comprising the AH8.1 are strongly associated with a large number of immune-driven diseases.\textsuperscript{27} For some diseases, the primary association with AH8.1 is confined to the HLA class I region, as seen in myasthenia gravis,\textsuperscript{28} or to the HLA class II region, as seen...
in coeliac disease and type 1 diabetes.\textsuperscript{29,30} For other diseases including PSC, associations have been reported for both HLA class I and class II alleles of the AH8.1.\textsuperscript{5} Our data suggest that studying admixed or multi-ethnic populations will likely aid in fine mapping the AH8.1 association in PSC to the HLA class I and/or HLA class II region. This is in agreement with a previous African American PSC study, in which an association with HLA-B8 but not HLA-DR3 was detected.\textsuperscript{31} As we could not dissociate the strong LD between HLA-DRB1*03:01 and HLA-DQB1*02:01, pinpointing the potential causative allele within the HLA class II region (i.e. HLA-DRB1*03:01, HLA-DQA1*05:01 or HLA-DQB1*02:01) might remain a challenge.

Every HLA-DRB1*04:01 and HLA-DRB1*04:04 haplotype in the admixed/non-European study population carried HLA-DQB1*03:01 and HLA-DQB1*03:02 alleles, respectively (Table S4). The HLA-DRB1*04:01 and HLA-DRB1*04:04 alleles were each observed in 3 patients of admixed or non-European ancestry. In the Scandinavian study population, HLA-DRB1*04:01 haplotypes carried either HLA-DQB1*03:01 or HLA-DQB1*03:02, and the HLA-DRB1*04:04 haplotype carried the HLA-DRB1*03:02 allele (Table S5). The HLA-DRB1*04:04 allele was observed only once in the Scandinavian study population because of the selection of Scandinavian PSC patients for this study: this patient was previously genotyped to have the HLA-DRB1*04:01 allele.\textsuperscript{18} Collectively, our data suggest that studying admixed or multi-ethnic populations might not help in identifying the causative allele in the protective HLA-DRB1*04-DQA1*03-DQB1*03 haplotype.

In conclusion, our data suggest that studying admixed or multi-ethnic populations could aid in fine mapping the causative HLA allele in the PSC-associated haplotype HLA-DRB1*13:01-DQA1*01:03-DQB1*06:03.

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Conflict of interest

The authors have declared no conflicting interests.

REFERENCES


**SUPPORTING INFORMATION**

Additional Supporting Information may be found online in the supporting information tab for this article.