

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Hirschfield GM, Liu X, Xu C, et al. Primary biliary cirrhosis associated with *HLA*, *IL12A*, and *IL12RB2* variants. *N Engl J Med* 2009;360:2544-55. DOI: 10.1056/NEJMoa0810440.

SUBJECTS

Canada-based cases had a mean age of 60.7 years (23-92) at recruitment and a diagnosis of PBC for an average of 8.9 years (1-30). 93% of cases were female, 95.5% AMA positive and 5.2% had received a liver transplant. Cases were recruited between 2005-2008 with the majority (372) ascertained from Toronto and the remaining from London (Ontario), Halifax, Edmonton and Calgary. US-based cases had a mean age at recruitment of 60.2 years (29-85) and a diagnosis of PBC for an average of 9.3 years (0-33). 92.4% of US cases were female and 87.6% AMA positive; 10.5% had received a liver transplant.

Canadian control subjects (n=1279, 87% female) were geographically matched healthy volunteers with no prior history of autoimmune disease (mean age 31.4 years, range 18-88) also ascertained between 2005 and 2008. A total of 1137 healthy subjects from the M.D. Anderson Cancer Center Lung Cancer Study, a case-control study ongoing since 1999, were also used as controls. These “historic” controls (mean age 61.1; SD 8.9) were ascertained from Kelsey-Seybold Clinics in the Houston Metropolitan area. Caucasian ancestry was ascertained first by self-report (questionnaire choices including white (Caucasian), Indian subcontinent, Japanese, other Asian, Black African, African-American, specified Other) and then by excluding any subjects whose genotypes clustered with other or mixed ancestry groups (i.e. we excluded African-Americans, Hispanics, Asians, and Caribbean Islanders).

Another 310 healthy “US-based” controls were from the Mayo Clinic PBC Genetic Epidemiology Registry and Biospecimen Repository. Controls were recruited from the Mayo

Clinic Division of General Internal Medicine during annual visits for preventative medical examination and were matched by age (± 2.5 years), gender, and state of residence to individual PBC patients. Control exclusion criteria included evidence of prior or current liver disease. 90% were female and the mean age 62.4 years (33-88).

GENOTYPING

DNA was prepared using standard methods. A total of 536 samples from PBC subjects and 399 control subjects collected in Canada were genotyped on Illumina HumanHap370 Genotyping BeadChips. This platform was selected because of the genome coverage provided, reflecting the almost exclusive use of tag SNPs with high minor allele frequencies (median=0.25), density (mean inter SNP distance=7.8kb) and LD (mean $r^2 > 0.8$). A total of 1137 samples from M.D. Anderson Cancer Center Lung Cancer Study (historic controls) were genotyped at the Centre for Inherited Disease Research (Johns Hopkins University) on the Illumina HumanHap300 chip. Cluster definitions for each SNP were determined using Illumina BeadStudio Genotyping Module v 2.3.41 and the combined intensity data from ~90% of study samples. The resulting cluster definition file was used on all study samples to determine genotype calls and quality scores. Genotype calls were made when a genotype yielded a quality score (Gencall value) of 0.25 or higher in Texas and 0.15 in the Canadian samples. Among these markers, 0.46% of cells were missing. For the lung control study, fifty-four blind duplicate pairs were included, the concordance of SNP genotype calls was 99.99%. Based upon 106 duplicate HapMap samples (56 unique individuals) from all 4 populations (Caucasian, Yoruban, Chinese and Japanese) the

concordance was 99.8%, and the Mendelian consistency rate calculated from 29 Hapmap trios (16 unique trios) was 99.96%.

A Sequenom MassARRAY iPLEX platform was used for both replication and fine-mapping genotyping (<http://www.sequenom.com/seq-genotyping.html>). In brief, allele-specific extension products were plated onto a SpectroCHIP (Sequenom), subjected to mass spectrometric analysis (MassARRAY system, Sequenom, San Diego, CA) and the genotypes identified using SpectroCALLER software.

In stage 2, we genotyped 16 SNPs in 14 genes/loci based on GWA signals. For fine-mapping studies, initiated coincident with the stage 2 analysis, a total of 93 SNPs from three different loci (*IL12A*, *IL12RB2-IL23R*, and *STAT4*) were selected based on at least one of the following criteria: 1) HapMap Phase II data (<http://www.hapmap.org/>) identifying the SNP as a TagSNP with minor allele frequency >0.05 or a higher minor allele frequency (>0.01) and r^2 threshold of 0.8 and/or 2) localization in potential functional regulatory gene regions (i.e. exon, exon-intron boundary, 5' or 3' flank). Out of 93 SNPs tested, 89 passed quality control filters including 25 across the *STAT4* gene and 200kb flanking regions at chromosome 2q32.2-q32.3, 25 in the *IL12A* region of chromosome 3q25.33-q26, and 39 in the *IL12RB2/IL23R* loci at chromosome 1p31.1-31.2.

POPULATION-BASED QUALITY FILTERS

Genotyping data from HumHap370 and HumanHap300 were filtered prior to data merging as indicated above. After setting Maximum per-person missing (MIND) to be no less than 0.05 and GENO (Maximum per-SNP missing) to be no less than 0.05, minor allele frequency to be no less than 0.01 and Hardy Weinberg equilibrium test to be no less than 0.0001, 929 samples and 334,444 SNPs from the HumanHap370 data set (including 536 PBC cases and 399 controls) and 1137 subjects and 312,547 SNPs from the HumanHap300 data set were retained. After merging, the same MIND and GENO settings were applied to all of the samples and SNPs. The final combined data retain 2012 subjects (505 PBC subjects and 1507 control subjects) and 305,724 common SNPs. Quantile–Quantile Plots of Test Statistics (P Values) for the PBC GWA are shown in supplementary Figure S1.

ASSESSMENT OF STATISTICAL POWER

To carry out power calculations for a GWA screen, a genotypic relative risk analysis (<http://statgen.iop.kcl.ac.uk/gpc/cc2.html>) was conducted with the following parameters: disease prevalence, 0.001 for PBC, D' between disease and SNP alleles, 1.0; genotypic heterozygous relative risk, 1.5 under a log-additive model; alpha, 5×10^{-5} (corresponding to 16/317,000 the probability to retain a marker from the first to second stage of analysis) marker allele frequency (A) = 0.20, as this is the mean minor allele frequency of the tested SNPs. The power to detect association was estimated as 81.7% based on the sample of 2072 subjects (536 cases and 1536 controls) in a GWA study (stage 1), and 90.7% based on the total sample of 3744 subjects (1031 PBC cases and 2713 healthy controls) in a combined analysis using a genome-wide significance

criterion of $p < 1 \times 10^{-7}$. For a heterozygous genotypic relative risk of 1.4, the corresponding powers are 50% in the first stage and 60% in the combined analysis.

HLA HAPLOTYPE ANALYSIS

For the HLA region for which linkage disequilibrium extends over a broad area, we used PLINK to jointly model the impact that inferred SNP haplotypes (derived from the 13 PBC-associated HLA SNPs identified in Table 1) have on disease risk. We first modeled the association of all haplotypes across the region with PBC risk, and then sequentially removed SNPs until the SNPs removed yielded significant haplotypic risks for disease. We then analyzed the remaining SNPs as haplotypes to evaluate the haplotypic effects on PBC risk.

POPULATION ATTRIBUTABLE FRACTION

Population attributable fraction (AF_p) for single DRB1 genotypes described in the introduction

were calculated as: $AF_p = \frac{PF(OR - 1)}{1 + PF(OR - 1)}$ where OR is the odds ratio associated with

DRB1*0801 genotype and PF is the genotype frequency of the risk genotype.

Population attributable fraction (AF_p) for a single SNP was calculated over genotypes i (indexed as 1, 2 for the heterozygous and homozygous risk genotypes respectively) and for the Canadian and American replication populations (indexed as j=1,2).

$$AF_p = \frac{\sum_{i=1}^2 \sum_{j=1}^2 PF_{ij}(OR_{ij} - 1)}{1 + \sum_{i=1}^2 \sum_{j=1}^2 PF_{ij}(OR_{ij} - 1)}$$

In this analysis, PF is the risk allele frequency and OR is the odds ratio associated with having a risk genotype. The population attributable risk fraction was computed over all possible combinations of genotypes among the three risk SNPs according to the formula:

$$AF_p = \frac{\sum_{i=1}^2 \sum_{j=1}^2 \sum_{k=1}^3 PF_{ijk}(OR_{ij}-1)}{1 + \sum_{i=1}^2 \sum_{j=1}^2 \sum_{k=1}^3 PF_{ijk}(OR_{ij}-1)}$$

where the factors are defined for each joint genotype (Hanley JA. A heuristic approach to the formulas for population attributable fraction. *J Epidemiol Community Health* 2001;55:508–14). The odds ratios and frequencies used for these computations are shown in supplementary table 11.

CONDITIONAL AND LINEAR TREND ANALYSES

To further investigate whether a single or multiple SNPs influence disease risk within the *IL12A*, *IL12RB2* and *STAT4* loci (extended Canada based cohort dataset), we conducted conditional analysis using multiple logistic regression with a stepwise selection procedure implemented in SAS version 9.1 (SAS Institute Inc, Cary, North Carolina, USA) for which the entry criterion allowed any SNP with $P < 0.15$ to enter the model for consideration but at each step, each SNP had to meet a $P < 0.05$ criterion to be retained. We tested for a linear trend in PBC risk using PLINK, with an additive genetic model, to identify gene dose-response effects for rs6441286/rs574808 and rs3790567 SNPs at the *IL12A* and *IL12RB2* loci. A gene dose effect was also evaluated using SAS version 9.1 and a general model for genotypic effects.

SUPPLEMENTARY FIGURE LEGEND

Supplementary Figure S1. Quantile–Quantile plot of test statistics for the genomewide association study

The $-\log P$ values from EIGENSTRAT analysis are plotted on the Y axis against the expected $-\log(P)$ values on the X axis after removing disqualified individuals, outliers, individuals with excess IBD sharing SNPs that failed HWE, and SNPs with MAF <0.01 . Under the null hypothesis of no association at any locus, the data points are expected to follow the green line. The lambda value is 1.056 after adjusting for eigenvectors and 1.085 without eigenvector adjustment.

AUTHOR CONTRIBUTIONS

This study was initiated in 2004 by KAS and EJH who were responsible for initial study design. ALM, RPM, KMP and CNG assisted in developing a clinical network and sample collection/processing framework required for case accrual, and GMH and XL assisted in design of the genetic analyses. Clinical data were gathered by GMH, ALM, RPM, KMP, CNG and CC under the supervision of EJH in Canada; BDJ, EJA and KNL were responsible for the US case series. Genetic data were obtained primarily by XL with assistance from GX, EJW and CX and the data analysis carried out by YL (MSc), YL (MD), XG and KJ under the supervision of CIA and KAS. GMH, XL, CIA, EJH and KAS vouch for the data and the analysis. The paper was written primarily by GMH, XL and KAS with assistance from CX, EJH and KNL. KAS, the senior and corresponding author, decided in collaboration with GMH and EJH, and in agreement with all other authors to publish this paper.

Supplementary Table S1. Associations between HLA haplotypes and primary biliary cirrhosis.

SNP Haplotype	Frequency Cases	Frequency Controls	χ^2	Degrees of Freedom	P
CGAG	0.0535	0.09816	18.54	1	1.66x10 ⁻⁵
CAAG	0.1239	0.1643	9.244	1	2.36 x10 ⁻³
AACA	0.04533	0.0119	41.65	1	1.09 x10 ⁻¹⁰
CACA	0.2954	0.2009	37.94	1	7.29 x10 ⁻¹⁰
CGAA	0.159	0.1871	3.945	1	0.047
CAAA	0.3229	0.3377	0.7315	1	0.3924
Overall	NA	NA	98.7	5	9.93 x10 ⁻²⁰

*Haplotype is comprised of the following SNPs in the order shown: rs2395148-rs3135363-rs2856683-rs9357152

Table S1 Legend: The impact that SNPs and haplotypes across the MHC have on risk for PBC was analysed using PLINK. For this analysis, we included the 13 most significant SNPs in the HLA region that are identified in Table 1 and jointly modeled the impact of the inferred haplotypes derived from these SNPs on disease risk.

Supplementary Table S2. Comparison of original stage 1 GWA analysis with an analysis restricted to Canadian controls alone.

SNP ID	Chr	Gene	Risk allele frequency		Original GWA analysis incorporating Canadian and historic controls n=505 cases and 1507 controls (383 Canadian and 1124 M.D. Anderson "historic")			Alternate GWA analysis incorporating only Canadian controls n=505 cases and 383 Canadian controls		
			US Controls	Canadian Controls	EIGENSTRAT P	PLINK P	OR (95% CI)	EIGENSTRAT P	PLINK P	OR (95% CI)
HLA regions										
rs2856683	6p21.3	<i>HLA-DQB1</i>	0.221	0.219	1.34 x 10 ⁻¹⁴	8.58 x 10 ⁻¹⁷	1.99 (1.69-2.34)	6.00 x 10 ⁻⁹	1.05 x 10 ⁻⁸	1.91 (1.53-2.39)
rs9275312	6p21.3	<i>HLA-DQB1</i>	0.128	0.121	1.99 x 10 ⁻¹¹	3.84 x 10 ⁻¹³	2.01(1.66-2.42)	1.05 x 10 ⁻⁶	1.15 x 10 ⁻⁶	1.93 (1.48-2.53)
rs9275390	6p21.3	<i>HLA-DQB1</i>	0.247	0.239	5.73 x 10 ⁻¹¹	1.13 x 10 ⁻¹³	1.81 (1.55-2.11)	8.81 x 10 ⁻⁷	1.69 x 10 ⁻⁶	1.69 (1.36-2.10)
rs7775228	6p21.3	<i>HLA-DQB1</i>	0.121	0.120	1.90 x 10 ⁻¹⁰	1.70 x 10 ⁻¹⁰	1.87 (1.54-2.27)	8.18 x 10 ⁻⁶	5.04 x 10 ⁻⁶	1.88 (1.43-2.47)
rs2395148	6p21.3	<i>C6orf10</i>	0.022	0.009	1.50 x 10 ⁻⁹	5.62 x 10 ⁻¹⁰	3.24 (2.20-4.77)	5.55 x 10 ⁻⁷	6.43 x 10 ⁻⁷	5.75 (2.61-12.66)
rs9277535	6p21.3	<i>HLA-DPB1</i>	0.232	0.249	7.26 x 10 ⁻⁹	8.28 x 10 ⁻⁹	1.60 (1.36-1.87)	3.24 x 10 ⁻⁴	4.88 x 10 ⁻⁴	1.46 (1.18-1.81)
rs3806156	6p21.3	<i>BTNL2</i>	0.350	0.365	1.31 x 10 ⁻⁸	1.27 x 10 ⁻⁹	1.58 (1.37-1.84)	0.001	2.00 x 10 ⁻⁴	1.46 (1.20-1.78)
rs9357152	6p21.3	<i>HLA-DQB1</i>	0.741	0.745	3.88 x 10 ⁻⁸	7.25 x 10 ⁻⁸	1.65 (1.37-1.97)	5.42 x 10 ⁻⁴	0.010	1.35 (1.08-1.72)
rs3135363	6p21.3	<i>BTNL2</i>	0.738	0.712	4.46 x 10 ⁻⁸	3.72 x 10 ⁻⁷	1.56 (1.30-1.85)	3.96 x 10 ⁻⁴	0.006	1.37 (1.10-1.72)
rs9277565	6p21.3	<i>HLA-DPB1</i>	0.193	0.213	5.94 x 10 ⁻⁸	8.32 x 10 ⁻⁸	1.58 (1.34-1.87)	0.001	0.001	1.44 (1.15-1.81)
rs2281389	6p21.3	<i>HLA-DPB1</i>	0.163	0.184	1.58 x 10 ⁻⁷	2.38 x 10 ⁻⁷	1.59 (1.33-1.90)	0.002	0.004	1.41 (1.12 -1.79)
rs660895	6p21.3	<i>HLA-DRB1</i>	0.200	0.184	3.41 x 10 ⁻⁷	4.68 x 10 ⁻⁸	1.60 (1.35-1.90)	7.10 x 10 ⁻⁵	8.60 x 10 ⁻⁵	1.60 (1.27-2.02)
rs9501626	6p21.3	<i>HLA-DRA</i>	0.117	0.092	1.34 x 10 ⁻¹⁴	8.58 x 10 ⁻¹⁷	1.99 (1.69-2.34)	1.30 x 10 ⁻⁵	6.57 x 10 ⁻⁶	1.99 (1.47-2.70)

Table S2 Legend: GWA analysis was repeated using Canadian control data only (“Alternate GWA analysis”). The results demonstrate that the associations detected in the initial GWA survey are not secondary to stratification between control sets.

Supplementary Table S3. Combined (stage 1 and 2) analysis restricted to AMA positive patients.

SNP ID	Chr.	Location	Gene	Combined		Combined AMA positive cases	
				n=1031 cases and 2713 controls		n=927 cases and 2713 controls	
				P	Odds Ratio (95% CI)	P	Odds Ratio (95% CI)
A. HLA regions							
rs2856683	6p21.3	32763196	<i>HLA-DQB</i>	1.78 x 10 ⁻¹⁹	1.75 (1.55-1.98)	2.8 x 10 ⁻²⁰	1.79 (1.58-2.03)
rs9275312	6p21.3	32773706	<i>HLA-DQB1</i>				
rs9275390	6p21.3	32777134	<i>HLA-DQB1</i>				
rs7775228	6p21.3	32766057	<i>HLA-DQB1</i>				
rs2395148	6p21.3	32429532	<i>C6orf10</i>	3.62 x 10 ⁻¹⁴	2.87 (2.16-3.82)	3.55 x 10 ⁻¹⁵	3.00 (2.25-4.00)
rs9277535	6p21.3	33162839	<i>HLA-DPB1</i>	3.92 x 10 ⁻¹¹	1.50 (1.33-1.70)	2.12 x 10 ⁻¹¹	1.52 (1.35-1.72)
rs3806156	6p21.3	32481676	<i>BTNL2</i>	1.11 x 10 ⁻⁹	1.42 (1.27-1.58)	1.25 x 10 ⁻⁹	1.43 (1.27-1.60)
rs9357152	6p21.3	32772938	<i>HLA-DQB1</i>				
rs3135363	6p21.3	32497626	<i>BTNL2</i>				
rs9277565	6p21.3	33164875	<i>HLA-DPB1</i>				
rs2281389	6p21.3	33167774	<i>HLA-DPB1</i>				
rs660895	6p21.3	32685358	<i>HLA-DRB1</i>				
rs9501626	6p21.3	32508322	<i>HLA-DRA</i>				
B. Non-HLA regions							
rs6441286	3q25.33-q26	161211572	<i>IL12A</i>	2.42 x 10 ⁻¹⁴	1.54 (1.38-1.72)	7.23 x 10 ⁻¹³	1.51 (1.35-1.70)
rs3790567	1p31.2	67594965	<i>IL12RB2</i>	2.76 x 10 ⁻¹¹	1.51 (1.33-1.70)	1.11 x 10 ⁻¹¹	1.53 (1.35-1.73)
rs574808	3q25.33-q26	161215677	<i>IL12A</i>	1.88 x 10 ⁻¹³	1.54 (1.37-1.73)	1.34 x 10 ⁻¹²	1.53 (1.36-1.73)
rs6838639	4q27	123118615	<i>TRPC3</i>	2.35 x 10 ⁻³	1.23 (1.08-1.40)	2.10 x 10 ⁻³	1.23 (1.08-1.41)
rs3790565	1p31.2	67583944	<i>IL12RB2</i>	1.24 x 10 ⁻⁸	1.46 (1.28-1.67)	4.56 x 10 ⁻⁹	1.49 (1.30-1.70)
rs9964104	18q21	50751695	<i>CCDC68</i>	2.13 x 10 ⁻⁴	1.25 (1.11-1.40)	1.59 x 10 ⁻⁴	1.26 (1.12-1.42)
rs3124607	9q34.3	138534660	<i>NOTCH1</i>				
rs16833239	2q32	191648505	<i>STAT4</i>	4.67 x 10 ⁻⁵	1.65 (1.30-2.10)	2.53 x 10 ⁻⁵	1.71 (1.33-2.19)
rs10222962	4p15	32036553	<i>PCDH7</i>				
rs2211312	13q33.3	107203928	<i>FAM155A</i>	0.608	1.05 (0.88-1.24)	0.38	1.08 (0.91-1.29)
rs907092	17q21	35175785	<i>IKZF3</i>	7.61 x 10 ⁻⁶	1.29 (1.15-1.44)	6.21 x 10 ⁻⁶	1.30 (1.16-1.45)
rs9303277	17q21	35229995	<i>IKZF3</i>				
rs4679904	3q26.1	161823590	<i>ARF7</i>	1.13 x 10 ⁻⁶	1.38(1.21-1.57)	4.76 x 10 ⁻⁶	1.36 (1.19-1.55)
rs2305480	17q12	35315722	<i>GSDML</i>				
rs6140113	20p13	691770	<i>C20orf54</i>	0.338	1.08 (0.93-1.25)	0.238	1.10 (0.94-1.28)
rs10488631	7q32.1	128381419	<i>IRF5/TNPO3</i>	1.52 x 10 ⁻⁷	1.52 (1.30-1.78)	6.83 x 10 ⁻⁷	1.50 (1.28-1.76)
rs6748358	2q33	204465150	<i>CTLA4</i>				

Table S3 Legend: A repeat of the combined analysis in Table 1, restricted to AMA positive cases only, reveals no significant effect of AMA status on the associations identified.

Supplementary Table S4. Linear trend for risk variants across genotypes at the *IL12A* and *IL12RB2* loci

Locus	SNP	Risk Allele	Effect	Odds Ratio (95% CI)	P
<i>IL12A</i>	rs6441286	G	GG vs GT vs TT	1.527 (1.323-1.762)	7.17 x 10 ⁻⁹
	rs574808	T	TT vs CT vs CC	1.497 (1.288-1.74)	1.43 x 10 ⁻⁷
<i>IL12RB2</i>	rs3790567	A	AA vs AG vs GG	1.511 (1.296-1.762)	1.41 x 10 ⁻⁷

Table S4 Legend: A gene dose effect can be demonstrated for the *IL12A* and *IL12RB2* loci: using PLINK and an additive model, a clear linear trend for risk variants is seen across all genotypes.

Supplementary Table S5. Genotypic effects for risk variants at the *IL12A* and *IL12RB2* loci

Locus / SNP	Risk allele	Genotype	Odds ratio (95% CI)
<i>IL12A</i> / rs6441286	G	GG vs TT	2.215 (1.677-2.926)
		GT vs TT	1.756 (1.402-2.2)
<i>IL12A</i> / rs574808	T	TT vs CC	2.31 (1.669-3.197)
		CT vs CC	1.68 (1.219-2.316)
<i>IL12RB2</i> / rs3790567	A	AA vs GG	2.373 (1.687-3.338)
		AG vs GG	1.497 (1.221-1.835)

Table S5 Legend: Using SAS and a general model for genotypic effects for *IL12A*, the homozygous genotype effect is less than log-additive, while for *IL12RB2* the effect is approximately log-additive.

Supplementary Table S6. Association data from fine-mapping of the *IL12RB2*, *STAT4*, and *IL12A* loci.

CHR	SNP	Location	Minor Allele	Major Allele	MAF-Cases	MAF-Control	P Value	Odds Ratio (95%CI)
1	IL12RB2_RS3762317	67545170	G	A	0.114	0.123	4.18X10 ⁻¹	1.097 (0.876 - 1.374)
1	IL12RB2_RS1890741	67555340	C	G	0.187	0.176	4.27X10 ⁻¹	1.076 (0.898 - 1.291)
1	IL12RB2_RS1546159	67561014	T	C	0.015	0.021	2.70X10 ⁻¹	1.360 (0.786 - 2.352)
1	IL12RB2_RS11209050	67564324	A	C	0.283	0.205	2.76X10 ⁻⁷	1.532 (1.301 - 1.804)
1	IL12RB2_RS6693065	67572606	G	A	0.179	0.218	7.13X10 ⁻³	1.278 (1.069 - 1.528)
1	IL12RB2_RS4655703	67573960	A	T	0.463	0.407	1.66X10 ⁻³	1.256 (1.089 - 1.447)
1	IL12RB2_RS746389	67575391	G	A	0.463	0.403	7.60X10 ⁻⁴	1.275 (1.107 - 1.469)
1	IL12RB2_RS1995147	67575647	G	C	0.462	0.403	8.41X10 ⁻⁴	1.273 (1.105 - 1.467)
1	IL12RB2_RS10889682	67576471	C	G	0.195	0.224	5.26X10 ⁻²	1.188 (0.998 - 1.414)
1	IL12RB2_RS17129823	67577357	C	T	0.076	0.055	1.30X10 ⁻²	1.425 (1.076 - 1.886)
1	IL12RB2_RS1908632	67578394	G	T	0.333	0.248	1.22X10 ⁻⁷	1.516 (1.299 - 1.769)
1	IL12RB2_RS3790564	67579042	T	G	0.133	0.164	1.57X10 ⁻²	1.281 (1.048 - 1.567)
1	IL12RB2_RS3790565	67583944	C	T	0.252	0.183	2.44X10 ⁻⁶	1.505 (1.269 - 1.785)
1	IL12RB2_RS10489625	67587008	G	A	0.046	0.038	2.64X10 ⁻¹	1.219 (0.861 - 1.726)
1	IL12RB2_RS946685	67588303	A	G	0.252	0.183	1.93X10 ⁻⁶	1.506 (1.271 - 1.783)
1	IL12RB2_RS6679356	67592782	C	T	0.270	0.190	7.02X10 ⁻⁸	1.574 (1.334 - 1.858)
1	IL12RB2_RS10889684	67594609	T	C	0.143	0.172	2.80X10 ⁻²	1.246 (1.024 - 1.516)
1	IL12RB2_RS10749775	67594675	C	A	0.192	0.125	1.88X10 ⁻⁷	1.655 (1.368 - 2.003)
1	IL12RB2_RS3790567	67594965	A	G	0.337	0.250	8.32X10 ⁻⁸	1.519 (1.303 - 1.771)
1	IL12RB2_RS6695348	67599604	T	C	0.333	0.247	9.87X10 ⁻⁸	1.519 (1.302 - 1.772)
1	IL12RB2_RS12564159	67603882	G	C	0.336	0.298	2.34X10 ⁻²	1.190 (1.024 - 1.383)
1	IL12RB2_RS2252596	67606089	A	G	0.015	0.022	1.23X10 ⁻¹	1.541 (0.886 - 2.681)
1	IL12RB2_RS2307145	67606115	C	G	0.046	0.035	1.32X10 ⁻¹	1.311 (0.921 - 1.865)
1	IL12RB2_RS2307153	67606231	A	G	0.019	0.024	2.99X10 ⁻¹	1.302 (0.791 - 2.146)
1	IL12RB2_RS3790568	67608648	A	G	0.052	0.052	9.69X10 ⁻¹	1.006 (0.734 - 1.379)
1	IL12RB2_RS3828069	67612161	C	T	0.141	0.173	1.60X10 ⁻²	1.273 (1.046 - 1.549)
1	IL12RB2_RS6676606	67615554	G	A	0.094	0.072	2.73X10 ⁻²	1.327 (1.032 - 1.707)
1	IL12RB2_RS4297265	67624923	G	A	0.476	0.418	1.20X10 ⁻³	1.264 (1.097 - 1.457)
1	IL12RB2_RS2270614	67628609	T	C	0.475	0.421	2.42X10 ⁻³	1.244 (1.080 - 1.433)
1	IL12RB2_RS2229546	67634108	C	A	0.354	0.325	8.41X10 ⁻²	1.139 (0.983 - 1.321)
2	STAT4_RS11676659	191627599	G	A	0.036	0.050	7.71X10 ⁻²	1.380 (0.964 - 1.975)
2	STAT4_RS11893432	191630119	G	C	0.227	0.205	1.52X10 ⁻¹	1.141 (0.953 - 1.366)
2	STAT4_RS3024866	191631086	C	T	0.275	0.264	5.14X10 ⁻¹	1.054 (0.899 - 1.236)
2	STAT4_RS3024851	191637067	A	T	0.036	0.048	1.08X10 ⁻¹	1.349 (0.935 - 1.947)
2	STAT4_RS3024847	191638535	A	T	0.432	0.410	2.35X10 ⁻¹	1.092 (0.944 - 1.264)
2	STAT4_RS1517352	191639709	A	C	0.425	0.396	9.34X10 ⁻²	1.13 (0.980 - 1.303)
2	STAT4_RS13017460	191640801	A	G	0.437	0.410	1.34X10 ⁻¹	1.115 (0.967 - 1.285)
2	STAT4_RS1400656	191643278	G	A	0.035	0.050	4.30X10 ⁻²	1.454 (1.01 - 2.092)
2	STAT4_RS10168266	191644049	T	C	0.226	0.196	4.15 x 10 ⁻⁵	1.196 (1.008 - 1.420)

2	STAT4_RS7594501	191646845	A	G	0.041	0.077	3.49X10 ⁻⁵	1.973 (1.422 - 2.737)
2	STAT4_RS2459611	191647432	C	T	0.116	0.108	4.60X10 ⁻¹	1.088 (0.870 - 1.361)
2	STAT4_RS16833238	191647744	T	A	0.109	0.099	3.33X10 ⁻¹	1.121 (0.890 - 1.411)
2	STAT4_RS16833239	191648505	A	G	0.041	0.077	6.32 X 10 ⁻⁵	1.932 (1.397 - 2.674)
2	STAT4_RS3024921	191651517	A	T	0.097	0.052	2.08 X 10 ⁻⁶	1.945 (1.488 - 2.543)
2	STAT4_RS11889341	191651987	T	C	0.277	0.229	1.98X10 ⁻³	1.288 (1.097 - 1.512)
2	STAT4_RS13010752	191654848	A	T	0.097	0.092	6.09X10 ⁻¹	1.064 (0.838 - 1.352)
2	STAT4_RS6434435	191662109	A	G	0.147	0.176	2.98X10 ⁻²	1.240 (1.021 - 1.505)
2	STAT4_RS10931480	191662292	G	A	0.153	0.187	1.23X10 ⁻²	1.275 (1.054 - 1.543)
2	STAT4_RS10931481	191663097	G	A	0.336	0.323	4.36X10 ⁻¹	1.061 (0.914 - 1.232)
2	STAT4_RS13011805	191664494	T	C	0.095	0.087	4.64X10 ⁻¹	1.096 (0.858 - 1.398)
2	STAT4_RS4274624	191666901	C	T	0.261	0.230	5.06X10 ⁻²	1.183 (1.000 - 1.399)
2	STAT4_RS7574865	191672878	T	G	0.272	0.222	1.21X10 ⁻³	1.309 (1.112 - 1.541)
2	STAT4_RS12463658	191673589	C	A	0.434	0.423	5.12X10 ⁻¹	1.049 (0.910 - 1.210)
2	STAT4_RS10181656	191678124	G	C	0.278	0.230	1.90X10 ⁻³	1.289 (1.098 - 1.514)
2	STAT4_RS10497711	191722166	G	A	0.049	0.069	2.11X10 ⁻²	1.437 (1.054 - 1.959)
3	IL12A_RS17810546	161147744	G	A	0.069	0.117	9.77X10 ⁻⁶	1.784 (1.376 - 2.312)
3	IL12A_RS9811792	161179692	C	T	0.416	0.424	6.30X10 ⁻¹	1.036 (0.898 - 1.195)
3	IL12A_RS16830960	161184588	G	A	0.051	0.029	1.51X10 ⁻³	1.766 (1.238 - 2.521)
3	IL12A_RS2243123	161192345	C	T	0.232	0.276	5.01X10 ⁻³	1.263 (1.073 - 1.488)
3	IL12A_RS583911	161193084	G	A	0.503	0.421	4.34X10 ⁻⁶	1.392 (1.208 - 1.603)
3	IL12A_RS2243133	161194967	T	C	0.045	0.028	9.49X10 ⁻³	1.662 (1.128 - 2.450)
3	IL12A_RS2243135	161195687	C	G	0.342	0.406	2.51X10 ⁻⁴	1.314 (1.135 - 1.521)
3	IL12A_RS568408	161196161	A	G	0.104	0.145	7.65X10 ⁻⁴	1.457 (1.169 - 1.816)
3	IL12A_RS668998	161198245	G	A	0.501	0.422	1.12X10 ⁻⁵	1.372 (1.191 - 1.581)
3	IL12A_RS2243154	161198936	A	G	0.108	0.081	8.25X10 ⁻³	1.375 (1.085 - 1.743)
3	IL12A_RS4608735	161199702	C	A	0.107	0.124	1.58X10 ⁻¹	1.177 (0.938 - 1.476)
3	IL12A_RS11927521	161199779	G	A	0.344	0.407	2.65X10 ⁻⁴	1.312 (1.134 - 1.519)
3	IL12A_RS17826053	161200323	G	T	0.104	0.145	7.91X10 ⁻⁴	1.459 (1.169 - 1.82)
3	IL12A_RS6441284	161200962	G	A	0.339	0.411	4.47X10 ⁻⁵	1.359 (1.173 - 1.575)
3	IL12A_RS485497	161201826	G	A	0.439	0.518	1.09X10 ⁻⁵	1.374 (1.192 - 1.583)
3	IL12A_RS4680536	161202965	G	A	0.340	0.421	4.08X10 ⁻⁶	1.410 (1.218 - 1.632)
3	IL12A_RS9852519	161203322	T	C	0.470	0.372	2.11X10 ⁻⁸	1.502 (1.302 - 1.732)
3	IL12A_RS598638	161203511	T	C	0.170	0.173	8.32X10 ⁻¹	1.021 (0.847 - 1.23)
3	IL12A_RS4679867	161206597	A	T	0.317	0.405	4.52X10 ⁻⁷	1.464 (1.262 - 1.699)
3	IL12A_RS4679868	161206848	A	G	0.494	0.387	1.58X10 ⁻⁹	1.546 (1.342 - 1.782)
3	IL12A_RS6441286	161211572	G	T	0.494	0.390	4.83X10 ⁻⁹	1.525 (1.324 - 1.757)
3	IL12A_RS574808	161215677	C	T	0.320	0.412	1.15X10 ⁻⁷	1.493 (1.287 - 1.733)
3	IL12A_RS589545	161216294	A	G	0.322	0.413	1.34X10 ⁻⁷	1.487 (1.283 - 1.724)
3	IL12A_RS2936298	161226577	A	G	0.170	0.193	9.97X10 ⁻²	1.167 (0.971 - 1.404)
3	IL12A_RS17217081	161229792	G	A	0.015	0.018	5.22X10 ⁻¹	1.205 (0.68 - 2.134)

Table S6 Legend: Association data are shown for 80 of 93 SNPs tested in the fine-mapping of Canada-based cases

and controls. Tests for association were carried out using PLINK. MAF corresponds to minor allele frequency. Data

for 9 *IL23R* region SNPs are not shown (all P>0.05).

Supplementary Table S7 *IL12A* Haplotype associations with PBC

Haplotype	Extended Canada-Based Collection			US-Based Collection			Combined Analysis			Odds Ratio (95% CI)
	Frequency Cases (N=621)	Frequency Controls (N=1279)	P	Frequency Cases (N=410)	Frequency Controls (N=310)	P	Frequency Cases (N=1031)	Frequency Controls (N=1589)	P	
TAGTG	0.491	0.305	4.82 x 10⁻²⁹	0.489	0.375	1.82 x 10⁻⁵	0.491	0.320	1.15 x 10⁻³⁴	2.01 (1.83-2.30)
AGTCA	0.309	0.318	0.569	0.326	0.424	2.00 x 10 ⁻⁴	0.317	0.340	0.0836	0.90 (0.80-1.01)
TGTTG	0.179	0.125	9.37 x 10 ⁻⁶	0.179	0.195	0.4378	0.179	0.141	3.00 x 10 ⁻⁴	1.33 (1.14-1.54)
Other haplotypes	0.021	0.252		0.006	0.006		0.013	0.199		

Table S7 Legend: *IL12A* haplotype analyses were performed in the extended Canada and US-based cases/control collections and a combined analysis then performed (Haploview v.4.1). The 5-locus haplotype analysis consisted in order of the rs4679867, rs4679868, rs6441286, rs574808 and rs589545 SNPs. Three haplotypes accounted for 80% of the overall haplotypes in controls. Haplotypes with an estimated frequency <20% in the control group were designated as ‘‘other haplotypes’’. The most significantly-associated haplotype is shown in bold.

Supplementary Table S8: Haplotype association results for *IL12RB2*

Haplotype	Frequency		P	Odds Ratio (95% CI)
	Cases (N=621)	Controls (N=1279)		
<i>10-Locus</i>				
TGTAGTCAGC	0.519	0.565	7.40 x 10 ⁻³	0.83 (0.72-0.95)
TTTAGTTAGC	0.129	0.158	0.020	0.79 (0.65-0.92)
GGCAACCCAT	0.192	0.119	2.72 x 10 ⁻⁹	1.75 (1.45-2.10)
GGTAGCCAAT	0.080	0.063	0.054	1.29 (1.00 -1.68)
GGCAATCAAT	0.018	0.040	3.00 x 10 ⁻⁴	0.43 (0.27-0.68)
GGCGATCAAT	0.042	0.021	4.00 x 10 ⁻⁴	1.98 (1.35-2.91)
<i>3-Locus</i>				
ACA	0.511	0.489	0.153	1.10 (0.96 – 1.26)
GTC	0.356	0.253	3.07 x 10⁻¹¹	1.53 (1.32 -1.77)
GTA	0.117	0.076	2.53 x 10 ⁻⁵	1.63 (1.30 -20.40)
GCA	0.009	0.092	6.02 x 10 ⁻²³	0.09 (0.05-0.161)
ATC	0.006	0.073	7.27 x 10 ⁻¹⁹	0.08 (0.04-0.162)

Table S8 Legend: Haplotype analysis for the *IL12RB2* gene was performed on the extended Canada-based collection using Haploview v.4.1. Odds ratios and 95% confidence intervals (CI) are shown. The 10-locus haplotype analysis includes (in order) SNPs rs1908632-rs790564-rs3790565-rs10489625-rs946685-rs6679356-rs10889684-rs10749775-rs3790567-rs6695348, and the 3-locus haplotype analysis includes (in order) SNPs rs4297265-rs2270614-rs2229546. The most significant haplotype association is shown in bold.

Supplementary Table S9. Replication and combined data analyses showing *STAT4* associations with PBC

Chr.	SNP ID (Location)	Physical Location	Minor/Major Alleles	US-Based Collection	Extended Canada-Based Collection	Combined Analysis	
				P	P	P	Odds Ratio (95% CI)
2	rs10168266 (Intron 5)	191644049	<u>T</u> /C	0.074	4.15 x 10 ⁻⁵	5.95 X 10 ⁻²	1.15 (0.99-1.32)
2	rs16833239 (Intron 4)	191648505	A/ <u>G</u>	0.482	6.32 x 10 ⁻⁵	3.69 X 10 ⁻⁴	1.53 (1.21-1.93)
2	rs3024921 (Intron 3)	191651517	<u>A</u> /T	0.013	2.08 x 10⁻⁶	5.76 X 10⁻⁸	1.81 (1.45-2.26)

Table S9 Legend: Association testing between SNPs in *STAT4* were carried out in the US-based collection and the extended Canada-based collection using PLINK. A combined analysis was performed and allelic odds ratios and the corresponding 95% confidence intervals (CI) were calculated. Genome positions are based on the NCBI database, build 36 (hg18). The risk allele for each SNP is underlined and the most significant data is bolded.

Supplementary Table S10. Stepwise selection/conditional analysis based on highest allelic associations identified as risk variants for PBC

Locus	SNP	Step	χ^2 (DF=2)	P
<i>IL12A</i>	rs6441286	1	24.0482	<0.001
	rs485497	2	10.247	0.006
	rs17810546	3	6.5781	0.0373
<i>IL12RB2</i>	rs10749775	1	28.2004	<0.001
	rs1908632	2	6.3627	0.0415
<i>STAT4</i>	rs3024921	1	24.8585	<0.001
	rs7574865	2	17.469	0.0002
	rs7594501	3	10.8064	0.0045

Table S10 Legend: Using stepwise forward selection, we demonstrate that multiple alleles across the loci *IL12A*, *IL12RB2* and *STAT4* are associated with PBC.

Supplementary Table S11: Population attributable risk for the *HLA*, *IL12A* and *IL12RB2* loci

SNP	Genotype			Average PAR		
<i>HLA</i> rs2856683		AA	CA	CC	Total	
	<i>Canada-based controls</i>	540	300	48	888	
	Frequency	0.451	0.251	0.040		
	OR	1	1.6	1.67		
	<i>US-based controls</i>	186	104	19	309	
	Frequency	0.155	0.087	0.016		
	OR	1	1.7985	2.41		0.212
<i>IL12A</i> rs6441286		GG	GT	TT		
	<i>Canada-based controls</i>	148	399	334	881	
	Frequency	0.124	0.335	0.281		
	OR	1.69	1.23	1		
	<i>US-based controls</i>	34	164	111	309	
	Frequency	0.029	0.138	0.093		
	OR	2.98	1.39	1		0.215
<i>IL12RB2</i> rs3790567		AA	AG	GG		
	<i>Canada-based controls</i>	53	329	509	891	
	Frequency	0.044	0.275	0.425		
	OR	2.67	1.35	1		
	<i>US-based controls</i>	22	107	178	307	
	Frequency	0.018	0.089	0.149		
	OR	1.53	1.51	1		0.184

Table S11 Legend: Population attributable risk fractions were calculated as described in the supplementary appendix, using control subject (US and Canadian) genotyping data for the three most significant variants identified.

PBC- Quantile-Quantile Plot

