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(54) Title: INTERLEUKIN-12 POLYMORPHISMS FOR IDENTIFYING RISK FOR PRIMARY BILIARY CIRRHOSIS

(57) Abstract: Primary biliary cirrhosis and related autoimmune disorders are characterized by polymorphisms in various loci including IL-12-related genes, and by reduced levels of IL-12A transcripts. Methods useful to screen for these defects are described for use in diagnosing these conditions. Methods useful to treat subjects afflicted with these conditions are also described.



INTERLEUKIN-12 POLYMORPHISMS FOR IDENTIFYING RISK FOR PRIMARY BILIARY CIRRHOSIS

Field of the Invention

This invention relates to diagnosis and treatment of liver disease. More particularly, the invention relates to detection of genetic markers of liver disease, and to the treatment of subjects presenting with such markers. Embodiments of the invention are applied particularly for diagnosis and treatment of primary biliary cirrhosis.

Background to the Invention

- 10** Primary biliary cirrhosis (PBC) is the most common autoimmune liver disease, affecting up to one in 1000 women over the age of 40 years ¹. Treatment for this chronic cholestatic condition remains limited and empiric ². The granulomatous destruction of interlobular bile ducts that characterizes PBC is almost always associated with anti-mitochondrial antibodies (AMA, specific for the E-2 subunit of the pyruvate dehydrogenase complex, PDC-E2) ³. Hepatic accumulation of autoreactive T lymphocytes in PBC, as well as data from animal models of autoimmune cholangitis, suggest an important role for T lymphocytes and particularly, CD4+ T helper subpopulations in PBC pathogenesis ⁴⁻⁶.
- 20** A genetic predisposition for PBC has been revealed by both family aggregation and twin concordance data. The monozygotic twin concordance rate in PBC is 60% ⁷ and the sibling relative risk is estimated to be 10.5 ⁸. The co-existence of other autoimmune diseases in patients and raised prevalence of such diseases in their families is also in keeping with an important genetic contribution to PBC ^{9,10}. Among the genes studied as PBC susceptibility candidates, only HLA has consistently shown an association with PBC, with the odds ratios associating PBC with *HLA-DRB1* *0801 in Caucasians estimated 2.4-3.3 and the derived population attributable fraction for HLA-DRB*08 ranging from 2.8 to 8.8%. ^{11,12} PBC has also been associated in some, but not all studies, with alleles in the gene encoding the cytotoxic T immunoregulatory lymphocyte antigen
- 30** 4 (CTLA-4) receptor. ¹³⁻¹⁵

Summary of the Invention

To explore the genetic basis of PBC, there was conducted a genomewide association (GWA) screen involving the analysis of 2072 (536 cases/1536 controls) North American Caucasian subjects. This GWA data together with replication and fine-mapping data confirm the association of PBC with *HLA* class II alleles and suggest important roles for the interleukin (IL) 12/IL12 receptor (IL12R) signaling axis in risk for PBC.

- 40** In one general aspect of the present invention, a subject presenting with, or at risk for, autoimmune disease including autoimmune liver disease and particularly including primary biliary cirrhosis (PBC) is identified by screening a biological sample obtained from the subject to identify at least one of the following characteristics:
- (a) at least one polymorphism within the gene for one or both of IL-12A and IL12-RB; and
 - (b) a level of IL-12A protein, IL-12A protein complex or IL-12A transcript that is reduced relative to a healthy individual,

the presence of either characteristic indicating the subject has, or is at risk for PBC.

In related aspects, the present invention provides kits comprising primers and instructions for the use thereof in genotyping the subject's DNA to reveal polymorphisms indicative of PBC.

10 In another general aspect of the present invention, there is provided a method useful to treat a subject presenting with, or at risk for, autoimmune disease including autoimmune liver disease such as PBC, the method comprising the step of administering to the subject a pharmaceutical composition comprising an agent effective to modulate, e.g., to stimulate or to inhibit, signaling via the IL-12/IL-12R pathway. In embodiments, the subject so treated is a subject that has tested positive for at least one polymorphism within a gene encoding one or both of IL-12A and IL-12RB, or for a reduced level of endogenous IL-12A protein or transcript.

These and other aspects of the present invention are now described with reference to the accompanying drawings in which:

Reference to the Figures

20 Figure 1 provides a flow chart demonstrating quality control and study design. Panel A depicts the genotyping quality control for SNPs and subjects whilst Panel B outlines the stages undertaken during the study from stage I GWA screening, through stage II replication analysis, to fine-mapping. SNP – single nucleotide polymorphism; HWE – Hardy Weinberg Equilibrium; MAF- Minor allele frequency;

30 Figure 2 illustrates genomewide association results, in which the y axis represents the level of significance for each single-nucleotide polymorphism ($\log_{10}$ -transformed P values; EIGENSTRAT) at the relative genomic position on each chromosome along the x axis. Chromosomes are shown in alternating colors. The dashed line shows the suggested GWA significance threshold.³⁹ The P values are after adjusting in EIGENSTRAT for the 10 eigenvectors having the highest eigenvalues;

40 Figure 3 depicts gene structures and LD blocks for *IL12A* (A) and *IL12RB2* (B). The top panels show the results of population-based genomewide, fine-mapping and combined association analyses. The results of chi-square analysis are presented and the $-\log_{10}$ transformation of P values are plotted, so that more significant P values appear higher. Middle panels show the organization of the human *IL12A* (A) and *IL12RB2* (B) genes and surrounding loci. Location of the genes is not drawn to exact scale. Bottom panels demonstrate the haplotype block structure, based on D² and r² values for 25 genotyped *IL12A* locus SNPs and 30 *IL12RB2* locus genotyped SNPs. Haplotype block structure was defined according to the criteria established by Gabriel et al.²⁰ using the pairwise estimates of standardized Lewontin's coefficient (D'), while the LD among pairs of SNPs was characterized using the square of the correlation coefficient (r²). The regions of high r² are shown in dark green and regions with lower r² are light grey, i.e. intensity decreases as r² values decrease;

Figure 4 is a reproduction of Table S1 herein, showing the complete association data for HLA region SNPs. Results are shown for SNPs in the HLA region yielding a stage I GWA significant association ($P < 1 \times 10^{-4}$) sorted according to EIGENSTRAT P values. Statistical tests for association were carried out with the use of EIGENSTRAT and PLINK (<http://pngu.mgh.harvard.edu/purcell/plink/>). Genome positions are based on the NCBI database build 36 (hg18);

10 Figure 5 is a reproduction of Table S2 herein, showing the complete associate for non-HLA region SNPs (GWA study). Results are shown for SNPs in non-HLA regions yielding a stage I GWA significant association ($P < 1 \times 10^{-4}$) sorted according to EIGENSTRAT P values. Statistical tests for association were carried out with the use of EIGENSTRAT and PLINK (<http://pngu.mgh.harvard.edu/purcell/plink/>). Genome positions are based on the NCBI database build 36 (hg18);

Figure 6 is a reproduction of Table 1 herein, showing results of the genomewide association study and stage II replication;

Figure 7 is a reproduction of Table 2 herein, showing fine mapping results from the IL-12A and IL-12RB2 loci, and

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Figure 8 is a histogram showing detection of IL-12A transcripts in human PBMs, with our without prior LPS stimulation, in subjects genotyped either with risk (TAGTG) or non-risk (AGTCA) haplotypes.

Detailed Description of the Invention

30 There has now been discovered an association between autoimmune disease and certain genetic polymorphisms present in one or more of the genes that produce proteins involved in the IL-12/IL-12R signaling axis. These genetic polymorphisms associate particularly with autoimmune liver disease, and especially with primary biliary cirrhosis (PBC). The particular genes harbouring these one or more polymorphisms are one or both of (1) the human IL-12A gene (UniProt reference P29459) that produces the interleukin-12 p35 protein (having UniProt reference P29459-1 and which, together with the IL-12 p40 protein also known as IL-12B, combine to form functional heterodimeric IL-12 protein), and (2) the human IL-12RB2 gene that produces the IL-12 receptor beta-2 protein, (having UniProt reference Q99665-1, which together with the IL-12RB1 protein combine to form functional IL-12 receptor).

40 In the case of the IL-12 genes, the one or more polymorphisms associated with PBC comprise the single nucleotide polymorphisms (SNPs) associated with the following SNP IDs:

For the IL-12A gene:

	<i>SNP ID</i>	<i>Location</i>	<i>Minor/Major</i>	<i>Physical Location</i>
			<i>Alleles</i>	
	rs17810546	5' flanking	G/ <u>A</u>	161147744
	rs583911	intron 2	<u>G</u> /A	161193084
	rs668998	3' flanking	<u>G</u> /A	161198245
	rs6441284	3' flanking	G/ <u>A</u>	161200962
	rs485497	3' flanking	<u>G</u> /A	161201826
	rs4680536	3' flanking	G/ <u>A</u>	161202965
10	rs9852519	3' flanking	T/ <u>C</u>	161203322
	rs4679867	3' flanking	A/ <u>T</u>	161206597
	rs4679868	3' flanking	<u>A</u> /G	161206848
	rs6441286	3' flanking	<u>G</u> /T	161211572
	rs574808	3' flanking	C/ <u>T</u>	161215677
	rs589545	3' flanking	A/ <u>G</u>	161216294

For the IL-12RB2 gene:

	<i>SNP ID</i>	<i>Location</i>	<i>Minor/Major</i>	<i>Physical Location</i>
			<i>Alleles</i>	
20	rs11209050	intron 3	<u>A</u> /C	67564324
	rs1908632	intron 8	<u>G</u> /T	67578394
	rs3790565	intron 8	<u>C</u> /T	67583944
	rs946685	intron 8	<u>A</u> /G	67588303
	rs6679356	intron 9	<u>C</u> /T	67592782
	rs10749775	intron 9	<u>C</u> /A	67594675
	rs3790567	intron 9	<u>A</u> /G	67594965
	rs6695348	intron 9	<u>T</u> /C	67599604

In the lists above, and elsewhere in this specification, the SNP ID refers to the identification number attached to a reference SNP cited and searchable in the single nucleotide polymorphism database provided by NCBI at <http://www.ncbi.nlm.nih.gov/SNP>. The "location" refers to a region within the subject gene, whereas the "physical location" refers to the location of the polymorphism within the whole of the human genomic sequence, as defined on the NCBI database, build 36 (hg18). The noted "minor/major alleles" identify the particular single nucleotide polymorphism, using underlining to identify the specific risk allele identified in subjects presenting with PBC, as revealed in greater detail in the examples herein.

Each of the alleles (polymorphisms) noted above is associated with PBC patients at a P value that is at least 10^{-6} . In embodiments of the present invention, the SNP targeted to identify subjects having, or at risk for, PBC is associated with PBC patients at a statistically more significant P value of at least 10^{-7} , preferably at least 10^{-8} and most preferably at least 10^{-9} . In this context, and according to preferred embodiments of the present invention, a subject presenting with PBC, or at risk therefor, is identified when the polymorphism revealed by analysis of the DNA thereof comprises at least one SNP selected from the group of IL-12A SNPs consisting of rs6441286 and rs574808 and the group of IL-12RB2 SNPs consisting of rs3790567 and rs3790565. In a particularly

preferred embodiment, the subject is so identified when the revealed polymorphism comprises the IL-12A SNP rs4679868 located in the 3'UTR and/or the IL-12RB2 SNP 6679356 located in intron 9.

It will be appreciated that the present method, useful in identifying subjects presenting with PBC, and those at risk therefor, is adapted to reveal at least one of the noted SNPs in the subject's DNA, and desirably is adapted to reveal two or more such SNPs in any combination, thereby to enhance the confidence with which the diagnosis is made, and to understand the potential severity with which the disease may affect the subject.

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In embodiments, the genotyping is applied to identify particular haplotypes or haplotype blocks, i.e. genetic sequences that incorporate at least two single nucleotide polymorphisms. In one embodiment, the method seeks to identify an IL-12A or IL-12RB2 haplotype, such as a haplotype including an IL-12A haplotype selected from the five locus haplotypes AGTCA (SEQ ID No.1), TGTTG (SEQ ID No. 2) and, preferably, TAGTG (SEQ ID No.3) (where the five loci consist in order of the rs4679867, rs4679868, rs6441286, rs574808 and rs589545 SNPs), and/or an IL-12RB2 haplotype that is either a 10-locus haplotype selected from TGTAGTCAGC (SEQ ID No.4), TTTAGTTAGC (SEQ ID No.5), GGCAACCCAT (SEQ ID No.6), GGTAGCCAAT (SEQ ID No.7), GGCAATCAAT (SEQ ID No.8), GCGATCAAT (SEQ ID No.9), (where the 10 loci consist in order of the rs1908632, rs790564, rs3790565, rs10489624, rs946685, rs6679356, rs10889684, rs10749775, rs3790567 and rs6695348 SNPs) or a 3 locus haplotype selected from ACA, GTC, GTA, GCA, ATC (where the 3 locus haplotypes consist of the ordered SNPs rs4297265, rs2270614 and rs2229546). In a preferred embodiment, the haplotype is a 3-locus haplotype, preferably a 3-locus IL-12RB2 haplotype, particularly including GTC.

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It should also be appreciated that detection of polymorphisms occurring within the IL-12 genes noted herein can be used alone or in combination with detection of polymorphisms within other genes for which PBC-related polymorphisms are identified. In particular embodiments, PBC diagnosis is made by detecting PBC-associated polymorphisms that occur either *per se* or also within the human leukocyte antigen (HLA) gene, the gene for signal transducer and activator of transcription 4 (STAT4, UniProt Q14765) and/or the gene for cytotoxic T-lymphocyte protein 4 (CTLA-4, UniProt P16410), as well as in one or more of the genes for other proteins noted herein as harbouring SNPs correlating with PBC, as set out in Tables 1 and 2 herein (see Figures 4 and 5, respectively).

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In embodiments, the HLA region-based polymorphisms that can usefully be detected to aid in PBC diagnosis are one or more of the following:

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<i>SNP ID</i>	<i>Location</i>	<i>Gene</i>	<i>Risk Allele</i>
Rs2856683	32763196	HLA-DQB1	C
Rs2395148	32429532	C6orf10	A
Rs9277535	33162839	HLA-DPB1	G
Rs3806156	32481676	BTNL-2	A

In a preferred embodiment, the polymorphism is the HLA-DQB1 risk allele C noted above, and haplotypes comprising this SNP.

In other embodiments, the STAT4 gene-based polymorphisms that can usefully be detected to aid in PBC diagnosis are one or more of the following:

10	<i>SNP ID</i>	<i>Location</i>	<i>Minor/Major</i>	<i>Physical Location</i>
			<i>Alleles</i>	
	Rs10168266	intron 5	<u>T</u> /C	191644049
	Rs16833239	intron 4	A/ <u>G</u>	191648505
	Rs3024921	intron 3	<u>A</u> /T	191651517

In other particular embodiments, PBC diagnosis is made by detecting PBC-associated polymorphisms that occur either *per se* or also, together with IL-12 gene-based polymorphism(s), within the 17q12-21 locus ($P = 3.50 \times 10^{-13}$), within the IRF5-TNPO3 risk locus ($P = 8.66 \times 10^{-13}$) and within the MMEL1 locus ($P = 2.28 \times 10^{-9}$).

20 Polymorphisms that correlate with PBC and lie within the 17q12-21 risk locus include the following. This locus includes four genes, i.e., zone pellucida-binding protein 2 (ZPBP2), the IKAROS family zinc finger 3 protein involved in leukocyte development and IgE production (IKZF3), Gasdermin-B (GSDMB) involved in epithelial barrier function, and ORM1-like protein 3, an uncharacterized protein (ORMDL3) belonging to a family of transmembrane proteins anchored in the endoplasmic reticulum. All eight SNPs across this region achieved significance in replication analysis:

SNP	Position	Gene	Risk Allele
rs907092	35175785	IKZF3	A
rs9303277	35229995	IKZF3	C
rs11557467	35282160	ZPBP2	G
rs8067378	35304874	GSDMB	A
rs2305480	35315722	GSDMB	T
rs2290400	35319766	GSDMB	A
rs7216389	35323475	GSDMB	T
rs4795405	35341943	ORMDL3	T

Of the 17q12-21 risk loci noted above, the strongest association signal came from the ZPBP2 SNP, rs11557467, which is thus a preferred marker for PBC diagnosis in this series.

30 Polymorphisms that correlate with PBC and lie within the MMEL1 locus, located on chromosome 1p36, include the following. This locus comprises the gene for membrane metallo-endopeptidase-like 1 (MMEL1).

SNP	Position	Gene	Risk Allele
rs3748816	2516606	MMEL1	C
rs3890745	2543484	MMEL1	G

Other polymorphisms that correlate with PBC lie within the *IRF5*-*TNPO3* locus. This locus comprises the genes for interferon regulatory factor 5-transportin 3).

SNP	Position	Risk Allele
rs35000415	128372852	T
<i>IRF5</i> _rs2070197	128376236	C
rs10488631	128381419	C
rs12539741	128384041	T

Among the associations identified, the strongest signals were provided by rs12539741 and rs2070197 alleles. These variants map just 3' to the *IRF5* coding region, are in tight linkage disequilibrium ($r^2 > 0.95$) with one another, and their associations with PBC reach P values of 1.65×10^{-10} (OR=1.63) and 3.74×10^{-10} (OR=1.62), respectively, in the combined cohort analysis. These polymorphisms are also among the SNPs at this locus highly associated with SLE and are both correlated with changes in *IRF5* expression in transformed B cells [4]. By contrast, an insertion-deletion polymorphism 64bp upstream of *IRF5* exon 1A representing a putative disease-causal variant for SLE, showed only modest association with PBC ($P=1.00 \times 10^{-3}$) and no associations were detected between PBC and another SLE-associated SNP, rs2004640, or a 3' UTR variant, rs10954213, identified as a key predictor of *IRF5* expression level [4]. Analyses also identified a number of haplotypes across the *IRF5*-*TNPO3* locus that were more strongly associated with PBC than any of the individual test SNPs. Among these, a two-allele haplotype involving the rs35000415 and rs12539741 SNPs showed the strongest association (omnibus $P=7.79 \times 10^{-31}$, OR=1.97 for the TT haplotype) with risk for PBC, as shown below:

Two SNP Haplotype

HAPLOTYPE	P	SNPs
TT	5.58×10^{-19}	rs35000415 rs12539741
TC	4.62×10^{-16}	rs35000415 rs12539741
CC	2.82×10^{-08}	rs35000415 rs12539741
OMNIBUS	7.79×10^{-31}	rs35000415 rs12539741

Three SNP Haplotype

HAPLOTYPE	P	SNPs
TCT	4.24×10^{-19}	rs35000415 <i>IRF5</i> _rs2070197 rs12539741
TTC	8.87×10^{-16}	rs35000415 <i>IRF5</i> _rs2070197 rs12539741
CTC	2.85×10^{-08}	rs35000415 <i>IRF5</i> _rs2070197 rs12539741
OMNIBUS	1.13×10^{-30}	rs35000415 <i>IRF5</i> _rs2070197 rs12539741

Thus, in further embodiments, the genotyping is applied to identify particular haplotypes or haplotype blocks, i.e. genetic sequences that incorporate at least two single nucleotide polymorphisms particularly in the *IRF5*-*TNPO3* locus, and particularly the two SNP

haplotype shown *supra*. Such haplotypes further include the 3-locus risk allele haplotypes within the IRF5-TNPO3 also shown *supra*.

In summary, with respect to PBC risk alleles, the present invention identifies numerous loci associated with this condition, and permits diagnosis of the condition through genotyping based on any one, two, three, four, five, six, seven, eight, nine, ten or more of the following risk alleles, and any combinations thereof, including combinations that consist only of a selection from among the alleles noted below, and combinations that comprise at least an IL-12A risk allele, or an IL-12RB risk allele, and any combination of one or more other risk alleles noted below:

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<i>SNP ID</i>	<i>Allele</i>	<i>Physical Location</i>	<i>Gene</i>
rs17810546	A	161147744	IL-12A
rs583911	G	161193084	IL-12A
rs668998	G	161198245	IL-12A
rs6441284	A	161200962	IL-12A
rs485497	G	161201826	IL-12A
rs4680536	A	161202965	IL-12A
rs9852519	C	161203322	IL-12A
rs4679867	T	161206597	IL-12A
rs4679868	A	161206848	IL-12A
rs6441286	G	161211572	IL-12A
rs574808	T	161215677	IL-12A
rs589545	G	161216294	IL-12A
rs11209050	A	67564324	IL-12RB2
rs1908632	G	67578394	IL-12RB2
rs3790565	C	67583944	IL-12RB2
rs946685	A	67588303	IL-12RB2
rs6679356	C	67592782	IL-12RB2
rs10749775	C	67594675	IL-12RB2
rs3790567	A	67594965	IL-12RB2
rs6695348	T	67599604	IL-12RB2
rs2856683	C	32763196	HLA-DQB1
rs2395148	A	32429532	C6orf10
rs9277535	G	33162839	HLA-DPB1
rs3806156	A	32481676	BTNL-2
rs10168266	T	191644049	STAT4
rs16833239	G	191648505	STAT4
rs3024921	A	191651517	STAT4
rs907092	A	35175785	IKZF3
rs9303277	C	35229995	IKZF3
rs11557467	G	35282160	ZPBP2
rs8067378	A	35304874	GSDMB
rs2305480	T	35315722	GSDMB
rs2290400	A	35319766	GSDMB
rs7216389	T	35323475	GSDMB
rs4795405	T	35341943	ORMDL3
rs3748816	C	2516606	MMEL1
rs3890745	G	2543484	MMEL1
rs35000415	T	128372852	IRF5-TNPO3
rs2070197	C	128376236	IRF5
rs10488631	C	128381419	IRF5-TNPO3
rs12539741	T	128384041	IRF5-TNPO3

It will thus be appreciated that the present invention relates to a method useful to identify subjects presenting with, or at risk for, autoimmune disease including PBC which comprises the step of genotyping DNA from the subject to identify one or more of the polymorphisms herein identified, wherein the presence thereof indicates the disease or that the subject is at risk for the disease. The ultimate diagnosis of the disease will of course consider other disease hallmarks such as clinical symptoms characteristic of the disease when manifest, knowledge of hereditary factors, histological examinations, the presence of protein markers, and the like. In the case of PBC, detection of one or more polymorphisms herein identified can be used to confirm a clinical diagnosis which is

10 based on the appearance in the subject of signs of altered liver function including such symptoms as the presence in blood of markers of deranged liver function, including anti-mitochondrial antibodies as well as antibodies to such nuclear proteins as glycoprotein 210 and p62. Patients suffering from PBC may also present with fatigue, itchy skin, jaundice resulting from bilirubin elevation, local collections of cholesterol in the skin and around the eyes (xanthoma) as well as symptoms generally associated with cirrhosis and portal hypertension.

Patients having one or more of these symptoms, and patients otherwise suspected for whatever medical reason of having or being at risk for PBC, are genotyped in accordance

20 with a method of the present invention to identify one or more of the polymorphisms described herein, as an aid or adjunct to diagnosing this disease.

The genotyping process is generally well established in the art. It entails an examination of the sequence or other physical properties of genomic DNA extracted from the subject to identify either the specific polymorphisms herein identified or the hallmarks thereof. Genotyping can be established by extracting DNA or RNA from a patient sample such as blood, tissue biopsy, and the like, and then analyzing the DNA by any one or more methods including hybridization-based techniques, enzyme-based methods, and other post-amplification methods based on physical properties of DNA.

Useful hybridization-based methods make use of DNA probes that are complementary in sequence to, and hence hybridize with, a target DNA region comprising a SNP of interest. Such methods include dynamic allele-specific hybridization, which takes advantage of the differences in the melting temperature in DNA that results from the instability of mismatched pairs. In this method, a segment of target DNA is amplified and attached to a bead through a PCR reaction with a biotinylated primer. The amplified product is then attached to a streptavidin column and washed to remove the unbiotinylated strand. An allele/SNP-specific oligonucleotide probe is then added in the presence of a molecule that fluoresces when bound to double stranded DNA. The

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40 intensity is then measured as temperature is increased, until the melting point can be determined. A SNP will result in a lower than expected melting point.

In another hybridization-based approach, oligonucleotide SNP arrays are used, in which probes are arrayed on a small chip. Several redundant probes are used to interrogate each SNP, to deal with mismatched hybridization, the probes being designed to have the SNP site in several different locations as well as containing mismatches to the SNP allele. By comparing the differential amount of hybridization of target DNA to each of these

redundant probes, it is possible to determine specific homozygous and heterozygous alleles. The Affymetrix Human SNP 5.0 GeneChip® system is particularly useful for this purpose.

10 Still other hybridization-based techniques are based on specifically engineered single stranded oligonucleotide probes which have complementary regions at each of end of a central probe sequence. One end of the probe is labeled with a fluorophor, and the other end is labeled with a fluorophor quencher. The probe takes on a hairpin structure in its isolated state, and accordingly does not fluoresce, unless and until the target sequence is encountered by and hybridized to the probe. This elegant design allows detection of a single SNP in a DNA sample.

20 Enzyme-based genotyping techniques include those based on restriction fragment length polymorphism (RFLP), which makes use of the many different endonucleases and their high affinity to unique and specific restriction sites within target DNA. In this approach, target DNA is subjected to a particular one or more endonucleases, and the resulting fragment pattern is compared against a control sample lacking the SNP of interest. A difference in gel-resolved fragments between the target DNA and control indicates that the target DNA incorporates an alteration, such as a SNP, that renders it either vulnerable or resistant to digestion. In a variation of this technique, a particular region of the target DNA being queried for the presence of a given SNP can be amplified, and the amplified DNA can then be digested, to reduce the complexity of the result and point more directly to the SNP of interest as being responsible for the difference in endonuclease-mediated fragmentation.

30 Still other enzyme-based genotyping methods include polymerase chain reaction (PCR) based methods, such as tetra primer ARMS-PCR, in which two pairs of primers are used to amplify two alleles in one PCR reaction. The primers are designed such that the two primer pairs overlap at a SNP location, but each match perfectly to only one of the possible SNPs. As a result, if a given allele is present in the PCR reaction, the primer pair specific to that allele will produce product but not to the alternative allele with a different SNP. The two primer products are designed so that their PCR products have lengths sufficiently different to allow their discrimination by gel electrophoresis.

40 Other methods use flap endonuclease (FEN), which catalyzes structure-specific cleavage that is highly sensitive to mismatches and can be used to interrogate SNPs with a high degree of specificity. In the basic assay, a FEN called cleavase is combined with two specific oligonucleotide probes that together with the target DNA form a tripartite structure recognized by cleavase. The first probe is complementary to the 3' end of the target DNA, and has a final base that is a non-matching base that overlaps the SNP nucleotide. The second probe is an allele-specific probe which is complementary to the 5' end of the target DNA, but also extends past the 3' side of the SNP nucleotide. The allele-specific probe will contain a base complementary to the SNP nucleotide. If the target DNA contains the desired allele, the probes will bind to the target DNA forming the tripartite structure which can then be cleaved by cleavase. If the SNP nucleotide in the target DNA is not complementary with the allele-specific probe, the correct structure

does not form and no cleavage occurs. The assay is usually coupled with a FRET system to detect the cleavage event.

The primer extension approach can also be used in genotyping. This is a two step reaction in which a probe that hybridizes upstream of a SNP nucleotide is used followed by a mini-sequencing reaction in which DNA polymerase extends the hybridized primer by adding a base complementary to the SNP nucleotide. This incorporated base is detected and determines the SNP allele. Various detection methods including mass spectrometry (e.g., MALDI-TOF) and ELISA-like formats are employed. Illumina Inc.'s Infinium® assay is an example of a whole genome genotyping pipeline that is based on primer extension, and allows for genotyping of over 100,000 SNPs. The assay uses hapten-labeled nucleotides in a primer extension reaction, wherein the hapten label is detected using antibodies which in turn are coupled to a detectable signal.

One particularly suitable approach entails PCR-based amplification of a DNA region in which a suspected SNP is located, using suitable forward and reverse primers to generate an amplicon having a size that is conveniently small to allow direct sequencing of the amplicon, and then comparison of the determined sequence against a wild type sequence, so that the presence of the risk allele can be revealed.

It will be appreciated that a wide variety of techniques thus are available for use in genotyping DNA from a subject to be diagnosed for PBC based on the SNPs and haplotypes herein described. In particular embodiments of the present invention, the DNA of a given subject can be interrogated to identify one or more of the present SNPs by implementing PCR-based sequencing methods. The IL-12 SNP sequences exemplified herein were all extracted from the NCBI SNP database, and annotation of each SNP position in the human genome is based on the March 2006 human reference sequence (NCBI Build 36.1) produced by the International Human Genome Sequencing Consortium. SNP genotyping is performed for instance using the Sequenom MassARRAY platform using the iPLEX method. SNP assay design can be performed with the Sequenom Assay Design v3.1, and eXTEND available at MySequenom.com under Tools. For each target SNP analysis, the proximal variation (ProxSNP) can be masked to ensure that PCR and extension primers can bind, amplify and extend a unique region. For this purpose, the table below provides primers that are suitable for producing amplicons that incorporate IL-12 SNPs of particular interest (the risk alleles), the specific presence of which can then be determined by amplicon sequencing according to well established methods.

In embodiments, genomic DNA is extracted from whole blood using standard phenol/chloroform extraction procedures and resuspended in 1X TE buffer (pH 8.0).

PCR amplification is carried out using 5-10 ng of template DNA, in 5 ul reactions containing 1.25X PCR Buffer (Qiagen), 1.625 mM MgCl₂ (Qiagen), 500 uM dNTP mix (Fermentas), 100 nM primer mix (IDT) and 0.5 U Hotstar Taq polymerase (Qiagen). The reactions are incubated in a standard thermocycler using the following cycling conditions: initial denaturation at 94°C for 15 minutes, followed by 45 cycles of 94°C

for 20 seconds, 56°C for 30 seconds, 72°C for 1 minute, followed by a final extension at 72°C for 3 minutes.

Unincorporated dNTPs are dephosphorylated by treatment with shrimp alkaline phosphatase following PCR. The 5 ul PCR reaction is incubated with 2 ul of SAP mix, containing 0.85X SAP buffer and 0.3 U SAP enzyme (Sequenom), in a standard thermocycler at 37°C for 20 minutes, followed by a 5 minute heat inactivation at 85°C.

10 For analysis on the Sequenom iPLEX system, for instance, 2 ul of iPLEX extension cocktail are added to the PCR reaction to a final concentration of 0.222X iPLEX buffer, 1X iPLEX termination mix, 0.625 uM, 0.833 uM, 1.04 uM or 1.25 uM of each primer, and 1X iPLEX enzyme (Sequenom). The primer concentrations in the multiplex reactions are adjusted based on the primer mass, with higher concentrations (1.04 uM or 1.25 uM) used for high mass primers. The reaction conditions for primer extension are as follows: initial denaturation at 94°C for 30 seconds, followed by a 40 cycle program consisting of a single denaturation at 94°C for 5 seconds and 5 cycles of 52°C for 5 seconds and 80°C for 5 seconds. A final extension is performed at 72°C for 3 minutes.

20 To remove salts from the iPLEX reaction products, the samples are diluted with 16 ul of water and 6 mg of Clean Resin (Sequenom) was added to each reaction. The reactions are rotated for at least 10 minutes, followed by centrifugation at 5000 rpm for 5 minutes. The reaction products are dispensed onto a 384-element SpectroCHIP bioarray (Sequenom) using the Sequenom RS-1000 MassARRAY Nanodispenser and analyzed using the Sequenom MassARRAY Analyzer Compact.

30 In another of its aspects, the present invention provides a kit useful in the diagnosis of primary biliary cirrhosis (PBC), the kit comprising nucleic acid primers useful to amplify DNA obtained from a subject having or suspected of being at risk for PBC, wherein the primers have a nucleic acid sequence adapted to amplify a region of said DNA comprising at least one PBC risk allele as defined herein. The primers typically are in the form of DNA oligonucleotides, and prime the region comprising the SNP/risk allele by hybridizing therewith to produce an amplicon that is then sequenced to reveal the presence of the SNP. The primers can be packaged in the kit within sealed vials comprising the DNA primers in lyophilized or other suitable form. The kit further comprises instructions for the use of such primers in detecting risk alleles associated with PBC. Primers suitable for amplifying patient DNA to reveal IL-12 SNPs associated with PBC are set out below:

40	SNP ID	IL-12A	rs17810546
	Genome Position	161147744	
	2 nd PCR Primer	ACGTTGGATGCAATGAGGTTTTCTGTATGGC	(SEQ ID No.10)
	1 st PCR Primer	ACGTTGGATGCAGATCAAGAACCCAACAAG	(SEQ ID No.11)
	Amplicon length	99	
	Extension Primer	GTATGGCTTTCTGTGGTA	(SEQ ID No.12)
	Ext1 call/Ext2 call	G/A	

	SNP ID	IL-12A	rs4679867
	Genome Position	161206597	
	2 nd PCR Primer	ACGTTGGATGGAAGTTCTGGCAATTCAGTC	(SEQ ID No.13)
	1 st PCR Primer	ACGTTGGATGCACAGGTTAAGGGCTCAGTC	(SEQ ID No.14)
	Amplicon length	97	
	Extension Primer	TTCTGGCAATTCAGTCTTATAAC	(SEQ ID No.15)
	Ext1 call/Ext2 call	A/T	
10	SNP ID	IL-12A	rs4680536
	Genome Position	161202965	
	2 nd PCR Primer	ACGTTGGATGGGTCCTGTTTTAAAGCATCC	(SEQ ID No.16)
	1 st PCR Primer	ACGTTGGATGCCAACTATTGCCAAAGAGCC	(SEQ ID No.17)
	Amplicon length	83	
	Extension Primer	AGCATCCTATTCCTTGG	(SEQ ID No.18)
	Ext1 call/Ext2 call	G/A	
20	SNP ID	IL-12A	rs9852519
	Genome Position	161203322	
	2 nd PCR Primer	ACGTTGGATGGCATTCTTCCCCATGAAAAG	(SEQ ID No.19)
	1 st PCR Primer	ACGTTGGATGCCTGGAGAAGTACAACTGGC	(SEQ ID No.20)
	Amplicon length	101	
	Extension Primer	CCCCATGAAAAGTAAATAATTCCAC	(SEQ ID No.21)
	Ext1 call/Ext2 call	C/T	
30	SNP ID	IL-12A	rs4679868
	Genome Position	161206848	
	2 nd PCR Primer	ACGTTGGATGTTTATGGGGCCAATGATTTG	(SEQ ID No.22)
	1 st PCR Primer	ACGTTGGATGGATTTTTGCACTCTATCTCC	(SEQ ID No.23)
	Amplicon length	95	
	Extension Primer	TGAATTGGTAGACATATATGCC	(SEQ ID No.24)
	Ext1 call/Ext2 call	G/A	
40	SNP ID	IL-12A	rs485497
	Genome Position	161201826	
	2 nd PCR Primer	ACGTTGGATGGATTTTCGAGGGCTTACCGAG	(SEQ ID No.25)
	1 st PCR Primer	ACGTTGGATGCTGATCTGCTGGTTTCCAAG	(SEQ ID No.26)
	Amplicon length	99	
	Extension Primer	TCTGACTTGCTCCTGG	(SEQ ID No.27)
	Ext1 call/Ext2 call	G/A	
	SNP ID	IL-12A	rs574808
	Genome Position	161215677	
	2 nd PCR Primer	ACGTTGGATGTCAACTGCTGTCAATCCCAC	(SEQ ID No.28)
	1 st PCR Primer	ACGTTGGATGGAAAGTAGCAAAGTAGACAG	(SEQ ID No.29)
	Amplicon length	100	
	Extension Primer	gCAGAAAGTGGGATTTGTAG	(SEQ ID No.30)
	Ext1 call/Ext2 call	C/T	

	SNP ID	IL-12A	rs583911
	Genome Position	161193084	
	2 nd PCR Primer	ACGTTGGATGAGCTTGTCTTAAGGGTTTGC	(SEQ ID No.31)
	1 st PCR Primer	ACGTTGGATGCAAGTATAACTTCTAAAGGG	(SEQ ID No.32)
	Amplicon length	100	
	Extension Primer	TTTGCATGTTTGTATATCCATCA	(SEQ ID No.33)
	Ext1 call/Ext2 call	A/G	
10	SNP ID	IL-12A	rs589545
	Genome Position	161216294	
	2 nd PCR Primer	ACGTTGGATGTCAAATAAGATAGGCCTCCC	(SEQ ID No.34)
	1 st PCR Primer	ACGTTGGATGACTGCGCCTGACTCCAATTT	(SEQ ID No.35)
	Amplicon length	97	
	Extension Primer	TAACTAATTTTCAGAGCAATTTTT	(SEQ ID No.36)
	Ext1 call/Ext2 call	G/A	
20	SNP ID	IL-12A	rs6441284
	Genome Position	161200962	
	2 nd PCR Primer	ACGTTGGATGCTGTCTAAGTCAAGGGAATG	(SEQ ID No.37)
	1 st PCR Primer	ACGTTGGATGAAGAGAGAGAAGGGAGATAC	(SEQ ID No.38)
	Amplicon length	100	
	Extension Primer	GAATGCTTTCCCTATAACC	(SEQ ID No.39)
	Ext1 call/Ext2 call	A/G	
30	SNP ID	IL-12A	rs6441286
	Genome Position	161211572	
	2 nd PCR Primer	ACGTTGGATGGGAGGTGTATCTGGTGTTTC	(SEQ ID No.40)
	1 st PCR Primer	ACGTTGGATGGAGAAGCTGGAGAGAGCTA	(SEQ ID No.41)
	Amplicon length	99	
	Extension Primer	CTCCCAGAGGAGGACCTCA	(SEQ ID No.42)
	Ext1 call/Ext2 call	G/T	
40	SNP ID	IL-12A	rs668998
	Genome Position	161198245	
	2 nd PCR Primer	ACGTTGGATGGGGCAAGAATTTGAAAAACG	(SEQ ID No.43)
	1 st PCR Primer	ACGTTGGATGTCTTTCTCCCTGTAAATGTG	(SEQ ID No.44)
	Amplicon length	99	
	Extension Primer	AATTTGAAAAACGAGAATGTTTG	(SEQ ID No.45)
	Ext1 call/Ext2 call	G/A	
	SNP ID	IL-12RB2	rs10749775
	Genome Position	67594675	
	2 nd PCR Primer	ACGTTGGATGGATGGGGTAGGAAATTCTGG	(SEQ ID No.46)
	1 st PCR Primer	ACGTTGGATGCTTCCTCCCCAGTGAGGTTC	(SEQ ID No.47)
	Amplicon length	94	
	Extension Primer	CAGAGTCACTGTCAAGG	(SEQ ID No.48)
	Ext1 call/Ext2 call	C/A	

	SNP ID	IL-12RB2	rs1908632
	Genome Position	67578394	
	2 nd PCR Primer	ACGTTGGATGGATCATTGCACACTTCTTCAG	(SEQ ID No.49)
	1 st PCR Primer	ACGTTGGATGTGTGTGTGTGAGAGAGACAG	(SEQ ID No.50)
	Amplicon length	98	
	Extension Primer	ACTTCTTCAGTTTTTAAGAAATGAGT	(SEQ ID No.51)
	Ext1 call/Ext2 call	G/T	
10	SNP ID	IL-12RB2	rs6679356
	Genome Position	67592782	
	2 nd PCR Primer	ACGTTGGATGAGCTCGGCTCTGAAGAATTG	(SEQ ID No.52)
	1 st PCR Primer	ACGTTGGATGGCTAAGAATGAGGTTGATGC	(SEQ ID No.53)
	Amplicon length	95	
	Extension Primer	CTTCTTCCCTCCTGAC	(SEQ ID No.54)
	Ext1 call/Ext2 call	T/C	
20	SNP ID	IL-12RB2	rs6695348
	Genome Position	67599604	
	2 nd PCR Primer	ACGTTGGATGAGCAAGCATCAGAACCATAC	(SEQ ID No.55)
	1 st PCR Primer	ACGTTGGATGATTCCTGGTCTGATAATTCC	(SEQ ID No.56)
	Amplicon length	80	
	Extension Primer	ctTCAGAACCATACTTGGATA	(SEQ ID No.57)
	Ext1 call/Ext2 call	C/T	
30	SNP ID	IL-12RB2	rs946685
	Genome Position	67588303	
	2 nd PCR Primer	ACGTTGGATGCATTTGCTGACTACAGTGTG	(SEQ ID No.58)
	1 st PCR Primer	ACGTTGGATGTTTCTTGCAAGGCTGTCCC	(SEQ ID No.59)
	Amplicon length	100	
	Extension Primer	ccccTGTGTCTTTCAGCAAAC	(SEQ ID No.60)
	Ext1 call/Ext2 call	A/G	
40	SNP ID	IL-12RB2	rs11209050
	Genome Position	67564324	
	2 nd PCR Primer	ACGTTGGATGTTCGTGGGAGGTACTCAATGC	(SEQ ID No.61)
	1 st PCR Primer	ACGTTGGATGTGCATTGTCTCTTGGAGCAC	(SEQ ID No.62)
	Amplicon length	99	
	Extension Primer	cACTCAATGCCTATGTACAC	(SEQ ID No.63)
	Ext1 call/Ext2 call	C/A	
	SNP ID	IL-12RB2	rs3790567
	Genome Position	67594965	
	2 nd PCR Primer	ACGTTGGATGACAGCTATAGCTCATGGTCC	(SEQ ID No.64)
	1 st PCR Primer	ACGTTGGATGTCTGGCCTGGAGCTTTCCAA	(SEQ ID No.65)
	Amplicon length	98	
	Extension Primer	CTCATGGTCCTCCATGTATG	(SEQ ID No.66)
	Ext1 call/Ext2 call	G/A	

- SNP ID IL-12RB2 rs3790565
 Genome Position 67583944
 2nd PCR Primer ACGTTGGATGCAGCAGATGGCACAATTTAG (SEQ ID No.67)
 1st PCR Primer ACGTTGGATGCTGTGATGGGCCAGGATTG (SEQ ID No.68)
 Amplicon length 98
 Extension Primer ATGGCACAATTTAGATTGTTT (SEQ ID No.69)
 Ext1 call/Ext2 call C/T
- 10** SNP ID IRF5 rs10488631
 PCR Primer 1: ACGTTGGATGATTCACTGCCTTGTAGCTCG (SEQ ID No. 70)
 PCR primer 2: ACGTTGGATGGTCTATCAGGTACCAAAGGC (SEQ ID No. 71)
 Extension Primer: TGTAGCTCGGAAATGGTTC (SEQ ID No. 72)
 Alleles: T/C
- SNP ID IRF5 rs35000415
 PCR Primer 1: ACGTTGGATGGGCACTTCCAGATTCAAACC (SEQ ID No. 73)
 PCR primer 2: ACGTTGGATGGTGTGATCTCTAAGGAGGAC (SEQ ID No. 74)
 Extension Primer: GGGTGCTCACCTTATCC (SEQ ID No. 75)
 Alleles: T/C
- 20** SNP ID IRF5 rs12539741
 PCR Primer 1: ACGTTGGATGTCAATTCATACCTCCTGCCC (SEQ ID No. 76)
 PCR primer 2: ACGTTGGATGCACTTTTATGACCTGAATGC (SEQ ID No. 77)
 Extension Primer: CCTGCCCTGGTCAAAA (SEQ ID No. 78)
 Alleles: T/C
- SNP ID MMEL1 rs3748816
 PCR Primer 1: ACGTTGGATGTTGGAGTACTCCTCGTCCAG (SEQ ID No. 79)
 PCR primer 2: ACGTTGGATGATGAGCATCCGGGAGCAGA (SEQ ID No. 80)
30 Extension Primer: CCTCGTCCAGGCGCCTGTTC (SEQ ID No. 81)
 Alleles: T/C
- SNP ID MMEL1 rs3890745
 PCR Primer 1: ACGTTGGATGGCATT TTTGTGACTGTAGCCC (SEQ ID No. 82)
 PCR primer 2: ACGTTGGATGGGAATCACCTGGGGAAATTG (SEQ ID No. 83)
 Extension Primer: CTGAGGGAGGGCCCAA (SEQ ID No. 84)
 Alleles: A/G
- 40** SNP ID Chr17 region rs11557467
 PCR Primer 1: ACGTTGGATGGATGTATTCTTTAGAGTGCTG (SEQ ID No. 85)
 PCR primer 2: ACGTTGGATGTGATGGCTCTATGACATGGC (SEQ ID No. 86)
 Extension Primer: GCTGAAGAAAATCTTGATA (SEQ ID No. 87)
 Alleles: G/T

A subject that presents with one or more of the polymorphisms noted herein is a subject that is at risk for PBC or, if other PBC signs and symptoms are manifest, is a subject presenting with PBC.

- 50** In other aspects, the present invention provides for the detection of IL-12A protein in a patient sample as an additional or alternative way to identify subjects presenting with or

at risk for PBC and related autoimmune disorders. Such subjects present with reduced levels of endogenous IL-12A protein and/or transcript, relative to normal subjects. Detection of endogenous IL-12A can be achieved using any of the methods established for protein species detection. This typically can utilize an antibody or other agent that binds selectively and specifically to the target protein. Antibodies that bind IL-12A selectively are available commercially. These antibodies can be used in any useful assay format, including radioimmunoassay (RIA), or an enzyme immunoassay (EIA) or enzyme-linked immunosorbent assay (ELISA) format or with the aid of flow cytometry, to capture and detect IL-12A and IL-12A complexes (such as IL-12 and IL-35) present in a patient sample. Measurement of IL-12A levels can then be achieved by quantifying the bound IL-12A, using for instance an agent that is labeled for detection.

Presently, there is no known cure for PBC but medication may slow the progression of the disease so that a normal lifespan may be attainable. Specific treatment for fatigue is unavailable, although ursodoxycholic acid is frequently used in treatment, to reduce cholestasis and to improve liver function and blood test results. Cholestyramine may also be prescribed to absorb bile acids. Multivitamins, including vitamin D and calcium, are also recommended as patients with PBC have poor lipid-dependent vitamin absorption. In advanced cases of PBC, liver transplant is indicated and can result in favourable prognosis.

The genotyping results presented herein, showing a clear and significant association between PBC and polymorphism in IL-12-related genes, suggest that PBC subjects can benefit from IL-12A-based therapy. It is expected in particular that these subjects will benefit from administration of agents that stimulate the IL-12/IL-12R signaling axis. As will be appreciated from the genotyping data, PBC subjects exhibit polymorphisms not only within the IL-12A gene, but also in the IL-12RB2 gene. The polymorphisms exist in gene regions that are unlikely to affect the sequences of the transcripts and proteins expressed from these genes. Rather, the polymorphisms reside in 5', 3' and intronic sequences suggesting that these patients will have difficulty with the levels at which they express the required proteins, relating to reduced or dysfunctional transcript production and other phenomena unrelated to proper protein function.

More particularly, further evidence, shown herein, indicates that the level of IL-12A transcripts in human peripheral blood mononuclear (PBM) cells is reduced in healthy individuals homozygous for the 5 locus PBC risk haplotype ((TAGTG) compared with healthy individuals homozygous for the non-risk haplotype (AGTCA). The applications of other techniques useful to assess the relative levels of IL-12A transcripts and/or translated protein in biological samples obtained from individuals thus form valuable additional aspects of the invention.

In one aspect, there is provided a method for identifying a subject having or at risk for PBC, or for an autoimmune condition related thereto such as an autoimmune liver disease, comprising measuring the level of an IL-12A RNA transcript, an expression product thereof or IL-12A complex, such as IL-12 and/or IL-35, in a biological sample obtained from that subject, and comparing the measured level with a corresponding

reference level for a healthy control subject, wherein a reduction in the measured level relative to the reference level indicates autoimmune disease, including PBC.

10 Assay methods useful to determine the presence or amount of the IL-12A-based analyte can be applied to biological samples that include tissues, cells and extracts thereof including RNA and protein. The biological sample can thus be blood, including whole blood, plasma and serum, as well as saliva, urine, cerebrospinal fluid, cells particularly including white cells such as leukocytes and lymphocytes, peripheral blood mononuclear cells generally including antigen presenting cells and monocytes, tissues including particularly spleen, marrow, and the like. The biological sample can also be extracted from such fluids, cells and tissues, particularly including protein and RNA components thereof.

20 When applied to detect IL-12A in tissue samples, the assay can be based on immunohistochemistry techniques established in the art, in which an IL-12A antibody or any other agent that binds selectively to IL-12A is used essentially to target a conjugated stain or label to the site at which IL-12A is localized on the tissue. The intensity with which the tissue is stained or labeled by the binding agent is then compared, either visually or preferably with instrumentation, against control tissue, and a reduction in the label intensity compared to the control tissue reveals tissue that produces a reduced level of IL-12A, and indicates the subject providing the tissue has or is at risk for PBC.

30 Similarly, agents that bind selectively to IL-12A can be used to assay the presence or amount of IL-12A protein that is present either in cell-bound or soluble form. The practice of flow cytometry can be applied to detect and measure the amount of IL-12A protein in solution or on the surface of cells. Similarly, the presence of soluble IL-12A protein can be detected using the binding agent and well established assay platforms and formats, such as the ELISA, RIA, and EIA formats including sandwich formats, or any other assay format, suitably one that is automated and adapted to quantify the amount of IL-12A detected by the assay.

A most useful reagent for use in these assays is an antibody that binds specifically to IL-12A, including antibodies that bind specifically to IL-12A when complexed to form IL-12 and IL-35. Such antibodies reveal the presence of free and complexed IL-12A, and are desirable for that reason. A variety of such antibodies are available commercially as reagents for this purpose.

40 The IL-12A specific antibodies can be provided as immunoconjugates in which the antibody is complexed with a detectable label, such as an enzyme label including peroxidase, a FITC label, gold sol, and the like to provide a means for revealing the presence and quantity of antibody that has accumulated at the desired target. In the alternative, the antibody without a label can be revealed using a labeled secondary antibody that binds to the IL-12A primary antibody.

The presence and amount of IL-12A present in a biological sample can also be determined using an assay for IL-12A transcripts. By this method, RNA extracted from

target cells, particularly liver cells or circulating immune cells including lymphocytes and leukocytes, is assayed to identify or quantify the presence of IL-12A encoding RNA or a signature fragment thereof. To achieve this, any standard method of amplifying RNA can be applied, in combination with primers specific for amplification of IL-12A-encoding RNA or any unique fragment thereof. One useful primer pair is described in the examples herein.

- 10 It will be appreciated that the assay is designed to reveal a relative reduction of IL-12A in samples obtained from test subjects relative to normal subjects, thereby to identify test subjects having or at risk for PBC. The relative reduction sought is one having statistical significance. The reduction is relative to normal subjects who, using the genotyping method herein described for instance, are confirmed to be free from PBC or the risk thereof. A statistically significant number of normal subjects will of course be tested to arrive at a known reference level for IL-12A in normal individuals for a given assay, and results from test subjects will be compared against these reference values when making a determination. In one study, comparing healthy subjects and subjects with alcoholic liver disease, it was revealed that the serum IL-12 levels in healthy subjects was on the order of 39.3 +/- 8.3 pg/ml ($p < 0.02$, $n = 35$) (see J. Chin. Med. Assoc., 2010 Feb; 73(2):67-71, abstract). Similarly, but in a study of asthma subjects, serum IL-12 levels in healthy controls, determined by ELISA, are reported as 67.42 +/- 20.58 ng/L ($P < 0.01$, $n = 30$) (see Zhongguo Dang Dai Er Ke Za Zhi, 2008 Apr; 10(2):146-8, abstract). These reports provide reference values for circulating IL-12, and are expected to correlate substantially with circulating levels of IL-12A. Thus, relative reductions in IL-12A can be determined by measuring levels of IL-12 in test and control subjects. A more complete assessment of IL-12A levels will also take into account the levels of IL-35, a cytokine that also incorporates IL-12A in a heterodimer. Accordingly, the determination of total IL-12A protein level can be accomplished either by measuring the amount of IL-12A detectable protein in a given sample, or by measuring the amount of IL-12A-complexed protein in the sample, such as by combining detectable levels of IL-12 and IL-35. Antibodies that detect IL-12 and IL-35 are also well known and available commercially, including the IL-12 antibody known as ustekinumab, a human monoclonal antibody that binds to the p40 component of IL-12. Whatever measurement is taken, it will be appreciated that levels of the same analyte will also be determined by the same method but in normal subjects, and a relative reduction by comparison will reveal a patient at risk for PBC.

Antibodies that are commercially available and useful in the present, protein-based assays are listed below:

Antibodies for p35 (IL-12A)

Vendor	Cat#	Epitop/specificity	source	Note
Sigma	GW22498	23-219aa	chicken	poly
Sigma	HPA001886	39-171	Rabbit	poly Mono, Clone
R&D	MAB1570	23-219aa	Mouse	27537
R&D	IC2191C	23-219	mouse	mono, for FC
Abcam	AB66462	c-terminal	Rabbit	poly
Abcam	ab37635	23-219	chicken	poly
Abnova	PAB1159	203-221	Rabbit	poly
Abnova	PAB8409	57-253	Sheep	poly
Novus Biologicals	NB100-81023	c-terminal	rabbit	poly
Novus Biologicals	NB100-75535	23-219	Chicken	poly
R & D Systems	IC2191A	IL-12 / IL-35 p35	Mouse	Mono, conjugated
R & D Systems	IC2191C	IL-12 / IL-35 p35	Mouse	Mono, conjugated
R & D Systems	IC2191P	IL-12 / IL-35 p35	Mouse	Mono, conjugated
Santa Cruz	sc-74149	IL-12A p35	Mouse	unconjugated
Santa Cruz	sc-65323	IL-12A p35	Mouse	unconjugated

Antibodies for p40 (IL-12B)

Vendor	Cat#	Epitop/specificity	source	Note
Sigma	GW21394	161-233	chicken	poly
Sigma	SAB2500536	278-291	Goat	poly
R&D	MAB1510	unknown	mouse	mono
Everest Biotech	EB07862	IL-12 / P40 IL12 / IL-23	Goat	poly, unconjugated
Cell Sciences	CSI10018	(p40)	mouse	mono, clone C8.6
Abcam	ab20196	unknown	mouse	mono
Abcam	ab62822	278-292	Goat	poly
Abnova	MAB2067	23-328	mouse	mono
Abnova	PAB7305	278-291	Goat	poly
Novus Biologicals	NB100-61661	278-291	Goat	poly
Novus Biologicals	NB100-64693	unknown	mouse	mono
Novus Biologicals	NB100-75276	IL12B chain	Chicken	poly
GeneTex	GTX82129	IL12 beta	Chicken	poly
GeneTex	GTX20050	IL12 p40	mouse	mono Conjugates -for FC
Biolegend	501809	IL-12/IL-23 p40	mouse	Conjugated -biotin
Biolegend	508801	IL-12/IL-23 p40	mouse	Conjugates -for FC
eBiosciences	46-7235-41	IL-12/IL-23 p40	mouse	Mono, purified
eBiosciences	14-7127	IL-12/IL-23 p40	mouse	mono, conjugates
BD BIOSCIENCES	559329	IL-12 (p40/p70)-	mouse	Mono, purified
BD Bioscience	554477	IL-12/IL-23 p40	mouse	mono, conjugates
BD Bioscience	554477	IL-12 P40/P70	mouse	Mono, purified
Santa Cruz Biotech	sc-53120	IL-12 P40/P70	mouse	mono
Santa Cruz Biotech	sc-53348	IL12 p40	mouse	mono
Santa Cruz Biotech	sc-1282	IL12B C-term	Goat	poly

Antibodies for IL-12

Vendor	Cat#	Epitop/specificity	source	Note
Santa Cruz	sc-74147	FL IL12	mouse	Mono, purified
Santa Cruz	sc-74150	FL IL12	mouse	Mono, purified
Biolegend	511001	IL12 P70		
LifeSpan BioSc	LS-C42972-50	IL12	Goat	poly, conjugated
	LS-C24859-			
LifeSpan BioSc	100	IL12	Goat	poly
	LS-C24876-			
LifeSpan Bio	100	IL12	mouse	Mono, purified
GeneTex	GTX82388	FL IL12	Chicken	poly
GeneTex	GTX20053	FL IL12	mouse	mono
Fisher/ ABR	PA1-86600	IL12		poly
eBiosciences	12-7235-71	IL12	mouse	mono,conjugates
eBioscience	14-7129	(IL-12) p40/70	mouse	mono
eBioscience	14-7128	IL12	mouse	mono
BD Bioscience	554658	IL12 P70	mouse	mono,purified
BD Bioscience	559325	IL12 P70	mouse	mono,conjugates
		Interleukin 12 (IL-		
Millipore	04-962	12)	mouse	clone 17.8
Invitrogen	A08E6E5	IL12		mono,purified

The reduced level of IL-12A protein and/or transcripts in PBC and related diseases suggests that IL-12A replacement will provide a therapy useful in their treatment.

Thus, in another aspect of the present invention, the present invention provides a method for treating a subject presenting with or at risk for PBC, optionally a subject identified by the genotyping or assay methods herein described, comprising the step of administering to the subject an agent effective to promote signaling via the IL-12/IL-12R axis. In related aspects, the invention provides the use of IL-12A or a complex thereof such as IL-12 and/or IL-35 in the preparation of a medicament for the treatment of PBC and related autoimmune liver conditions.

Agents that are effective to promote signaling via the IL-12/IL-12R axis particularly include the IL-12A protein and the IL-12RB2 protein, i.e., the proteins encoded by the genes in which PBC-associated polymorphisms have been identified as described herein. The amounts of such agents effective in the treatment of PBC are those amounts, delivered by any suitable treatment regimen, that result in the reduction or elimination of any one or more PBC symptoms, as identified hereinabove.

In one embodiment, the method comprises the step of administering an effective amount of IL-12A protein, or a polynucleotide encoding an IL-12A protein, particularly to subjects presenting with a genotype that includes any one or more of the IL-12A polymorphisms herein associated with PBC, or subjects presenting with reduced IL-12A protein or transcript levels. In embodiments, the IL-12A protein is the human IL-12A protein, which is the mature form of the protein having UniProt reference P29459. In the alternative, the administered protein can be a variant of the IL-12A protein that

incorporates 1, 2, 3, 4, 5 or more amino acid additions, substitutions and/or deletions, particularly including natural variations, and further including functional groups including additional glycosylation, polyethyleneglycolation or other depot means, yet retains the activity of IL-12A, i.e., associates with wild type IL-12B (UniProt reference 29460) to form a heterodimer that stimulates IL-12R, or associates with wild type Ebi3 to form IL-35 (where Ebi3 is a protein expressed from Epstein-Barr virus induced gene 3, and is also the IL-27B chain, having UniProt reference Q14213).

10 In another embodiment, the method comprises the step of administering an effective amount of IL-12RB2 protein, particularly to subjects presenting with a genotype that includes any one or more of the IL-12RB2 polymorphisms herein associated with PBC. In embodiments, the IL-12RB2 protein is the human IL-12RB2 protein, which is the mature form of the protein having UniProt reference Q99665). In the alternative, the administered protein can be a variant of the IL-12RB2 protein that incorporates 1,2,3,4,5 or more amino acid additions, substitutions and/or deletions yet retains the activity of IL-12RB2, i.e., associates with wild type IL-12RB1 (UniProt reference P42701) to form a heterodimer that is stimulated by IL-12.

20 In another embodiment, the method comprises the step of administering an effective amount of a polynucleotide encoding IL-12A, or encoding IL-12RB2, in the form of gene therapy using established administration and dosing regimens.

Also provided by the present invention is the use of IL-12A, a protein complex comprising IL-12A or a polynucleotide encoding IL-12A in the preparation of a medicament for the treatment of PBC. Similarly, the present invention also provides the use of IL-12RB2, a protein complex comprising IL-12RB2 or a gene encoding IL-12RB2 in the preparation of a medicament for the treatment of PBC.

30 Patients presenting with PBC also frequently present with other autoimmune and/or infectious diseases and disorders, the presence of which can be associated with an altered IL-12 signaling axis which may result from the polymorphisms herein identified. Thus, in a related aspect of the present invention, the present invention relates to a method for treating an autoimmune disease or disorder, which may or may not be associated with PBC in the treatment recipient, by administering an amount of IL-12A or complex and/or IL-12RB2 effective to reduce or eliminate one or more symptoms thereof, wherein the autoimmune disease or disorder is selected from ulcerative colitis, Crohn's disease, rheumatoid arthritis, autoimmune cholangitis, diabetes mellitus, multiple sclerosis, psoriasis, myasthenia gravis, autoimmune uveitis, systemic lupus erythematosus, Sjogren's syndrome and ankylosing spondylitis, and the like. In a further related aspect
40 of the present invention, there is provided a method for treating an infectious disease or disorder, by administering an amount of IL-12A and/or IL-12RB2 effective to reduce or eliminate one or more symptoms thereof, wherein the infectious disease or disorder is selected from example from HSV, HIV, hepatitis B, hepatitis C, papilloma, as well as bacterial infections including tuberculosis, salmonellosis, listeriosis and parasite infection such as malaria, leishmaniasis and schistosomiasis.

In still another of its aspects, the present invention provides a pharmaceutical composition comprising a pharmaceutically acceptable carrier and an agent effective to stimulate the IL-12/IL-12R axis, wherein the agent is selected preferably from human IL-12A or a complex thereof, and human IL-12RB2.

As proteins, both IL-12A and IL-12RB2 can be produced routinely for medical use by application of recombinant DNA methods, in which DNA coding for the desired protein is introduced, together with operably linked expression controlling elements, into a selected production host that can then be cultured to produce the desired protein.

- 10 Production of IL-12RB2 is described, for instance, in US 5,919,903, incorporated herein by reference, where gene expression is accomplished in Ba/F3 cells. Production of IL-12A is described, for instance, in WO92/005256 incorporated herein by reference, wherein expression of the IL-12A gene is described in a variety of hosts and expression systems.

It will also be appreciated that the genes encoding these proteins can be administered *per se* to drive endogenous expression of the protein products. The genes can be in the form of genomic DNA, in which case the wild type gene is used instead of the polymorphic forms associated with PBC, or the RNA or cDNA forms thereof, wherein the genes are

- 20 delivered using protocols established in the art of gene therapy.

Pharmaceutical compositions of IL-12 protein or complex, such as IL-12 or IL-35 and other p35-containing complexes, are prepared for storage and for administration by mixing the protein having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers (Remington's Pharmaceutical Sciences, 16th edition, Osol, A. Ed. [1980]), in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives

30 (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl, or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins such as serum, albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagines, histidine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g., Zn-protein complexes); and/or non-ionic surfactants such

40 as TWEEN, PLURONICS or polyethylene glycol (PEG).

The active ingredients to be used for *in vivo* administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes.

Sustained-release preparations may be prepared. Suitable examples of sustained-release include semipermeable matrices of solid hydrophobic polymers containing the antibody,

which matrices are in the form of shapes articles, e.g., films or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly (2-hydroxyethyl-methacrylate), polyactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate, and poly-D-(-)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods. Rational strategies can be devised for stabilization depending on the mechanism involved. For example, if the aggregation mechanism is discovered to be intermolecular S-S bond formation through thio-disulfide interchange, stabilization may be achieved by modifying sulfhydryl residues, lyophilizing from acidic solutions, controlling moisture content, using appropriate additives, and developing specific polymer matrix compositions.

A protein therapeutic according to the invention may be administered with a pharmaceutically-acceptable diluent, carrier, or excipient, in unit dosage form.

Any appropriate route of administration can be employed, for example, parenteral, intravenous, subcutaneous, intramuscular, intracranial, intraorbital, ophthalmic, intraventricular, intracapsular, intraspinal, intracisternal, intraperitoneal, intranasal, aerosol, or oral administration.

For the treatment of subjects presenting with PBC, the appropriate dosage will depend on the severity and course of the disease, whether the agent is administered for preventative or therapeutic purposes, previous therapy, the patients clinical history and response to the agent, and the discretion of the attending physician. The agent is suitably administered to the patient at one time or over a series of treatments. For example, depending on the type and severity of the disease, about 1 $\mu\text{g/kg}$ to 15 mg/kg (e.g., 0.1-20 mg/kg) of protein is a candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. A typical daily dosage might range from about 1 $\mu\text{g/kg}$ to 100 mg/kg or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment is sustained until a desired suppression of disease symptoms occurs. However, other dosage regimens may be useful. The effect of the agent can be seen as an increase in the endogenous levels, e.g., blood levels, of interferon gamma or STAT4. This effect is expected to be seen for example when a unit dose of about 1-50 μg of the agent is administered subcutaneously. The progress of this therapy is easily monitored by conventional techniques and assays used currently in the care of PBC subjects.

Other agents useful to stimulate the IL-12/IL-12 axis can be identified, for example, by screening combinatorial chemical libraries or small molecule or peptide/protein libraries including particularly IL-12A analog libraries and IL-12RB2 analog libraries to identify agents having the ability to stimulate IL-12 receptor signaling. Such libraries may be derived from natural products, synthetic (or semi-synthetic) extracts, or chemical libraries according to methods known in the art. Those skilled in the field of drug discovery and development will understand that the precise source of compounds is not critical to the

screening procedure(s) of the invention. Examples of natural compound sources include, but are not limited to plant, fungal, prokaryotic, or animal sources, as well as modification of existing compounds. Numerous methods are also available for generating random or directed synthesis (e.g., semi-synthesis or total synthesis) of any number of chemical compounds, including, but not limited to, saccharide-, lipid-, peptide-, and nucleic acid-based compounds. Synthetic compound libraries may be obtained commercially or may be produced according to methods known in the art. Furthermore, if desired, any library or compound is readily modified using standard chemical, physical, or biochemical methods. These agents can usefully be screened using standard bioassays that report IL-12 activity, such as the NK cell-based assay described in US20080138833 published June 12, 2008 incorporated herein by reference, wherein the effect of a given agent is determined by detecting production of interferon gamma by NK cells having a functional IL-12/IL-12R axis, an increase indicating that the agent stimulates that axis.

It will thus be appreciated that the present invention provides a novel means for identifying subjects that present with or are at risk for autoimmune disease particularly including PBC. The genotyping of such subjects reveals polymorphisms within proteins responsible for the signaling that occurs via the IL-12/IL-12R axis. The present invention accordingly also provides a method for the treatment of such subjects, particularly including subjects that are first genotyped by the present method and found to have one or more risk alleles, by administering thereto an agent effective to modulate signaling via this axis, including agents such as IL-12A and IL-12RB2 proteins and genes encoding them.

Examples

SUBJECTS AND CONTROLS

A two stage design was implemented (Figure 1) beginning with a GWA survey (stage I) incorporating Canada-based PBC cases and controls and additional US (M.D. Anderson) historic controls. This analysis was followed by replication analyses (stage II) incorporating US-based (IIa) and additional Canada-based (IIb) PBC cases and controls, as well as fine-mapping studies on the Canada-based GWA-screened and replication cohorts. Institutional ethics committee approval was obtained for all study participants. All patients fulfilled AASLD criteria for PBC and all subjects were Caucasian of European origin. 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 and then by excluding any subjects whose genotypes clustered with other or mixed racial groups (i.e. African- Americans, Hispanics, Asians, and Caribbean Islanders were excluded).

- 10 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).

- 20 A total of 536 PBC cases and 1536 healthy controls (399 Caucasian healthy volunteers with no prior history of autoimmune diseases collected in Toronto and an additional 1137 historic control samples collected from the M.D. Anderson Medical Center Lung Cancer Study¹⁷) were included in the stage I GWA analysis. Stage IIa replication included 410 PBC cases and 310 controls from the Mayo Clinic PBC Genetic Epidemiology Registry¹⁴ and Stage IIb included additional Canada-based subjects (116 PBC cases and 896 controls). All Canada-based cases and controls meeting quality control in stage I as well as the added 116 cases and 896 controls ascertained in Canada were included in the combined and fine-mapping analyses.

GENOTYPING AND QUALITY-CONTROL FILTERING

- 30 DNA purification and genotyping methods are described as follows. 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.
- 40 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 finemapping genotyping (<http://www.sequenom.com/seq-genotyping.html>). In brief, allelespecific 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, all in accordance with the manufacturer's instructions.

10 In stage II, we genotyped 16 SNPs in 14 genes/loci based on GWA signals. For finemapping studies, initiated coincident with the stage II 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.

20 In stage I the PBC cases and Canadian controls samples were genotyped for 373,400 SNPs using the Illumina HumanHap370 BeadChip. Control samples from the M.D. Anderson Cancer Center (historic controls) were genotyped on the Illumina HumanHap300 BeadChip. Genotyping data were filtered prior to data analysis. 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
30 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.

In brief, genotypes were called if they exceeded minimum quality control metrics. Individual samples with genotype call rates less than 95% and SNPs with call rates less than 95%, minor allele frequencies less than 1%, and/or deviating from Hardy-Weinberg equilibrium with $P < 0.0001$, were removed. Replication and fine-mapping were performed using the Sequenom MassArray iPLEX genotyping platform.

40 STATISTICAL ANALYSIS

For GWA association analysis, pairwise identity-by-state analysis was performed with PLINK¹⁸ to identify individuals with excess identity-by-descent-sharing ($PI_HAT > 0.25$). One subject from each of 15 pairs exceeding this threshold was removed from the association analysis (the individual in each pair with the higher SNP call rate was retained). Hierarchical cluster analysis was performed with PLINK to identify individuals

showing similar genotypes, over the entire genome. From this analysis, 39 outlier samples showed more than 4 standard deviations from a nearest neighbor and were removed from the analysis. Subsequent association analyses were conducted using PLINK with a conditional analysis that adjusted for stratifications among groups of individuals identified by hierarchical clustering. To alternatively control for the potential confounding influence of population stratification, association analysis was also performed based on a principal-component analysis implemented in EIGENSTRAT¹⁹ with default parameters, in which we adjusted for the 10 eigenvectors having the highest eigenvalues (-k 10, -m 10, -t 10, and -s 6.0). The lambda values showed minimal inflation, being 1.085 and 1.056 before and after eigenvector adjustment; using PLINK the lambda values were 1.14 without, and 1.09 with adjustment for clusters.

Allele and genotype associations were assessed using PLINK software (<http://pngu.mgh.harvard.edu/purcell/plink/>) and linkage disequilibrium (LD) between pairs of SNPs and haplotypes were evaluated by Haploview v.4.1 (<http://www.broad.mit.edu/mpg/haploview>). Haplotype block structure was defined according to the criteria established by Gabriel et al.²⁰ using the pairwise estimates of standardized Lewontin's coefficient (D'), while the LD among pairs of SNPs was characterized using the square of the correlation coefficient (r^2).

Combined analyses were carried out using SAS to perform Cochran Mantel-Haenszel tests allowing for potential confounding of the case/control frequency with genotype frequencies among groups of individuals at different stages/centers (analyses were stratified into GWA samples, US replication samples and Canada replication samples).

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 I), 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, see Figure 6) 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:

$$AF_p = \frac{PF(OR - 1)}{1 + PF(OR - 1)}$$

10

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).

$$AF_p = \frac{\sum_{i=1}^m PF_i(OR_i - 1)}{1 + \sum_{i=1}^m PF_i(OR_i - 1)}$$

20 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 Table S13

30

Table S13: 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 S13 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.

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.

RESULTS

STAGE I- GWA SCREEN

- 10 The most significant results of the stage I GWA survey are shown in Table 1 (full dataset provided in Supplementary Tables S1 and S2) and Figures 2 and 3, based on analysis of the 305,724 SNPs that passed quality control filters and the 505 PBC and 1507 control subjects retained after pairwise IBS estimation and population stratification correction. Thirteen SNPs in the *HLA/HLA* boundary regions on chromosome 6p21.3 and three SNPs from two distinct non-*HLA* regions at the *IL12A* (3q25.33-q26) and *IL12RB2* (1p31.2) loci, respectively, met a GWA significance threshold of $P < 5.0 \times 10^{-7}$ (based on EIGENSTRAT P values). The most highly associated markers, rs2856683, rs9275312, rs9275390 and rs7775228, map to the region between the *HLA-DQB1* and *HLA-DQA2* genes, the PLINK P values for these associations ranging between 1.70×10^{-10} and 8.58×10^{-17} with odds ratios varying between 1.81-2.01. Nine other SNPs mapping in or near the *C6orf10* ($P = 5.62 \times 10^{-10}$), *HLA-DPB1* ($P = 8.28 \times 10^{-9}$), *BTNL2* ($P = 1.27 \times 10^{-9}$) and
- 20 *HLA-DRA* ($P = 6.83 \times 10^{-7}$) genes within the *HLA* region also gave highly significant association signals, the *C6orf10* locus having the greatest association with risk (OR=3.24). Haplotype analysis, however, revealed that 4 of the 13 SNPs (rs2395148, rs3135363, rs2856683, and rs9357152 in the *C6orf10*, *BTNL2*, *HLA-DQB1* and *HLADQB1* genes, respectively) were sufficient to explain all of the *HLA*-associated risk for PBC (see Table S3 below).

Table S3 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	1.66×10^{-5}
CAAG	0.1239	0.1643	9.244	1	2.36×10^{-3}
AACA	0.04533	0.0119	41.65	1	1.09×10^{-10}
CACA	0.2954	0.2009	37.94	1	7.29×10^{-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	9.93×10^{-20}

- 30 *Table S3 Legend:* The impact that SNPs and haplotypes across the MHC have on risk for PBC was analyzed using PLINK. For this analysis, we included the 13 most significant SNPs in the HLA region that are identified in Table 1, Figure 6 and jointly modeled the impact of the inferred haplotypes derived from these SNPs on disease risk.

Similar levels of association were also found in a separate analysis of the genome-wide data incorporating only Canadian controls (Table S4).

Table S4. Comparison of original stage I GWA analysis with an analysis restricted to Canadian controls alone.

			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		
SNP ID	Chr	Gene	US Controls	Canadian Controls	EIGENSTRAT P	PLINK P	OR (95% CI)	EIGENSTRAT P	PLINK P	OR (95% CI)
HLA regions										
rs2856683	6p21.3	HLA-DQB1	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	HLA-DQB1	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	HLA-DQB1	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	HLA-DQB1	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	C6orf10	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	HLA-DPB1	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	BTNL2	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	HLA-DQB1	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	BTNL2	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	HLA-DPB1	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	HLA-DPB1	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	HLA-DRB1	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	HLA-DRA	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)

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.

Among the non-*HLA* loci, the strongest PBC-association signals observed were from SNPs in the *IL12A* (rs6441286 and rs574808, $P=3.25 \times 10^{-8}$; OR=1.51 and $P=5.34 \times 10^{-7}$; OR=1.47 respectively) and *IL12RB2* (rs3790567, $P=8.60 \times 10^{-8}$; OR=1.54) genes. A significant association with disease was also seen with a second *IL12RB2* region SNP, rs3790565 ($P=1.41 \times 10^{-6}$). Twelve other loci with association signals at $P < 5 \times 10^{-5}$ were identified, including *STAT4* (rs16833239, $P=2.60 \times 10^{-5}$), a locus associated with risk for several other autoimmune diseases, and a downstream biological mediator of IL12 signalling,²¹ *CTLA-4* (rs6748358, $P=1.41 \times 10^{-5}$), a locus previously associated with risk for PBC,^{14,15} and *IRF5/TNPO3* (rs10488631, $P=2.14 \times 10^{-5}$), a locus associated with risk for systemic lupus erythematosus (SLE)²² and inflammatory bowel disease (IBD).²³ These latter loci were more modestly associated with PBC, but their associations with risk were comparable to the *IL12A* and *IL12RB2* loci, odds ratios ranging from 1.39-2.13.

STAGE II – REPLICATION AND COMBINED ANALYSIS

To validate the GWA results (Table 1), sixteen of the most strongly-associated SNPs were tested (stage II replication) in two independently-collected case/control sample sets from the US (IIa) and Canada (IIb). Results of this analysis replicated ($P < 0.05$ in each of the two replication cohorts; Bonferroni corrected P -values < 0.05 in a joint analysis of the two replication datasets) the GWA results for the rs2856683, rs2395148 and rs9277535 SNPs at three distinct sites within the *HLA* region as well as the rs6441286/rs574808 and rs3790567 SNPs at the *IL12A* and *IL12RB2* loci, respectively. A combined analysis of the GWA and replication datasets was also undertaken and the results again provided strong evidence of PBC association with the rs2856683/*HLA-DQB1* ($P=1.78 \times 10^{-19}$; OR=1.75), *C6orf10* ($P=3.62 \times 10^{-14}$; OR=2.87), *HLA-DPB1* ($P=3.92 \times 10^{-11}$), *BTNL2* ($P=1.11 \times 10^{-9}$; OR=1.42), *IL12A* ($P=2.42 \times 10^{-14}$; OR=1.54) and *IL12RB2* ($P=2.76 \times 10^{-11}$;

OR=1.51) loci. A repeat of this analysis using a PBC case subgroup stratified by AMA status revealed no significant effect of AMA status on these associations (Table S5).

Table S5. Combined (stage I and II) analysis restricted to AMA positive patients.

				Combined		Combined AMA positive cases	
				n=1031 cases and 2713 controls		n=927 cases and 2713 controls	
SNP ID	Chr.	Location	Gene	P	Odds Ratio (95% CI)	P	Odds Ratio (95% CI)
A. HLA regions							
rs2856683	6p21.3	32763196	HLA-DQB	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	HLA-DQB1				
rs9275390	6p21.3	32777134	HLA-DQB1				
rs7775228	6p21.3	32766057	HLA-DQB1				
rs2395148	6p21.3	32429532	C6orf10	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	HLA-DPB1	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	BTNL2	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	HLA-DQB1				
rs3135363	6p21.3	32497626	BTNL2				
rs9277565	6p21.3	33164875	HLA-DPB1				
rs2281389	6p21.3	33167774	HLA-DPB1				
rs660895	6p21.3	32685358	HLA-DRB1				
rs9501626	6p21.3	32508322	HLA-DRA				
B. Non-HLA regions							
rs6441286	3q25.33-q26	161211572	IL12A	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	IL12RB2	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	IL12A	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	TRPC3	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	IL12RB2	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	CCDC68	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	NOTCH1				
rs16833239	2q32	191648505	STAT4	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	PCDH7				
rs2211312	13q33.3	107203928	FAM155A	0.608	1.05 (0.88-1.24)	0.38	1.08 (0.91-1.29)
rs907092	17q21	35175785	IKZF3	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	IKZF3				
rs4679904	3q26.1	161823590	ARF7	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	GSDML				
rs6140113	20p13	691770	C20orf54	0.338	1.08 (0.93-1.25)	0.238	1.10 (0.94-1.28)
rs10488631	7q32.1	128381419	IRF5/TNPO3	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	CTLA4				

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.

Copy number effect was also assessed for the two major non-*HLA* associated loci, *IL12A* and *IL12RB2*, and a gene dose effect identified for both loci (Tables S6 and S7).

Table S6. 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

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.

Table S7. 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)

- 10 *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.

STAGE III – FINE-MAPPING AND HAPLOTYPE ANALYSIS: *IL12A*, *IL12RB2* & *STAT4*

To refine and further validate the two major non-*HLA* PBC-associated loci emerging from the GWA screen (*IL12A* and *IL12RB2*), the entire set of Canadian PBC cases and controls were genotyped with tag SNPs across these loci (Table 2 in Figure 5, and Table S8).

- 20 Table S8. Association data from fine-mapping of the *IL12RB2*, *STAT4*, and *IL12A* loci.

SNP	Location	Minor Allele	Major Allele	MAF-Cases	MAF-Control	P Value	Odds Ratio (95%CI)
IL12RB2_RS3762317	67545170	G	A	0.114	0.123	4.18X10 ⁻¹	1.097 (0.876 - 1.374)
IL12RB2_RS1890741	67555340	C	G	0.187	0.176	4.27X10 ⁻¹	1.076 (0.898 - 1.291)
IL12RB2_RS1546159	67561014	T	C	0.015	0.021	2.70X10 ⁻¹	1.360 (0.786 - 2.352)
IL12RB2_RS11209050	67564324	A	C	0.283	0.205	2.76X10 ⁻⁷	1.532 (1.301 - 1.804)
IL12RB2_RS6693065	67572606	G	A	0.179	0.218	7.13X10 ⁻³	1.278 (1.069 - 1.528)

IL12RB2_RS4655703	67573960	A	T	0.463	0.407	1.66X10 ⁻³	1.256 (1.089 - 1.447)
IL12RB2_RS746389	67575391	G	A	0.463	0.403	7.60X10 ⁻⁴	1.275 (1.107 - 1.469)
IL12RB2_RS1995147	67575647	G	C	0.462	0.403	8.41X10 ⁻⁴	1.273 (1.105 - 1.467)
IL12RB2_RS10889682	67576471	C	G	0.195	0.224	5.26X10 ⁻²	1.188 (0.998 - 1.414)
IL12RB2_RS17129823	67577357	C	T	0.076	0.055	1.30X10 ⁻²	1.425 (1.076 - 1.886)
IL12RB2_RS1908632	67578394	G	T	0.333	0.248	1.22X10 ⁻⁷	1.516 (1.299 - 1.769)
IL12RB2_RS3790564	67579042	T	G	0.133	0.164	1.57X10 ⁻²	1.281 (1.048 - 1.567)
IL12RB2_RS3790565	67583944	C	T	0.252	0.183	2.44X10 ⁻⁶	1.505 (1.269 - 1.785)
IL12RB2_RS10489625	67587008	G	A	0.046	0.038	2.64X10 ⁻¹	1.219 (0.861 - 1.726)
IL12RB2_RS946685	67588303	A	G	0.252	0.183	1.93X10 ⁻⁶	1.506 (1.271 - 1.783)
IL12RB2_RS6679356	67592782	C	T	0.270	0.190	7.02X10 ⁻⁸	1.574 (1.334 - 1.858)
IL12RB2_RS10889684	67594609	T	C	0.143	0.172	2.80X10 ⁻²	1.246 (1.024 - 1.516)
IL12RB2_RS10749775	67594675	C	A	0.192	0.125	1.88X10 ⁻⁷	1.655 (1.368 - 2.003)
IL12RB2_RS3790567	67594965	A	G	0.337	0.250	8.32X10 ⁻⁸	1.519 (1.303 - 1.771)
IL12RB2_RS6695348	67599604	T	C	0.333	0.247	9.87X10 ⁻⁸	1.519 (1.302 - 1.772)
IL12RB2_RS12564159	67603882	G	C	0.336	0.298	2.34X10 ⁻²	1.190 (1.024 - 1.383)
IL12RB2_RS2252596	67606089	A	G	0.015	0.022	1.23X10 ⁻¹	1.541 (0.886 - 2.681)
IL12RB2_RS2307145	67606115	C	G	0.046	0.035	1.32X10 ⁻¹	1.311 (0.921 - 1.865)
IL12RB2_RS2307153	67606231	A	G	0.019	0.024	2.99X10 ⁻¹	1.302 (0.791 - 2.146)
IL12RB2_RS3790568	67608648	A	G	0.052	0.052	9.69X10 ⁻¹	1.006 (0.734 - 1.379)
IL12RB2_RS3828069	67612161	C	T	0.141	0.173	1.60X10 ⁻²	1.273 (1.046 - 1.549)
IL12RB2_RS6676606	67615554	G	A	0.094	0.072	2.73X10 ⁻²	1.327 (1.032 - 1.707)
IL12RB2_RS4297265	67624923	G	A	0.476	0.418	1.20X10 ⁻³	1.264 (1.097 - 1.457)
IL12RB2_RS2270614	67628609	T	C	0.475	0.421	2.42X10 ⁻³	1.244 (1.080 - 1.433)
IL12RB2_RS2229546	67634108	C	A	0.354	0.325	8.41X10 ⁻²	1.139 (0.983 - 1.321)
STAT4_RS11676659	191627599	G	A	0.036	0.050	7.71X10 ⁻²	1.380 (0.964 - 1.975)
STAT4_RS11893432	191630119	G	C	0.227	0.205	1.52X10 ⁻¹	1.141 (0.953 - 1.366)
STAT4_RS3024866	191631086	C	T	0.275	0.264	5.14X10 ⁻¹	1.054 (0.899 - 1.236)
STAT4_RS3024851	191637067	A	T	0.036	0.048	1.08X10 ⁻¹	1.349 (0.935 - 1.947)
STAT4_RS3024847	191638535	A	T	0.432	0.410	2.35X10 ⁻¹	1.092 (0.944 - 1.264)
STAT4_RS1517352	191639709	A	C	0.425	0.396	9.34X10 ⁻²	1.13 (0.980 - 1.303)
STAT4_RS13017460	191640801	A	G	0.437	0.410	1.34X10 ⁻¹	1.115 (0.967 - 1.285)
STAT4_RS1400656	191643278	G	A	0.035	0.050	4.30X10 ⁻²	1.454 (1.01 - 2.092)
STAT4_RS10168266	191644049	T	C	0.226	0.196	4.15 x 10 ⁻⁵	1.196 (1.008 - 1.420)
STAT4_RS7594501	191646845	A	G	0.041	0.077	3.49X10 ⁻⁵	1.973 (1.422 - 2.737)
STAT4_RS2459611	191647432	C	T	0.116	0.108	4.60X10 ⁻¹	1.088 (0.870 - 1.361)
STAT4_RS16833238	191647744	T	A	0.109	0.099	3.33X10 ⁻¹	1.121 (0.890 - 1.411)
STAT4_RS16833239	191648505	A	G	0.041	0.077	6.32 X 10 ⁻⁵	1.932 (1.397 - 2.674)
STAT4_RS3024921	191651517	A	T	0.097	0.052	2.08 X 10 ⁻⁶	1.945 (1.488 - 2.543)
STAT4_RS11889341	191651987	T	C	0.277	0.229	1.98X10 ⁻³	1.288 (1.097 - 1.512)
STAT4_RS13010752	191654848	A	T	0.097	0.092	6.09X10 ⁻¹	1.064 (0.838 - 1.352)
STAT4_RS6434435	191662109	A	G	0.147	0.176	2.98X10 ⁻²	1.240 (1.021 - 1.505)
STAT4_RS10931480	191662292	G	A	0.153	0.187	1.23X10 ⁻²	1.275 (1.054 - 1.543)
STAT4_RS10931481	191663097	G	A	0.336	0.323	4.36X10 ⁻¹	1.061 (0.914 - 1.232)
STAT4_RS13011805	191664494	T	C	0.095	0.087	4.64X10 ⁻¹	1.096 (0.858 - 1.398)

STAT4_RS4274624	191666901	C	T	0.261	0.230	5.06X10 ⁻²	1.183 (1.000 - 1.399)
STAT4_RS7574865	191672878	T	G	0.272	0.222	1.21X10 ⁻³	1.309 (1.112 - 1.541)
STAT4_RS12463658	191673589	C	A	0.434	0.423	5.12X10 ⁻¹	1.049 (0.910 - 1.210)
STAT4_RS10181656	191678124	G	C	0.278	0.230	1.90X10 ⁻³	1.289 (1.098 - 1.514)
STAT4_RS10497711	191722166	G	A	0.049	0.069	2.11X10 ⁻²	1.437 (1.054 - 1.959)
IL12A_RS17810546	161147744	G	A	0.069	0.117	9.77X10 ⁻⁶	1.784 (1.376 - 2.312)
IL12A_RS9811792	161179692	C	T	0.416	0.424	6.30X10 ⁻¹	1.036 (0.898 - 1.195)
IL12A_RS16830960	161184588	G	A	0.051	0.029	1.51X10 ⁻³	1.766 (1.238 - 2.521)
IL12A_RS2243123	161192345	C	T	0.232	0.276	5.01X10 ⁻³	1.263 (1.073 - 1.488)
IL12A_RS583911	161193084	G	A	0.503	0.421	4.34X10 ⁻⁶	1.392 (1.208 - 1.603)
IL12A_RS2243133	161194967	T	C	0.045	0.028	9.49X10 ⁻³	1.662 (1.128 - 2.450)
IL12A_RS2243135	161195687	C	G	0.342	0.406	2.51X10 ⁻⁴	1.314 (1.135 - 1.521)
IL12A_RS568408	161196161	A	G	0.104	0.145	7.65X10 ⁻⁴	1.457 (1.169 - 1.816)
IL12A_RS668998	161198245	G	A	0.501	0.422	1.12X10 ⁻⁵	1.372 (1.191 - 1.581)
IL12A_RS2243154	161198936	A	G	0.108	0.081	8.25X10 ⁻³	1.375 (1.085 - 1.743)
IL12A_RS4608735	161199702	C	A	0.107	0.124	1.58X10 ⁻¹	1.177 (0.938 - 1.476)
IL12A_RS11927521	161199779	G	A	0.344	0.407	2.65X10 ⁻⁴	1.312 (1.134 - 1.519)
IL12A_RS17826053	161200323	G	T	0.104	0.145	7.91X10 ⁻⁴	1.459 (1.169 - 1.82)
IL12A_RS6441284	161200962	G	A	0.339	0.411	4.47X10 ⁻⁵	1.359 (1.173 - 1.575)
IL12A_RS485497	161201826	G	A	0.439	0.518	1.09X10 ⁻⁵	1.374 (1.192 - 1.583)
IL12A_RS4680536	161202965	G	A	0.340	0.421	4.08X10 ⁻⁶	1.410 (1.218 - 1.632)
IL12A_RS9852519	161203322	T	C	0.470	0.372	2.11X10 ⁻⁸	1.502 (1.302 - 1.732)
IL12A_RS598638	161203511	T	C	0.170	0.173	8.32X10 ⁻¹	1.021 (0.847 - 1.23)
IL12A_RS4679867	161206597	A	T	0.317	0.405	4.52X10 ⁻⁷	1.464 (1.262 - 1.699)
IL12A_RS4679868	161206848	A	G	0.494	0.387	1.58X10 ⁻⁹	1.546 (1.342 - 1.782)
IL12A_RS6441286	161211572	G	T	0.494	0.390	4.83X10 ⁻⁹	1.525 (1.324 - 1.757)
IL12A_RS574808	161215677	C	T	0.320	0.412	1.15X10 ⁻⁷	1.493 (1.287 - 1.733)
IL12A_RS589545	161216294	A	G	0.322	0.413	1.34X10 ⁻⁷	1.487 (1.283 - 1.724)
IL12A_RS2936298	161226577	A	G	0.170	0.193	9.97X10 ⁻²	1.167 (0.971 - 1.404)
IL12A_RS17217081	161229792	G	A	0.015	0.018	5.22X10 ⁻¹	1.205 (0.68 - 2.134)

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 $P > 0.05$).

- 10 To study the *IL12A* locus, 25 SNPs spanning a 80 kb region encompassing the entire gene, 38 kb 5' flank and 32 kb 3' flank segments, were genotyped. Significant associations were observed for multiple SNPs across the region, the strongest signal ($P = 1.58 \times 10^{-9}$; OR=1.55) coming from rs4679868 at the 3' flank, a variant not assessed in the stage I survey. Of note, rs17810546 ($P = 9.77 \times 10^{-6}$), located in the *IL12A* 5' flank, has also been reported as a risk variant for celiac disease.²⁴ Haplotype analyses (Table S9) across the region also identified a five-allele 3' flank-located haplotype containing the rs4679867, rs4679868, rs6441286, rs574808, and rs589545 TAGTG alleles as a major risk haplotype for PBC ($P = 4.82 \times 10^{-29}$). A combined analysis of the Canada and US-based samples revealed this haplotype to be present in 49.1% of all PBC cases (as compared to 32.0% in controls) and confirmed a highly significant ($P = 1.15 \times 10^{-34}$; OR=2.01) association with risk for PBC.

Table S9 *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	4.82×10^{-27}	0.489	0.375	1.82×10^{-5}	0.491	0.320	1.15×10^{-34}	2.01 (1.83-2.30)
AGTCA	0.309	0.318	0.569	0.326	0.424	2.00×10^{-4}	0.317	0.340	0.0836	0.90 (0.80-1.01)
TGTTG	0.179	0.125	9.37×10^{-6}	0.179	0.195	0.4378	0.179	0.141	3.00×10^{-4}	1.33 (1.14-1.54)
Other haplotypes	0.021	0.252		0.006	0.006		0.013	0.199		

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.

For fine-mapping of the *IL12RB2* locus, the Canadian sample set was genotyped with 39 tag SNPs spanning 176.1kb across the *IL23R-IL12RB2* region, and including previously identified *IL23R* risk variants associated with Crohn’s disease and psoriasis.^{25,26}

Significant associations derived primarily from SNPs in the *IL12RB2* 3’ flank and intronic regions, the rs6679356 SNP showing the most significant association ($P=7.02 \times 10^{-8}$) with PBC. In addition, a three locus risk haplotype identified in the 3’ flank (GTC; present in 35.6% of cases and 25.3% of controls) was significantly associated with PBC, $P=3.07 \times 10^{-11}$; OR=1.53 (Table S10). No significant associations were seen for the *IL23R* SNPs.

Table S10: Haplotype association results for *IL12RB2*

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

Haplotype	Frequency		P	Odds Ratio (95% CI)
	Cases (N=621)	Controls (N=1279)		
10-Locus				
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)
3-Locus				
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)

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.

10

Although association with the *STAT4* rs16833239 SNP was not replicated in the stage II analysis, this association remained significant ($P=4.67 \times 10^{-5}$; OR=1.65) in the combined analysis and is of particular potential relevance to the *IL12A/IL12RB2* associations. Additional genotyping was thus also undertaken across the 94.6kb *STAT4* locus, the results revealing several intronic SNPs as being modestly associated with disease, the strongest association emanating from an intron 3 SNP, rs3024921, $P=5.76 \times 10^{-8}$ in the combined sample set (Tables S8 and S11).

Table S11. Replication & combined 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)

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.

- 10 Joint analysis of the fine-mapping SNPs across each of the *IL12A*, *IL12RB2* and *STAT4* loci (Table S12) was also undertaken and revealed that for each region multiple SNPs are required for the observed associations.

Table S12. 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

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

- 20 From these results, it will be appreciated that the GWA data identify the *HLA*, *IL12A* and *IL12RB2* loci as susceptibility loci for PBC, with multiple SNPs across the *HLA* region and several *IL12A* and *IL12RB2* locus SNPs achieving genomewide significance. The successful replication in independent cohorts of the most strongly-associated SNP at each of these loci, the high significance levels achieved in the combined analysis, and the identification by ancillary genotyping of multiple other PBC-associated SNPs across the *IL12A* and *IL12RB2* loci, provide compelling evidence for involvement of all three loci in PBC.

Associations between PBC and class II major histocompatibility complex (MHC) alleles have been previously reported,¹² but inconsistently replicated across independent studies. The data provide conclusive evidence for involvement of this region in PBC, revealing variants in the four class II *HLA* genes (*DQB1*, *DPB1*, *DRB1*, *DRA*) and the *C6orf10* and *BTNL2* genes at this locus to be highly associated with disease. Among these genes, *DQB1*, *DPB1* and *DRB1* have been previously implicated in PBC in some association studies²⁷ and association of a *BTNL2* truncating mutation identified initially in patients with sarcoidosis²⁸ (another granulomatous disease) has also been observed in several autoimmune diseases. This latter association may reflect strong linkage disequilibrium of the *BTNL2* mutation with disease-predisposing *HLA DQB1-DRB1* haplotypes.^{29,30} While strong LD across this region also confounds identity of the PBC-causal allele(s) underpinning the association signals, haplotype analyses (Table S3) suggest that most of the PBC risk associated with this region derives from two common haplotypes across the *C6orf10*, *BTNL2* and *HLA-DQB1* genes, *AACA* ($p=1.09 \times 10^{-10}$) and *CACA* ($P=7.29 \times 10^{-10}$). By validating the class II MHC region as a major contributor to risk for PBC, our data indicate that further dissection of this region is warranted and will enable definition of allele(s) etiologically relevant to PBC.

While clearly important in PBC pathogenesis, *HLA* contributions to PBC risk are proportionately less significant than for other autoimmune diseases,³¹ the association of the *HLA-DQB1* locus on risk (OR=1.75) being comparable to the associations of *IL12A* (OR=1.54) and *IL12RB2* (OR=1.51), and the population attributable fraction (PAF) fraction (Table S13) for the *IL12A* rs6441286 GT and GG risk genotypes (21.5%) being slightly higher than that for the *HLA-DQB1* rs2856683 CC and CA risk genotypes (21.2%). The *IL12RB2* AA risk genotype also provides a relatively high PAR of 18.4%. These estimates are in keeping with substantive contributions of all three of these loci to risk for PBC.

The data also suggest important contributions of the *IL12A* and *IL12RB2* loci to PBC susceptibility. This possibility is consistent with the major immunoregulatory roles of their protein products, IL12p35 and IL12RB2, which respectively associate with the IL12p40 and IL12RB1 chains to generate the IL12 cytokine and its receptor, IL12R. Understanding of the pathways whereby IL12 is induced and functions is continuing to evolve, but IL12 binding to IL12R is thought to modulate autoimmune responses by evoking interferon gamma production and thereby inhibiting interleukin 23/IL23R-mediated induction of proinflammatory IL17-producing T helper 17 cells.^{32,33} Relevance of this pathway to PBC is also suggested by the finding of PBC association with several SNPs across the *STAT4* gene, which encodes an effector integral to IL12/IL12R signaling. Interestingly, a *STAT4* intron 3 SNP, rs7574865, associated with risk for rheumatoid arthritis, SLE²¹ and type 1 diabetes,³⁴ was also associated with PBC in the current study ($P=1.21 \times 10^{-3}$; OR=1.31), although the strongest association signals at this locus came from intronic SNPs outside the LD block tagged by rs7574865.

Similarly, another PBC-associated SNP, rs178105416 in the *IL12A* gene, has been previously implicated in risk for celiac disease,²⁴ while SNPs in two other key components of the IL12/IL12R and related IL23 immunomodulatory axis, IL12B and

IL23R, are associated with risk for psoriasis, psoriatic arthritis and/or IBD, although not for PBC.^{25,26, 35}

The GWA study was well-powered (82%) to detect associations with an OR of 1.5 (Supplementary Appendix) and, as such, provided strong evidence for PBC association with a number of *HLA*, *IL12A* and *IL12RB2* variants. However, lower power to detect associations with more modest effects, even in the combined analysis (power estimated, for example, to be 60% for an OR of 1.4) may have impeded discovery of some PBC risk alleles. Larger and prospectively-followed sample sets will be required to identify non-*HLA* loci influencing risk and to elucidate the relevance of the PBC-associated loci to other clinically-relevant disease subphenotypes such as disease severity/progression. Population stratification appeared to have a minimal impact in this analysis given little inflation of genome-wide chi-square tests, and the similar levels of association provided by an analysis restricted to Canadian cases and controls.

At present, the disease-causal alleles at the PBC-associated *HLA* and non-*HLA* loci identified here remain unknown. Interestingly, the strongest association signals at the *IL12A* and *IL12RB2* loci emanated from SNPs in the 3' flank and intronic regions, suggesting these variants may influence *IL12/IL12RB2* expression. This possibility is consistent with the development of autoimmune/lymphoproliferative disease in *IL12RB2* knock-out mice³⁶ and a recent report describing the development of biliary cirrhosis in a child with IL12 deficiency.³⁷

These observations as well as the cumulative data linking inherited IL12, IL12R and IFN γ deficiency to increased susceptibility and severity of mycobacterial and other infectious disease,³⁸ suggest that PBC-associated *IL12A/IL12RB2* variants engender both an impaired response to infection, as well as a potentiated risk for autoimmunity, the former possibly driving the latter.

Precise characterization of the nature and functional sequelae of the *HLA* and *IL12A/IL12RB2* variants conferring risk for PBC is required, but the association of PBC with variants at these loci confirms the critical role for immunogenetic factors in genesis of this disease. These data also indicate that IL12/IL12R modulation can be of benefit in the treatment of PBC.

Evaluation of IL-12A Gene Expression Levels in Healthy Subjects

Patient samples were assayed for IL-12A transcript levels. More particularly, human peripheral blood mononuclear cells were purified from healthy donors and genotyped so as to identify individuals homozygous for the primary biliary cirrhosis risk (TAGTG) and non-risk (AGTCA) IL12A haplotypes. Representative data from two subjects with non-risk genotype (1 and 2) and from 2 with the risk genotype (3 and 4) are shown. Cells were stimulated with lipopolysaccharide (LPS) and interferon- γ for 8 hours and harvested for total RNA purification using with Qiagen kit. A two-step qPCR were performed to detect the expression of IL12A (p35) and the housekeeping gene, glyceraldehyde 3-phosphate dehydrogenase (GAPDH).

Primers for IL12A:

5'TATTGATGAGCTGATGCAGGC3' (SEQ ID No.88)

5'GTCAGTCCCCGAATTCTGAAA3' (SEQ ID No.89)

Primers for GAPDH:

5'ACCTCAACTACATGGTTTAC3' (SEQ ID No.90)

5'GAAGATGGTGATGGGATTTTC3' (SEQ ID No.91)

- 10 The results are presented in Figure 8 as IL12A relative to GAPDH expression levels. It will be appreciated from these results that subjects at risk for PBC, as determined by the genotyping method herein, also present with reduced IL-12A transcript levels, and correspondingly reduced IL-12A protein levels, relative to subjects having a non-risk genotype.

Further genotyping analysis

- Continued replication and fine mapping studies have also revealed association of primary biliary cirrhosis with the *17q12-21* and *MMEL1* loci and a high risk two-allele haplotype at the *IRF5-TNPO3* locus. More particularly, genome-wide association data have confirmed HLA, IL12A and IL12RB2 as risk loci for primary biliary cirrhosis (PBC). By genotyping additional subjects for SNPs showing suggestive GWA significance ($P < 1 \times 10^{-4}$), we identified *17q12-21* ($P = 3.50 \times 10^{-13}$), *IRF5-TNPO3* ($P = 8.66 \times 10^{-13}$), and *MMEL1* ($P = 2.28 \times 10^{-9}$) as PBC risk loci and defined a high risk two-allele haplotype at the *IRF5-TNPO3* locus ($P = 7.79 \times 10^{-31}$). These results expand identified PBC risk loci and the evidence for genetic overlap among autoimmune diseases.
- 20

- In particular, we have now tested another panel of 857 PBC cases of Caucasian European descent, together with an additional 3198 controls of Caucasian European descent (including 1860 historic control subjects from the New York Cancer Project [43]) for PBC associations with 36 SNPs, across 24 loci, in which association signals at $P < 1 \times 10^{-4}$ were detected in the initial GWA survey. The combination of these replication data and our prior genome-wide association data provided a genetic dataset derived from 1351 PBC cases and 4700 controls (See Supplementary Methods).
- 30

The following methods were applied in this subsequent study:

Subjects

- The study was approved by local institutional ethics committees at each participant clinical center. All cases were of Caucasian European descent, and were diagnosed with PBC according to American Association for the Study of Liver Disease guidelines [1, 2]. Overall 92% of patients were female, with an average age of 60 years, and 6.5% of patients were AMA negative. All cases were Caucasian of European descent who were
- 40

ascertained via: a) the original Canadian GWAS case collection, n=494; b) an additional recruitment of North American cases, n=720 or; c) from Europe, n=137. Controls were all healthy adults of Caucasian European descent and included the original GWAS control collection (n=1502), a second set of Canadian controls (n=972), and additional controls ascertained from Europe (124) and the USA (2102), the latter of which include 1860 “historic” controls from the New York Cancer Project previously genotyped at the Feinstein Institute using Illumina 500K BeadChips.

SNP genotyping and marker selection

- 10 Individual samples with genotype call rates of less than 95% and SNPs with call rates of less than 95%, minor allele frequencies of less than 1%, or deviation from the Hardy–Weinberg equilibrium at $P < 0.001$, were removed from the analysis.

Genotyping for the replication studies was performed using a Sequenom MassARRAY iPLEX platform and SNP annotation was based on NCBI dbSNP Build 129. 36 SNPs were genotyped across 24 loci in which association signals at $P < 1 \times 10^{-4}$ in the initial GWA survey had been identified. GWA data were obtained using the Illumina Human Hap370 BeadChip as previously reported [2]. Genotype data quality was assessed by genotype clustering using Sequenom Typer Analyzer, discordance in the duplicate

- 20 genotyping samples and genotyping call rate ($>95\%$). To delineate the genetic variation across the *IRF5* locus, resequencing of this gene (including intronic and exonic regions and 5' and 3' flanking regions) was performed in genomic DNA pools prepared from 100 subjects. Four long-range PCRs were designed with Primer3 (<http://frodo.wi.mit.edu/primer3/input.htm>) so as to cover the 24.8-kb *IRF5* genomic region (chr7: 128,361,145-128,385,948), the DNA then amplified using the SequalPrep™ Long PCR Kit (Invitrogen) and the purified PCR products pooled for sequencing. DNA sequencing library preparation, cluster generation were performed using DNA library preparation and cluster generation kits from Illumina and sequencing then undertaken on the Illumina/Solexa GAII platform using a 36 bp sequencing protocol. Sequence
- 30 alignment and polymorphism identification, performed using Velvet and SoftGenetics SeqAlign program (SoftGenetics), confirmed prior reports of 44 polymorphisms across this region, but failed to identify any novel variants. Of these 5 SNPs failed repeatedly in genotyping assays and 4 did not show polymorphisms (MAF $< 0.001\%$). Thus the fine-mapping studies incorporated 35 test polymorphisms, including two the *IRF5* promoter CGGGG (4/5) and 30-bp (rs60343456) indel polymorphisms that were assayed using an ABI3700 as previously reported [45].

Auto-antibody assays

Titres of anti-sp100 and gp210 antibodies were evaluated by QUANTA Lite™ (ELISA) assays using frozen serum samples, and the recommended protocol (INOVA Diagnostics, Inc. CA, USA).

Statistical analyses

- Allelic associations were assessed by means of PLINK software (v1.07), as were the haplotype associations. To identify a parsimonious model for association of haplotypes with risk for PBC, we used a forward-selection approach adding one SNP at a time to the top SNP or combination of SNPs. Tests of haplotypes included comparing the frequency of the presence of each specific haplotype versus its absence, which yielded a 1-degree of freedom test, and an omnibus test which jointly compares the frequencies of each haplotype in the cases to the frequency in the controls. This yields a chi-square test having degrees of freedom equal to the number of haplotypes minus 1. An R-script (www.r-project.org/) was used to generate the upper panel of Figure 1. Linkage disequilibrium between pairs of SNPs was determined with the use of Haploview software, version 4.1 (www.broad.mit.edu/mpg/haploview). Haplotype block structure was defined according to the criteria established by Gabriel and the pairwise estimates of standardized Lewontin's disequilibrium coefficient (D'), whereas the linkage disequilibrium among pairs of SNPs was characterized according to the square of the correlation coefficient (r^2). Combined analyses were carried out with Cochran–Mantel–Haenszel tests, with adjustment for potential confounding of the case or control frequency with genotype frequency among groups of subjects according to the stage of analysis or center. For replication analysis the North American and European samples were stratified, and for the combined analyses we stratified by North American, European and GWA samples. Conditional logistic regression analyses of multiple markers were performed using SAS v9.13 (SAS Institute Inc.).

- We used an *in silico* approach to check if the candidate genes directly interact with each other. Pathway Studio software (www.ariadnegenomics.com) was used to construct a network of interaction between genes (direct regulation of gene expression, protein/protein binding or promoter binding).

Results

Fifteen SNPs replicated ($P < 1.4 \times 10^{-3}$) in this cohort after Bonferroni adjustment. In addition to *HLA*, *IL12A* and *IL12RB*, genes at the *IRF5-TNPO3*, *MMEL1* and chromosome 17q12-21 (*IKZF3*, *ZBPB2*, *GSDMB* and *ORMDL3*) loci showed significant association with PBC in both the replication analysis and in an analysis combining the replication and initial GWA datasets, as shown below:

SNP	Chr.	Position	Gene	Risk Allele
rs3748816	1p36	2516606	<i>MMEL1</i>	C
rs3890745	1p36	2543484	<i>MMEL1</i>	G
rs11588497	1p35.1	32803048	<i>ZBTB8</i>	G
rs3790565	1p31.2	67583944	<i>IL12RB2</i>	C
rs3790567	1p31.2	67594965	<i>IL12RB2</i>	A
rs16833239	2q32.2-q32.3	191648505	<i>STAT4</i>	A
rs231735	2q33	204402121	<i>CTLA4</i>	G
rs6748358	2q33	204465150	<i>CTLA4</i>	A
rs1869241	3p26.1	5729732	<i>EDEM1</i>	T
rs619481	3p26-p24	8182706	<i>LMCD1</i>	C
rs1025818	3p24.3	16940025	<i>PLCL2</i>	G
rs6441286	3q25.33-q26	161211572	<i>IL12A</i>	G
rs4679904	3q25.33	161823590	<i>ARL14</i>	T
rs9277535	6p21.3	33162839	<i>HLA-DPB1</i>	G
rs13216266	6q23.3	140059450	<i>CITED2</i>	T
rs10250643	7p15	24502175	<i>MPP6</i>	G
rs10488631	7q32.1	128381419	<i>IRF5-TNPO3</i>	C
rs1760	8p23.3	432079	<i>C8orf42</i>	G
rs7834444	8q22	99455892	<i>KCNS2</i>	T
rs7838130	8q22	99456091	<i>KCNS2</i>	T
rs4259391	8q22	99460461	<i>KCNS2</i>	T
rs10504989	8q22.2	100501854	<i>VPS13B</i>	T
rs7477689	10p11.2	30055806	<i>SVIL</i>	G
rs3858359	11q21-q22	91148975	<i>MTNR1B</i>	C
rs2211312	13q33-q34	107203928	<i>LIG4</i>	C
rs907092	17q12-21	35175785	<i>IKZF3</i>	A
rs9303277	17q12-21	35229995	<i>IKZF3</i>	C
rs11557467	17q12-21	35282160	<i>ZPBP2</i>	G
rs8067378	17q12-21	35304874	<i>GSDMB</i>	A
rs2305480	17q12-21	35315722	<i>GSDMB</i>	T
rs2290400	17q12-21	35319766	<i>GSDMB</i>	A
rs7216389	17q12-21	35323475	<i>GSDMB</i>	T
rs4795405	17q12-21	35341943	<i>ORMDL3</i>	T
rs9964104	18q21	50751695	<i>CCDC68</i>	C
rs3745516	19q13.3-q13.4	55618554	<i>SPIB</i>	A
rs6140113	20p13	691770	<i>C20orf54</i>	T

Among the PBC loci confirmed by this analysis, the *IRF5-TNPO3* (interferon regulatory factor 5-transportin 3) locus is of particular interest because of the integral immunoregulatory roles for *IRF5*, prior association of this locus with systemic lupus erythematosus [44, 45], systemic sclerosis [46] and Sjögrens syndrome [47], and the strong effect on risk of the disease-associated SNP at this locus in the replication ($P_{\text{REP}}=1.13 \times 10^{-8}$) and combined datasets ($P_{\text{COM}}=8.66 \times 10^{-13}$; OR 1.57). Fine-mapping studies were thus initiated, wherein 1330 cases and 1833 controls were genotyped for 35 SNPs across this locus. Among the associations identified, as shown below, the strongest signals were provided by rs12539741 and rs2070197 alleles.

10

SNP	Position	Risk allele
rs729302	128356196	C
IRF5_rs11768806	128360364	T
rs4728142	128361203	A
rs1874330	128362460	G
rs3778754	128362788	C
rs6968563	128364967	C
STR_CGGGG	128365169	G
IRF5_rs2004640	128365537	G
rs3807307	128366438	G
IRF5_rs752637	128366656	A
rs3823536	128366902	T
rs3778752	128367283	A
rs3778751	128367284	A
rs55643109	128367411	T
rs3807306	128367916	C
rs11767834	128368512	T
rs11761199	128369071	G
rs6975315	128369721	A
rs7808907	128371320	T
rs1874328	128372340	C
rs1874327	128372612	T
rs35000415	128372852	T
Indel30	128374500	A
IRF5_rs2070197	128376236	C
rs10954213	128376663	G
rs11770589	128376724	A
rs10954214	128376869	C
IRF5_rs13242262	128378600	A
rs10488630	128381184	G
rs10488631	128381419	C
rs2280714	128381961	G
rs3847098	128382385	G
rs11761242	128382842	C
rs12539741	128384041	T
rs10236569	128384823	T

These variants map just 3' to the *IRF5* coding region, are in tight linkage disequilibrium ($r^2 > 0.95$) with one another, and their associations with PBC reach P values of 1.65×10^{-10} (OR=1.63) and 3.74×10^{-10} (OR=1.62), respectively, in the combined cohort analysis. These polymorphisms are also among the SNPs at this locus highly associated with SLE and are both correlated with changes in *IRF5* expression in transformed B cells [44]. By contrast, an insertion-deletion polymorphism 64bp upstream of *IRF5* exon 1A representing a putative disease-causal variant for SLE, showed only modest association with PBC ($P=1.00 \times 10^{-3}$) and no associations were detected between PBC and another SLE-associated SNP, rs2004640, or a 3' UTR variant, rs10954213, identified as a key

predictor of *IRF5* expression level [44]. The analyses also identified a number of haplotypes across the *IRF5-TNPO3* locus that were more strongly associated with PBC than any of the individual test SNPs. Among these, a two-allele haplotype involving the rs35000415 and rs12539741 SNPs showed the strongest association (omnibus $P=7.79 \times 10^{-31}$, OR=1.97 for the TT haplotype) with risk for PBC.

Haplotype analysis across the *IRF5-TNPO3* locus reveals a 2 SNP and 3 SNP haplotype strongly associated with PBC

2-SNP Haplotype			
HAPLOTYPE	DF	P	SNPs
TT	1	5.58×10^{-19}	rs35000415 rs12539741
TC	1	4.62×10^{-16}	rs35000415 rs12539741
CC	1	2.82×10^{-08}	rs35000415 rs12539741
OMNIBUS	2	7.79×10^{-31}	rs35000415 rs12539741

3-SNP Haplotype			
HAPLOTYPE	DF	P	SNPs
TCT	1	4.24×10^{-19}	rs35000415 IRF5_rs2070197 rs12539741
TTC	1	8.87×10^{-16}	rs35000415 IRF5_rs2070197 rs12539741
CTC	1	2.85×10^{-08}	rs35000415 IRF5_rs2070197 rs12539741
OMNIBUS	2	1.13×10^{-30}	rs35000415 IRF5_rs2070197 rs12539741

10

PLINK was used to evaluate haploype association by a forward-selection approach adding one SNP at a time to the top SNP or combination of SNPs. The omnibus test evaluated deviation from an equal frequency of haplotypes in cases and controls, using a goodness of fit chi-square test. The two SNP haplotype rs35000415-rs12539741 showed greatest association with PBC, when compared with controls, with an odds ratio of 1.97. CHISQ= χ^2 and DF=degrees of freedom.

20

A second region of interest emerging from these studies is the *17q12-21* locus, with all 8 of the tested SNPs across this region achieving significance in the replication analysis (P_{REP} values between 1.78×10^{-9} and 1.88×10^{-5}). This chromosomal region has also been associated with asthma [48], Crohn's disease [49] and type 1 diabetes [50] and contains four genes, *ZPBP2*, *IKZF3*, *GSDMB*, and *ORMDL3*, which respectively encode the zona pellucida-binding protein 2, the *IKAROS* family zinc finger 3 protein involved in leukocyte development and IgE production, Gasdermin-B involved in epithelial barrier function and *ORMI*-like protein 3, an uncharacterized protein belonging to a family of transmembrane proteins anchored in the endoplasmic reticulum. All 8 of the SNPs tested at this locus were in linkage disequilibrium (pair-wise r^2 range of 0.66-0.96), but the strongest association signal came from a *ZPBP2* SNP, rs11557467 ($P_{\text{COM}}=3.50 \times 10^{-13}$).

The replication and combined association data also reveal a strong association of PBC with two SNPs (rs3890745 and rs3748816) at the *MMEL1* (membrane metallo-

endopeptidase-like 1) locus on chromosome 1p36 (combined $P=2.28 \times 10^{-9}$ and $P=3.15 \times 10^{-8}$, respectively). These SNPs are in linkage disequilibrium with one another ($r^2 > 0.88$) and one (rs3748816) is a non-synonymous SNP in exon 16 that encodes a potentially functional Met to Thr substitution, while the other (rs3890745) maps within intron 2 of the *MMEL1* gene and has been associated with risk for rheumatoid arthritis and for celiac disease [51,52].

10 A suggestive association signal ($P_{\text{COM}}=9.12 \times 10^{-7}$; OR=1.27) was also observed in the combined case/control cohort for rs3745516, an intronic SNP in *SPIB*, a gene encoding the Spi-B transcription factor. This SNP did not achieve significance in the replication cohort after Bonferroni correction ($P_{\text{REP}}=6.21 \times 10^{-3}$), but the strength of the association in the combined analysis and Spi-B roles in dendritic cell development [53] and B-cell receptor signalling, are in keeping with a potential relevance of this locus to PBC pathogenesis.

20 Anti-mitochondrial antibodies (AMA) are found in most patients with PBC, but no correlation of specific PBC genotypes with AMA status was observed in our prior [2] or current association studies. PBC is also associated with specific anti-nuclear antibodies, some 20% of patients manifesting anti-glycoprotein-210 antibodies (anti-gp210) directed against the human nuclear pore complex, or anti-sp100 antibodies that recognize a 53-kDa nuclear antigen [14]. Evaluation of genotype status in the subset of cases (462) typed for anti-nuclear antibodies revealed a strong association of the *HLA* locus (rs9277535 at *HLA-DP1*) with disease ($P=4.25 \times 10^{-8}$; OR=2.25) in anti-sp100 antibody-positive patients. By contrast, no genetic distinctions were apparent for the anti-gp210 positive subgroup.

30 In conclusion, the current data confirm associations of PBC with the *HLA*, *IL12A* and *IL12RB2* loci and identify *IRF5-TNPO3*, *17q12-21*, and *MMEL1* as three additional risk loci for PBC. Our data also identify *IRF5-TNPO3* haplotypes that are highly associated with PBC and suggest that some genetic substructure may exist in PBC in relation to anti-sp100 antibody status. A search for functional relationships between the various PBC-associated loci was also undertaken using Pathway Studio software to construct a network of interactions between candidate genes identified in our study (data not shown). This revealed potential interactions between only 3 of the disease-associated genes (*IL12A*, *IL12RB2* and *STAT4*). Thus, it appears that many of the candidate genes identified here does not directly interact, but instead, act independently in conferring risk for PBC.

All references cited herein, including all database references and the sequence information referenced therein, are hereby incorporated herein in their entirety.

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CLAIMS:

1. A method for identifying a subject having or at risk for primary biliary cirrhosis (PBC), wherein a biological sample obtained from the subject is assessed to identify at least one of the following characteristics:
 - (a) at least one polymorphism within a gene for one or both of IL-12A and IL12-RB; and
 - (b) a level of IL-12A protein, IL-12A protein complex or IL-12A transcript that is reduced relative to a healthy individual,

10 the presence of said at least one characteristic indicating the subject has, or is at risk for PBC.

2. The method according to claim 1, wherein the biological sample is assessed by a method comprising genotyping DNA from said subject to identify at least one polymorphism within a gene encoding one or both of IL-12A and IL-12RB, wherein the presence thereof is indicative of PBC.

3. The method according to claim 2, wherein the at least one polymorphism occurs within a gene encoding IL-12A.

20

4. The method according to claim 3, wherein the at least one polymorphism in the IL-12A gene is selected from the group of risk alleles consisting of:

30

<i>SNP ID</i>	<i>Location</i>	<i>Risk Allele</i>	<i>Physical Location</i>
rs17810546	5' flanking	A	161147744
rs583911	intron 2	G	161193084
rs668998	3' flanking	G	161198245
rs6441284	3' flanking	A	161200962
rs485497	3' flanking	G	161201826
rs4680536	3' flanking	A	161202965
rs9852519	3' flanking	C	161203322
rs4679867	3' flanking	T	161206597
rs4679868	3' flanking	A	161206848
rs6441286	3' flanking	G	161211572
rs574808	3' flanking	T	161215677
rs589545	3' flanking	G	161216294

5. The method according to claim 4, wherein the at least one polymorphism in the IL-12A gene is selected from the group of IL-12A SNPs consisting of risk allele G at location 161211572 and risk allele T at location 161215677.
6. The method according to claim 5, wherein the at least one polymorphism in the IL-12A gene is any one or more of the polymorphisms in the 5-locus haplotype represented by SNPs rs4679867, rs4679868, rs6441286, rs574808 and rs589545 SNPs.

10

7. The method according to claim 6, wherein the at least one polymorphism is comprises the 5 locus haplotype TAGTG.
8. The method according to claim 1, wherein the at least one polymorphism occurs within a gene encoding IL-12RB2.
9. The method according to claim 8, wherein the at least one polymorphism in the IL-12RB2 gene is selected from the group consisting of:

20

<i>SNP ID</i>	<i>Location</i>	<i>Risk Allele</i>	<i>Physical Location</i>
rs11209050	intron 3	A	67564324
rs1908632	intron 8	G	67578394
rs3790565	intron 8	C	67583944
rs946685	intron 8	A	67588303
rs6679356	intron 9	C	67592782
rs10749775	intron 9	C	67594675
rs3790567	intron 9	A	67594965
rs6695348	intron 9	T	67599604

30

10. The method according to claim 8, wherein the at least one polymorphism in the IL-12RB2 gene is selected from the group of IL-12RB2 SNPs consisting of risk allele A at location 67594675 and risk allele C at location 67583944.

11. The method according to any preceding claim, comprising the further step of genotyping DNA from said subject to identify at least one polymorphism within a locus for one or more of an HLA region, STAT4 and CTLA4, IRF5-TNOP3, 17q12-21, and MMEL1, wherein the presence thereof is indicative of PBC.
12. A method for identifying a subject having or at risk for primary biliary cirrhosis (PBC) according to claim 1, wherein a biological sample obtained from the subject is assayed to determine the level of IL-12A protein or IL-12A transcript, the presence of a reduced level thereof indicating the subject has, or is at risk for PBC.
13. The method according to claim 12, wherein the biological sample is assayed to determine the level of IL-12A protein.
14. The method according to claim 13, wherein the level of IL-12A protein is determined using an antibody that binds selectively therewith.
15. The method according to claim 13, wherein the biological sample is assayed to determine the level of IL-12A protein by determining the level of IL-12A protein complex.
16. The method according to claim 15, wherein the level of IL-12A complex is determined by assaying for IL-12 and IL-35.
17. The method according to claim 12, wherein the biological sample is assayed to determine the level of IL-12A transcript.
18. A kit useful to identify a subject having or at risk for primary biliary cirrhosis (PBC), the kit comprising primers useful to amplify an IL-12A gene locus comprising at least one risk allele for PBC, and instructions for the use thereof in identifying at least one polymorphism indicative of PBC.

19. The use of an agent effective to stimulate the IL-12/IL-12R axis in the treatment of an autoimmune disease characterized by reduced levels of IL-12A protein or IL-12A transcripts.
20. The use according to claim 19, wherein the agent is IL-12A.
21. The use according to claim 20, wherein the agent is an IL-12A protein complex selected from IL-12 and IL-35.

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22. The use according to any one of claims 19, 20 and 21, wherein the autoimmune condition is primary biliary cirrhosis.
23. A method for treating a subject having or at risk for primary biliary cirrhosis, comprising administering to a subject in need thereof a treatment effective amount of a composition comprising IL-12A.
24. A method for identifying a subject having or at risk for primary biliary cirrhosis (PBC), wherein a biological sample obtained from the subject is assessed to identify at least one of the following alleles:

20

<i>SNP ID</i>	<i>Allele</i>	<i>Physical Location</i>	<i>Gene</i>
rs17810546	A	161147744	IL-12A
rs583911	G	161193084	IL-12A
rs668998	G	161198245	IL-12A
rs6441284	A	161200962	IL-12A
rs485497	G	161201826	IL-12A
rs4680536	A	161202965	IL-12A
rs9852519	C	161203322	IL-12A
rs4679867	T	161206597	IL-12A
rs4679868	A	161206848	IL-12A
rs6441286	G	161211572	IL-12A
rs574808	T	161215677	IL-12A
rs589545	G	161216294	IL-12A
rs11209050	A	67564324	IL-12RB2
rs1908632	G	67578394	IL-12RB2
rs3790565	C	67583944	IL-12RB2
rs946685	A	67588303	IL-12RB2
rs6679356	C	67592782	IL-12RB2
rs10749775	C	67594675	IL-12RB2
rs3790567	A	67594965	IL-12RB2
rs6695348	T	67599604	IL-12RB2

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rs2856683	C	32763196	HLA-DQB1
rs2395148	A	32429532	C6orf10
rs9277535	G	33162839	HLA-DPB1
rs3806156	A	32481676	BTNL-2
rs10168266	T	191644049	STAT4
rs16833239	G	191648505	STAT4
rs3024921	A	191651517	STAT4
rs907092	A	35175785	IKZF3
rs9303277	C	35229995	IKZF3
rs11557467	G	35282160	ZPBP2
rs8067378	A	35304874	GSDMB
rs2305480	T	35315722	GSDMB
rs2290400	A	35319766	GSDMB
rs7216389	T	35323475	GSDMB
rs4795405	T	35341943	ORMDL3
rs3748816	C	2516606	MMEL1
rs3890745	G	2543484	MMEL1
rs35000415	T	128372852	IRF5-TNPO3
rs2070197	C	128376236	IRF5
rs10488631	C	128381419	IRF5-TNPO3, and
rs12539741	T	128384041	IRF5-TNPO3,

the presence of said at least one allele indicating the subject has, or is at risk for PBC.

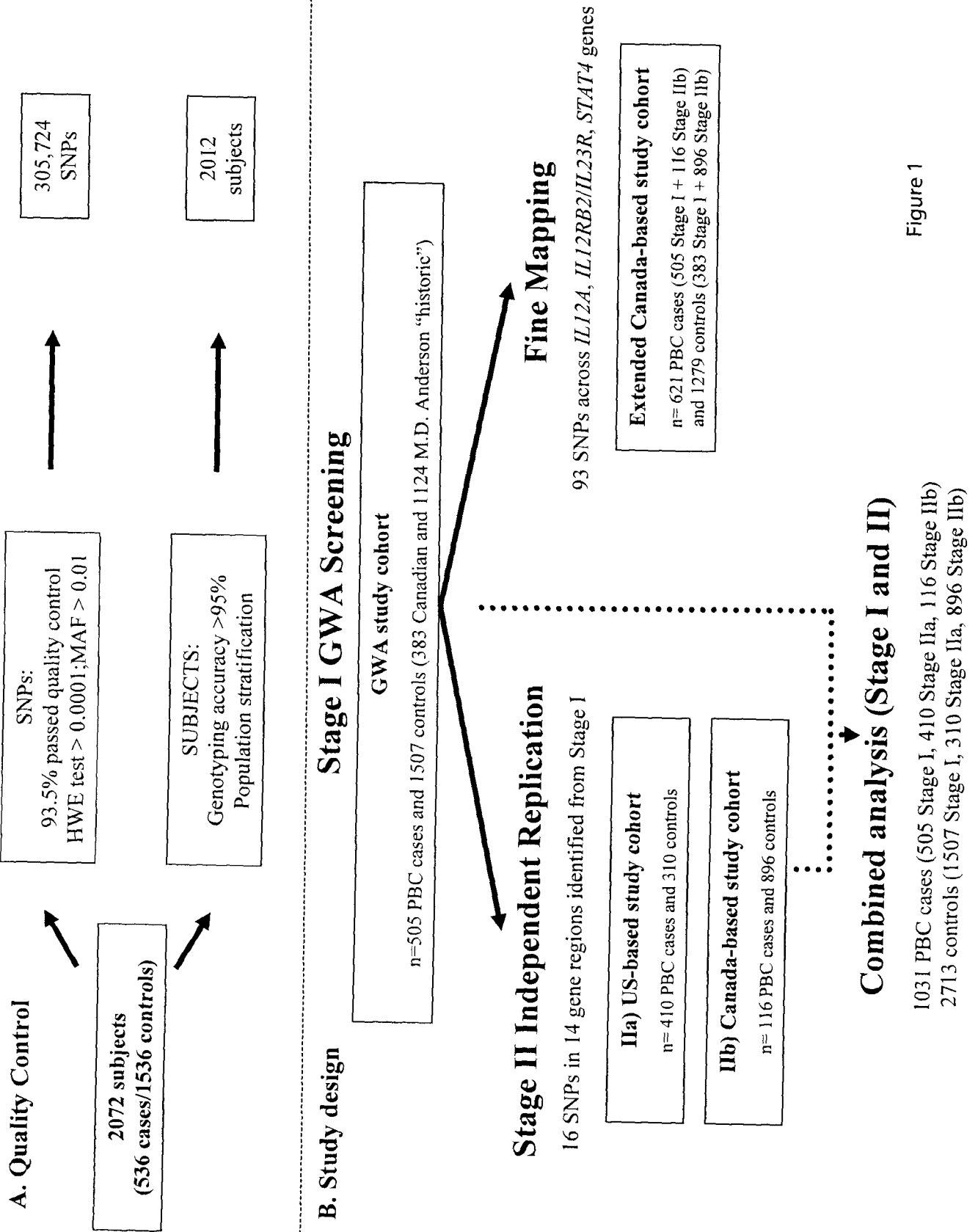
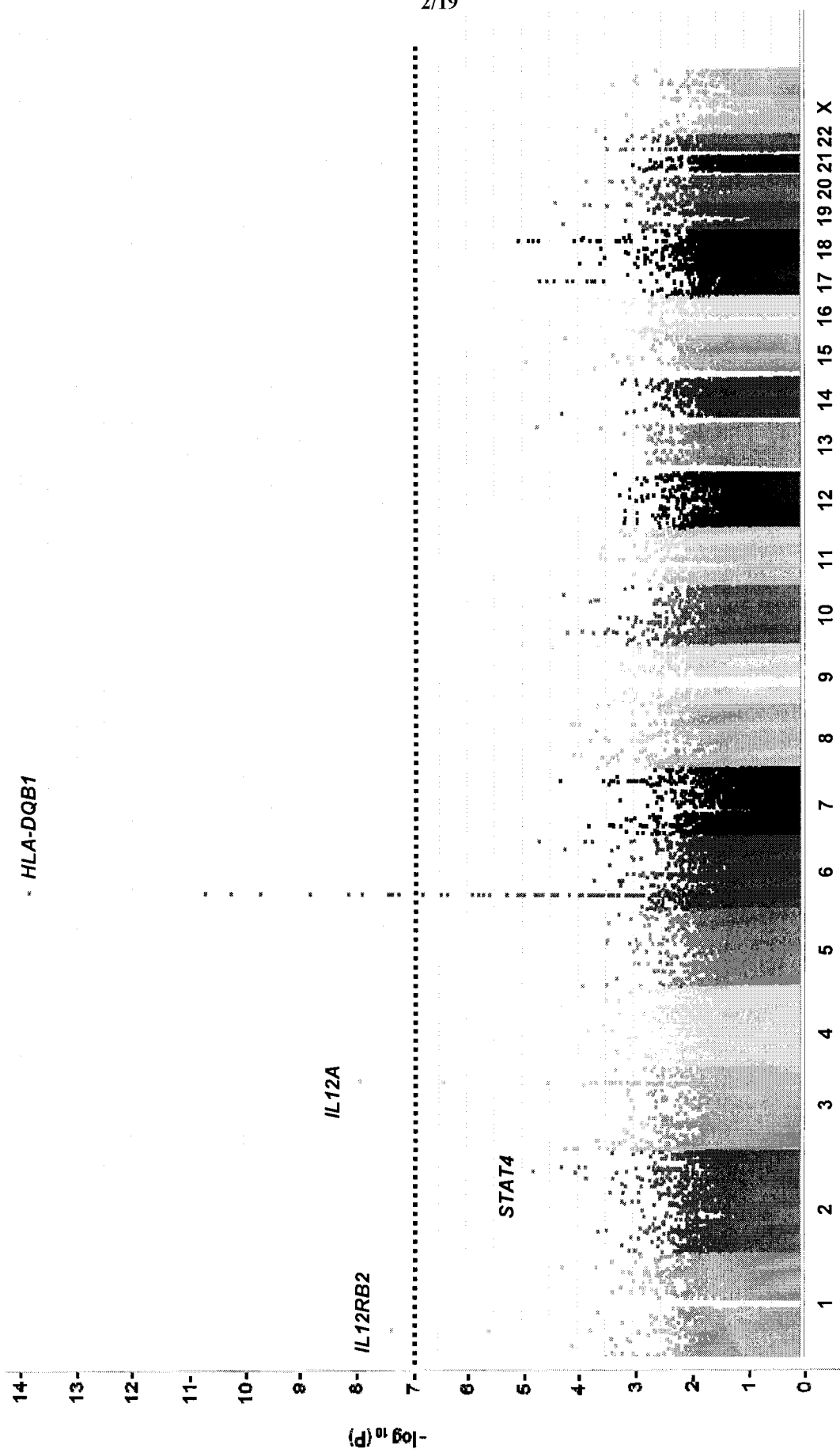


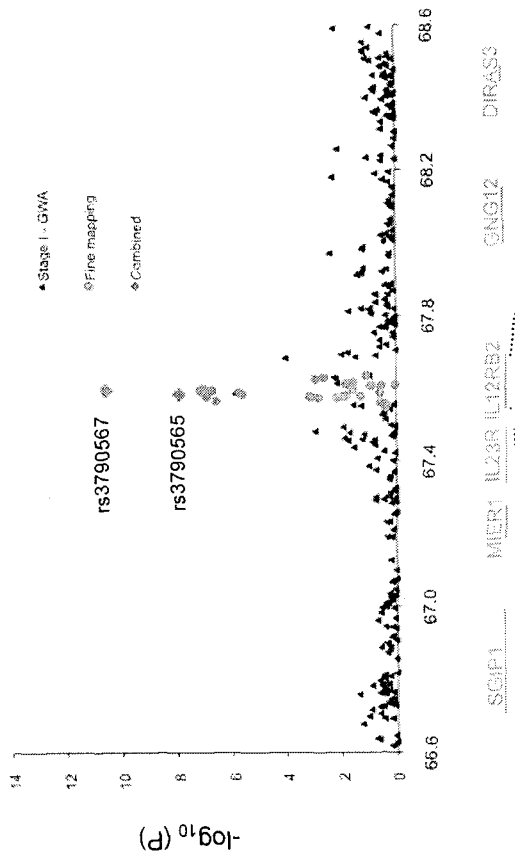
Figure 1



Chromosome Location

Figure 2

B. Association of the *IL12RB2* region on chr. 1p31.2



A. Association of the *IL12A* region on chr. 3q25.33-q26

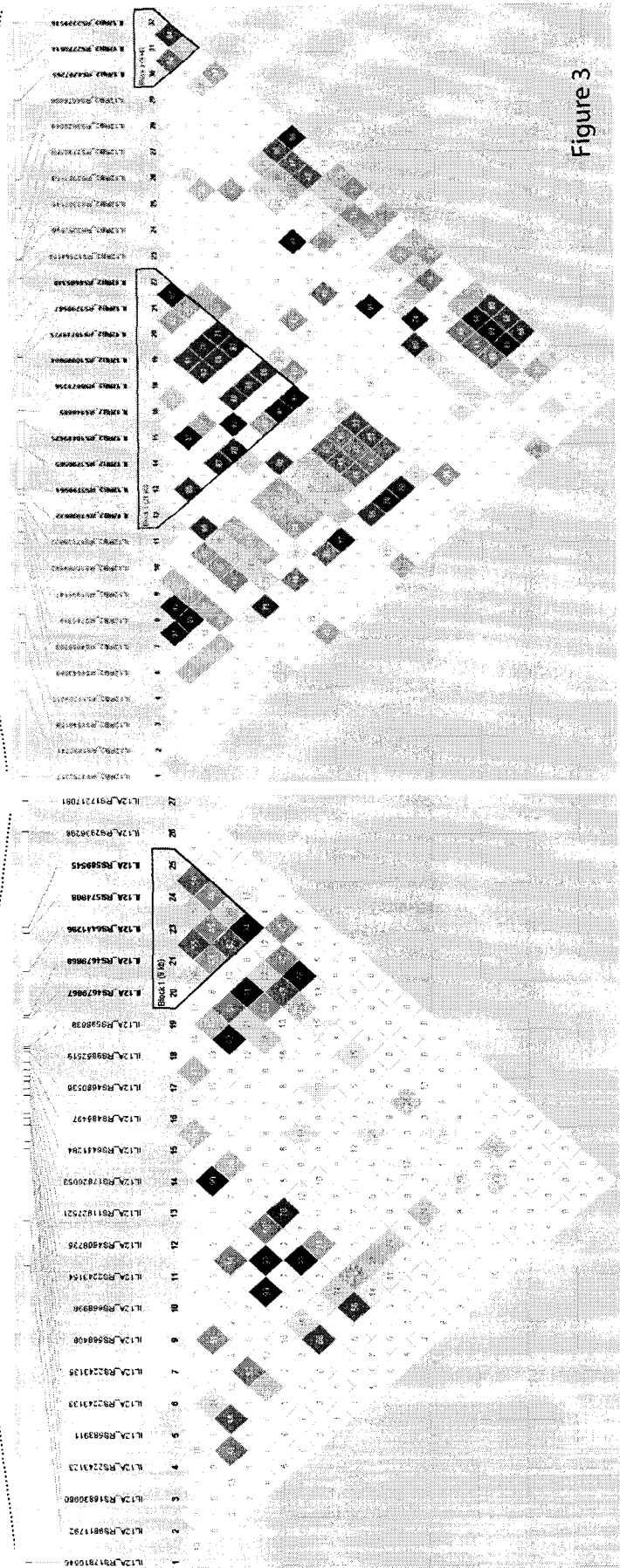
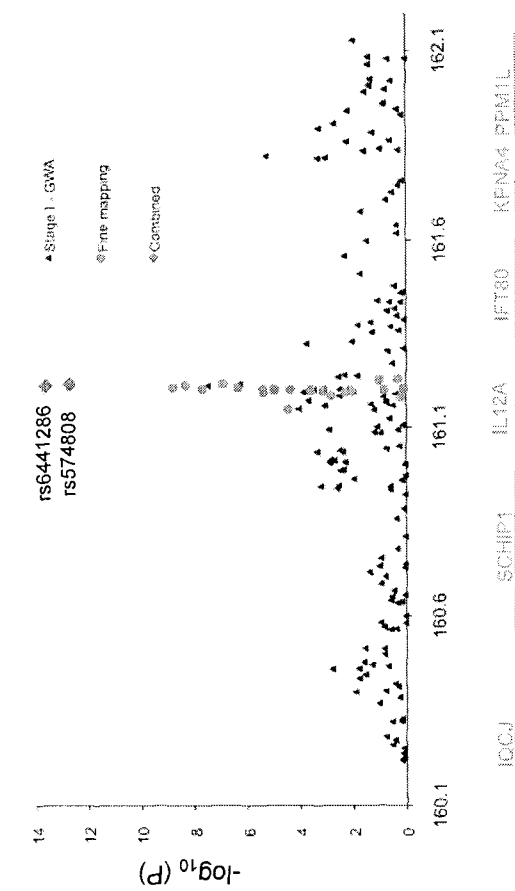


Figure 4 - Table S1

CHR	SNP	Location	Gene name	Genomewide association (stage I)			
				MAF		EIGENSTRAT P	PLINK P
				Cases	Controls		
6	rs7765810	30171475	TRIM31	0.171	0.190	1.24×10^{-2}	4.24×10^{-2}
6	rs1116221	30179309	TRIM31	0.238	0.262	3.70×10^{-2}	1.40×10^{-1}
6	rs2523987	30187972	TRIM31	0.147	0.134	1.42×10^{-2}	1.15×10^{-1}
6	rs3130350	30435818	RPP21	0.106	0.103	2.96×10^{-2}	3.80×10^{-1}
6	rs3094054	30441484	RPP21	0.116	0.108	4.75×10^{-3}	1.61×10^{-1}
6	rs2429657	30579499	HLA-E	0.221	0.243	3.18×10^{-2}	6.23×10^{-2}
6	rs3132610	30652380	ABCF1	0.128	0.119	8.56×10^{-3}	1.57×10^{-1}
6	rs9262143	30760760	NRM	0.123	0.112	4.29×10^{-3}	1.35×10^{-1}
6	rs12660883	30872399	IER3	0.151	0.168	1.94×10^{-2}	2.87×10^{-2}
6	rs886424	30889981	IER3	0.137	0.127	3.65×10^{-2}	2.62×10^{-1}
6	rs4713391	30900214	DDR1	0.148	0.115	1.55×10^{-2}	3.42×10^{-3}
6	rs12192704	30900249	DDR1	0.146	0.114	1.80×10^{-2}	3.91×10^{-3}
6	rs3132580	31028103	DPCR1	0.150	0.140	2.75×10^{-2}	2.33×10^{-1}
6	rs3871466	31091662	C6orf205	0.093	0.123	1.80×10^{-2}	1.66×10^{-2}
6	rs9262495	31094506	C6orf205	0.115	0.137	2.91×10^{-2}	5.68×10^{-2}
6	rs4713411	31095155	C6orf205	0.359	0.414	5.10×10^{-4}	1.27×10^{-3}
6	rs4713412	31095190	C6orf205	0.304	0.353	2.76×10^{-3}	4.69×10^{-3}
6	rs7758976	31095765	C6orf205	0.364	0.414	1.42×10^{-3}	4.47×10^{-3}
6	rs12528584	31119824	C6orf205	0.226	0.179	2.44×10^{-2}	5.39×10^{-3}
6	rs1265099	31213392	PSORS1C2	0.468	0.417	7.58×10^{-3}	3.93×10^{-3}
6	rs9263871	31278507	HCG27	0.403	0.350	4.94×10^{-2}	1.44×10^{-2}
6	rs11967684	31307745	HCG27	0.405	0.364	4.83×10^{-2}	1.51×10^{-2}
6	rs2394963	31359441	HLA-C	0.346	0.298	1.75×10^{-2}	4.62×10^{-3}
6	rs3873379	31370148	HLA-C	0.348	0.302	2.52×10^{-2}	7.56×10^{-3}
6	rs3915971	31377327	HLA-C	0.212	0.247	5.54×10^{-3}	1.59×10^{-2}
6	rs6931332	31382224	HLA-C	0.419	0.454	2.77×10^{-2}	1.51×10^{-1}
6	rs2523554	31439808	HLA-B	0.362	0.367	4.81×10^{-2}	5.88×10^{-1}
6	rs2244839	31546347	HCP5	0.334	0.267	5.61×10^{-3}	2.01×10^{-3}
6	rs2844505	31547042	HCP5	0.208	0.254	3.64×10^{-2}	9.76×10^{-3}
6	rs1055569	31548061	HCP5	0.410	0.335	1.24×10^{-3}	5.82×10^{-4}
6	rs2516440	31548476	HCP5	0.412	0.340	2.20×10^{-3}	1.03×10^{-3}
6	rs2395488	31553888	HCP5	0.421	0.350	3.59×10^{-4}	1.04×10^{-4}
6	rs2248372	31554445	HCP5	0.295	0.322	2.94×10^{-2}	6.59×10^{-2}
6	rs2516424	31556294	HCP5	0.422	0.350	2.78×10^{-4}	6.19×10^{-5}
6	rs2523647	31557757	MICB	0.290	0.222	1.19×10^{-3}	9.89×10^{-5}
6	rs3828903	31572718	MICB	0.298	0.310	2.86×10^{-2}	9.95×10^{-2}
6	rs2269475	31691910	AIF1	0.164	0.115	1.02×10^{-4}	1.99×10^{-5}

6	rs2242660	31705732	BAT2	0.421	0.370	1.92×10^{-2}	4.93×10^{-3}
6	rs1046089	31710946	BAT2	0.392	0.339	2.78×10^{-3}	1.09×10^{-3}
6	rs805303	31724345	BAT3	0.417	0.368	3.38×10^{-2}	9.40×10^{-3}
6	rs2280800	31754377	LY6G5C	0.166	0.115	4.12×10^{-5}	7.68×10^{-6}
6	rs805274	31773173	BAT5	0.315	0.259	3.19×10^{-2}	4.19×10^{-3}
6	rs2242653	31783744	C6orf21	0.184	0.130	3.38×10^{-5}	9.87×10^{-6}
6	rs3749953	31821103	MSH5	0.142	0.110	2.59×10^{-3}	1.68×10^{-3}
6	rs2299851	31826581	MSH5	0.112	0.082	5.85×10^{-3}	2.57×10^{-3}
6	rs2227956	31886251	HSPA1L	0.119	0.173	2.70×10^{-2}	7.66×10^{-4}
6	rs2763979	31902571	HSPA1B	0.394	0.347	4.88×10^{-3}	5.04×10^{-3}
6	rs9368699	31910520	C6orf48	0.029	0.042	2.59×10^{-2}	5.27×10^{-2}
6	rs3115673	31918064	C6orf48	0.169	0.132	5.13×10^{-3}	3.22×10^{-3}
6	rs9267649	31932807	NEU1	0.118	0.173	1.78×10^{-2}	6.61×10^{-4}
6	rs535586	31968316	EHMT2	0.266	0.326	4.74×10^{-2}	6.66×10^{-3}
6	rs4151672	32027809	CFB	0.059	0.040	2.12×10^{-3}	3.41×10^{-3}
6	rs429608	32038441	SKIV2L	0.150	0.139	4.98×10^{-2}	1.20×10^{-1}
6	rs6941112	32054593	STK19	0.312	0.321	3.14×10^{-2}	1.73×10^{-1}
6	rs2269425	32231617	PPT2	0.181	0.150	2.12×10^{-2}	4.50×10^{-3}
6	rs3134603	32233980	PPT2	0.087	0.128	2.30×10^{-2}	1.10×10^{-3}
6	rs3134943	32255739	RNF5	0.083	0.132	6.00×10^{-3}	2.16×10^{-4}
6	rs2070600	32259421	AGER	0.029	0.053	8.01×10^{-3}	6.10×10^{-3}
6	rs206015	32290737	NOTCH4	0.096	0.126	2.10×10^{-2}	1.68×10^{-2}
6	rs404860	32292323	NOTCH4	0.154	0.188	1.68×10^{-2}	2.04×10^{-2}
6	rs2854050	32293583	NOTCH4	0.055	0.070	3.18×10^{-2}	5.58×10^{-2}
6	rs3132946	32298006	NOTCH4	0.080	0.129	6.22×10^{-3}	3.36×10^{-4}
6	rs482759	32302995	NOTCH4	0.212	0.181	4.08×10^{-2}	1.75×10^{-2}
6	rs9267873	32307330	NOTCH4	0.377	0.371	4.35×10^{-2}	1.68×10^{-1}
6	rs382259	32317005	LOC401252	0.274	0.280	3.52×10^{-3}	2.18×10^{-2}
6	rs411326	32319295	LOC401252	0.305	0.254	1.52×10^{-4}	1.46×10^{-4}
6	rs9296015	32326967	LOC401252	0.224	0.190	4.26×10^{-2}	2.04×10^{-2}
6	rs4959089	32327703	LOC401252	0.224	0.189	4.04×10^{-2}	1.93×10^{-2}
6	rs9267992	32328375	LOC401252	0.089	0.132	2.95×10^{-2}	1.53×10^{-3}
6	rs2076537	32425613	C6orf10	0.386	0.350	1.06×10^{-2}	8.56×10^{-3}
6	rs2395148	32429532	C6orf10	0.055	0.019	1.50×10^{-9}	5.62×10^{-10}
6	rs6907322	32432923	C6orf10	0.240	0.172	8.01×10^{-6}	1.16×10^{-6}
6	rs2395150	32434023	C6orf10	0.449	0.408	1.06×10^{-3}	7.64×10^{-3}
6	rs2073048	32443411	C6orf10	0.175	0.120	9.48×10^{-6}	1.77×10^{-6}
6	rs3129939	32444744	C6orf10	0.142	0.175	3.24×10^{-2}	5.43×10^{-2}
6	rs2050190	32447054	C6orf10	0.293	0.322	3.27×10^{-2}	3.13×10^{-2}
6	rs2050189	32447625	C6orf10	0.199	0.227	1.80×10^{-2}	2.41×10^{-2}
6	rs2395157	32456123	C6orf10	0.343	0.285	1.90×10^{-4}	4.64×10^{-5}
6	rs4424066	32462406	BTNL2	0.497	0.425	1.70×10^{-5}	1.74×10^{-6}
6	rs3117098	32466491	BTNL2	0.222	0.305	1.94×10^{-6}	2.89×10^{-8}
6	rs3817973	32469089	BTNL2	0.498	0.425	2.22×10^{-5}	2.30×10^{-6}
6	rs2076530	32471794	BTNL2	0.492	0.434	4.05×10^{-5}	5.13×10^{-6}

6	rs4248166	32474399	BTNL2	0.223	0.153	2.15×10^{-5}	4.93×10^{-7}
6	rs3817963	32476065	BTNL2	0.351	0.291	1.61×10^{-4}	3.80×10^{-5}
6	rs3763305	32477466	BTNL2	0.082	0.047	1.14×10^{-4}	2.97×10^{-5}
6	rs3806156	32481676	BTNL2	0.457	0.354	1.31×10^{-8}	1.27×10^{-9}
6	rs3763309	32483951	BTNL2	0.259	0.214	4.99×10^{-3}	9.88×10^{-4}
6	rs3763313	32484449	BTNL2	0.249	0.185	7.33×10^{-4}	3.90×10^{-5}
6	rs3129963	32488186	BTNL2	0.142	0.181	7.18×10^{-3}	1.54×10^{-2}
6	rs2395163	32495787	BTNL2	0.262	0.210	1.10×10^{-3}	2.23×10^{-4}
6	rs3135363	32497626	BTNL2	0.216	0.292	4.46×10^{-8}	3.72×10^{-7}
6	rs9501626	32508322	HLA-DRA	0.173	0.110	4.43×10^{-7}	6.83×10^{-7}
6	rs2395175	32513004	HLA-DRA	0.192	0.165	1.22×10^{-2}	4.43×10^{-3}
6	rs3129882	32517508	HLA-DRA	0.363	0.409	7.30×10^{-3}	1.66×10^{-3}
6	rs6903608	32536263	HLA-DRA	0.236	0.306	1.52×10^{-4}	7.68×10^{-6}
6	rs2395185	32541145	HLA-DRA	0.386	0.336	6.80×10^{-3}	2.47×10^{-3}
6	rs2516049	32678378	HLA-DRB1	0.367	0.319	4.47×10^{-3}	1.17×10^{-3}
6	rs660895	32685358	HLA-DRB1	0.277	0.195	3.41×10^{-7}	4.68×10^{-8}
6	rs9271366	32694832	HLA-DQA1	0.090	0.136	8.35×10^{-3}	4.92×10^{-4}
6	rs3129763	32698903	HLA-DQA1	0.189	0.243	1.59×10^{-6}	3.93×10^{-5}
6	rs9275184	32762692	HLA-DQB1	0.151	0.101	2.75×10^{-4}	2.19×10^{-5}
6	rs2856683	32763196	HLA-DQB1	0.360	0.222	1.34×10^{-14}	8.58×10^{-17}
6	rs7775228	32766057	HLA-DQB1	0.207	0.122	1.90×10^{-10}	1.70×10^{-10}
6	rs2157051	32766602	HLA-DQB1	0.131	0.198	1.00×10^{-3}	2.22×10^{-5}
6	rs2647012	32772436	HLA-DQB1	0.311	0.393	1.06×10^{-3}	1.61×10^{-4}
6	rs9357152	32772938	HLA-DQB1	0.184	0.259	3.88×10^{-8}	7.25×10^{-8}
6	rs9275312	32773706	HLA-DQB1	0.227	0.128	1.99×10^{-11}	3.84×10^{-13}
6	rs9275390	32777134	HLA-DQB1	0.373	0.246	5.73×10^{-11}	1.13×10^{-13}
6	rs2647050	32777745	HLA-DQB1	0.317	0.366	5.08×10^{-4}	7.25×10^{-4}
6	rs2856718	32778233	HLA-DQB1	0.316	0.365	4.25×10^{-4}	5.89×10^{-4}
6	rs2856705	32778934	HLA-DQB1	0.133	0.106	1.78×10^{-2}	2.49×10^{-2}
6	rs3873444	32790702	HLA-DQA2	0.088	0.109	4.42×10^{-2}	2.07×10^{-2}
6	rs3916765	32793528	HLA-DQA2	0.143	0.113	9.21×10^{-3}	6.09×10^{-3}
6	rs9276644	32853021	HLA-DQB2	0.286	0.351	6.90×10^{-5}	5.53×10^{-5}
6	rs719654	32860117	HLA-DQB2	0.285	0.218	7.42×10^{-4}	4.57×10^{-5}
6	rs7758736	32866372	HLA-DOB	0.146	0.179	3.92×10^{-2}	4.09×10^{-2}
6	rs3948793	32867426	HLA-DOB	0.293	0.355	1.74×10^{-4}	1.41×10^{-4}
6	rs1383261	32873429	HLA-DOB	0.310	0.346	1.66×10^{-2}	2.01×10^{-2}
6	rs2219893	32877641	HLA-DOB	0.377	0.315	7.68×10^{-3}	8.82×10^{-4}
6	rs2856997	32889754	HLA-DOB	0.463	0.401	3.73×10^{-2}	3.96×10^{-3}
6	rs7383287	32891064	HLA-DOB	0.182	0.219	1.46×10^{-2}	1.52×10^{-2}
6	rs13501	32901501	TAP2	0.373	0.315	4.27×10^{-2}	3.40×10^{-3}
6	rs4576294	32906526	TAP2	0.132	0.106	3.74×10^{-2}	6.37×10^{-3}
6	rs9357155	32917826	PSMB8	0.150	0.115	1.46×10^{-2}	3.46×10^{-3}
6	rs2071543	32919607	PSMB8	0.157	0.125	3.08×10^{-2}	9.43×10^{-3}
6	rs2071482	32924678	TAP1	0.182	0.138	7.76×10^{-3}	6.11×10^{-3}
6	rs4713600	32930836	PSMB9	0.384	0.424	6.10×10^{-3}	6.71×10^{-3}

6	rs2071477	32933326	PSMB9	0.384	0.424	6.18×10^{-3}	6.74×10^{-3}
6	rs6929078	32946642	PSMB9	0.322	0.374	2.28×10^{-4}	6.27×10^{-4}
6	rs7757767	32953851	PSMB9	0.322	0.374	2.46×10^{-4}	6.68×10^{-4}
6	rs241407	32970718	PSMB9	0.125	0.096	4.75×10^{-2}	1.44×10^{-2}
6	rs241404	32973975	HLA-DMB	0.399	0.431	3.76×10^{-2}	8.92×10^{-2}
6	rs9296057	32977893	HLA-DMB	0.096	0.074	1.41×10^{-2}	2.46×10^{-2}
6	rs241398	32983428	HLA-DMB	0.398	0.430	3.51×10^{-2}	8.68×10^{-2}
6	rs154986	32987211	HLA-DMB	0.091	0.073	3.57×10^{-2}	5.52×10^{-2}
6	rs3129299	33008765	HLA-DMB	0.216	0.164	2.83×10^{-3}	1.89×10^{-3}
6	rs2071556	33012579	HLA-DMB	0.364	0.394	4.44×10^{-2}	5.46×10^{-2}
6	rs1042337	33012959	HLA-DMB	0.190	0.225	3.96×10^{-2}	3.97×10^{-2}
6	rs2395296	33019792	HLA-DMB	0.257	0.320	2.26×10^{-4}	2.27×10^{-4}
6	rs194682	33022591	HLA-DMA	0.381	0.323	8.74×10^{-4}	6.41×10^{-4}
6	rs209473	33030886	HLA-DMA	0.400	0.453	1.48×10^{-3}	2.94×10^{-3}
6	rs209474	33032562	HLA-DMA	0.453	0.394	5.34×10^{-4}	1.18×10^{-3}
6	rs565876	33058028	BRD2	0.413	0.355	3.41×10^{-4}	9.07×10^{-4}
6	rs3135034	33059640	BRD2	0.050	0.079	7.00×10^{-3}	7.71×10^{-3}
6	rs188245	33063954	BRD2	0.499	0.456	2.02×10^{-3}	5.07×10^{-3}
6	rs206767	33070398	HLA-DOA	0.425	0.476	7.86×10^{-4}	3.33×10^{-3}
6	rs423639	33095752	HLA-DOA	0.111	0.075	3.93×10^{-3}	1.61×10^{-3}
6	rs6457702	33096027	HLA-DOA	0.483	0.442	4.58×10^{-3}	2.56×10^{-3}
6	rs7774158	33115730	HLA-DPA1	0.332	0.368	1.29×10^{-2}	1.12×10^{-2}
6	rs9277535	33162839	HLA-DPB1	0.326	0.236	7.26×10^{-9}	8.28×10^{-9}
6	rs9277554	33163516	HLA-DPB1	0.358	0.293	3.29×10^{-5}	3.16×10^{-5}
6	rs9277565	33164875	HLA-DPB1	0.275	0.198	5.94×10^{-8}	8.32×10^{-8}
6	rs2281389	33167774	HLA-DPB1	0.236	0.168	1.58×10^{-7}	2.38×10^{-7}
6	rs3128917	33167974	HLA-DPB1	0.308	0.254	1.77×10^{-4}	1.78×10^{-4}
6	rs3117222	33168927	HLA-DPB1	0.308	0.255	2.06×10^{-4}	2.20×10^{-4}
6	rs2064478	33180244	HLA-DPB2	0.270	0.226	7.22×10^{-4}	7.71×10^{-4}
6	rs3130215	33182941	HLA-DPB2	0.337	0.403	2.35×10^{-3}	1.41×10^{-3}
6	rs3117230	33183613	HLA-DPB2	0.269	0.226	9.24×10^{-4}	9.94×10^{-4}
6	rs1883414	33194426	HLA-DPB2	0.376	0.306	1.90×10^{-5}	1.56×10^{-5}
6	rs4713607	33198814	HLA-DPB2	0.460	0.495	3.69×10^{-2}	3.61×10^{-2}
6	rs3129274	33202847	HLA-DPB2	0.268	0.317	1.65×10^{-2}	2.02×10^{-2}
6	rs3117016	33203494	HLA-DPB2	0.466	0.399	2.58×10^{-3}	9.93×10^{-4}
6	rs3117008	33204252	HLA-DPB2	0.457	0.496	3.75×10^{-2}	3.30×10^{-2}
6	rs6901221	33206254	HLA-DPB2	0.208	0.155	1.07×10^{-5}	2.17×10^{-5}
6	rs2294478	33206944	HLA-DPB2	0.452	0.483	2.15×10^{-2}	1.11×10^{-2}
6	rs4713610	33215933	HLA-DPB2	0.149	0.168	1.43×10^{-2}	2.77×10^{-2}
6	rs7754200	33218911	HLA-DPB2	0.248	0.222	9.33×10^{-3}	2.29×10^{-2}
6	rs3129234	33219325	HLA-DPB2	0.248	0.222	1.09×10^{-2}	2.64×10^{-2}
6	rs2395365	33224200	COL11A2	0.420	0.394	2.13×10^{-2}	5.28×10^{-2}
6	rs756441	33230149	COL11A2	0.420	0.394	1.99×10^{-2}	5.11×10^{-2}
6	rs756440	33230309	COL11A2	0.246	0.222	1.20×10^{-2}	3.01×10^{-2}
6	rs9368758	33245999	COL11A2	0.087	0.070	4.75×10^{-2}	8.84×10^{-2}

6	rs9277932	33249231	COL11A2	0.470	0.447	2.28×10^{-2}	1.71×10^{-2}
6	rs2855430	33249258	COL11A2	0.182	0.136	8.81×10^{-6}	1.11×10^{-5}
6	rs3762013	33250517	COL11A2	0.471	0.447	2.01×10^{-2}	1.50×10^{-2}
6	rs2855459	33262634	COL11A2	0.183	0.135	5.22×10^{-6}	5.73×10^{-6}
6	rs2269346	33266876	COL11A2	0.087	0.070	4.03×10^{-2}	8.50×10^{-2}
6	rs439205	33281820	HSD17B8	0.308	0.241	1.22×10^{-6}	1.07×10^{-6}
6	rs421446	33282761	HSD17B8	0.349	0.278	2.50×10^{-6}	4.80×10^{-6}
6	rs2854028	33287667	RING1	0.222	0.244	3.60×10^{-2}	4.24×10^{-2}
6	rs213220	33310618	VPS52	0.469	0.455	3.14×10^{-2}	6.66×10^{-2}
6	rs464921	33348484	RPS18	0.140	0.159	4.14×10^{-2}	4.98×10^{-2}
6	rs213207	33349147	RPS18	0.464	0.452	3.36×10^{-2}	5.66×10^{-2}
6	rs3106192	33434228	KIFC1	0.458	0.421	1.14×10^{-2}	1.25×10^{-2}
6	rs9278019	33434412	KIFC1	0.123	0.143	2.90×10^{-2}	3.42×10^{-2}
6	rs3130276	33444908	KIFC1	0.379	0.345	4.11×10^{-2}	4.41×10^{-2}
6	rs461964	33468552	KIFC1	0.400	0.356	8.83×10^{-3}	9.75×10^{-3}
6	rs9394145	33507756	SYNGAP1	0.310	0.262	2.19×10^{-3}	3.80×10^{-3}
6	rs6914422	33642858	BAK1	0.294	0.259	2.13×10^{-2}	4.48×10^{-2}
6	rs649775	33792291	C6orf125	0.107	0.081	2.35×10^{-3}	1.13×10^{-3}
6	rs6457740	33805103	IHPK3	0.296	0.271	4.92×10^{-2}	3.58×10^{-2}
6	rs9461924	33962794	MLN	0.235	0.262	3.87×10^{-2}	3.90×10^{-2}
6	rs9461926	33964881	MLN	0.339	0.369	4.12×10^{-2}	2.28×10^{-2}
6	rs2495972	34040420	GRM4	0.449	0.416	3.18×10^{-2}	2.69×10^{-2}
6	rs4713755	34243213	GRM4	0.470	0.445	4.61×10^{-2}	4.64×10^{-2}
6	rs10947494	34371721	NUDT3	0.491	0.464	4.75×10^{-2}	8.17×10^{-2}
6	rs206937	34440157	NUDT3	0.269	0.251	3.15×10^{-2}	6.09×10^{-2}

Figure 5 - Table 2

CHR	SNP	Location	Gene name	Genomewide Association (stage I)			
				MAF		Eigenstrat P	Plink P
				Cases	Control		
1	rs3748816	2516606	MELL1	0.411	0.342	5.58X10 ⁻⁴	8.48X10 ⁻⁵
1	rs4417082	5248130	AJAP1	0.467	0.403	3.20X10 ⁻⁴	2.58X10 ⁻⁴
1	rs6660541	11370392	UBIAD1	0.495	0.442	4.53X10 ⁻⁴	3.44X10 ⁻⁴
1	rs407829	13807728	PDPN	0.287	0.346	6.30X10 ⁻⁴	1.22X10 ⁻⁴
1	rs904910	16072750	SPEN	0.347	0.289	4.24X10 ⁻⁴	4.44X10 ⁻⁴
1	rs11588497	32803048	ZBTB8	0.147	0.197	7.79X10 ⁻⁵	3.59X10 ⁻⁵
1	rs360490	32993869	KIAA1522	0.253	0.299	4.81X10 ⁻⁴	1.25X10 ⁻³
1	rs783622	42139575	HIVEP3	0.406	0.459	8.48X10 ⁻⁴	9.98X10 ⁻⁴
1	rs12742768	47837606	FOXD2	0.090	0.124	4.04X10 ⁻⁴	6.30X10 ⁻⁴
1	rs6660568	47855078	FOXD2	0.074	0.103	7.79X10 ⁻⁴	1.47X10 ⁻³
1	rs3790565	67583944	IL12RB2	0.258	0.183	2.50X10 ⁻⁶	1.41X10 ⁻⁶
1	rs3790567	67594965	IL12RB2	0.341	0.240	4.51X10 ⁻⁸	8.60X10 ⁻⁸
1	rs10889687	67682370	SERBP1	0.211	0.160	1.67X10 ⁻⁴	9.91X10 ⁻⁵
1	rs1199673	87360437	LOC339524	0.413	0.469	6.28X10 ⁻⁴	2.06X10 ⁻³
1	rs1418426	111299634	C1orf103	0.319	0.383	2.24X10 ⁻⁴	3.98X10 ⁻⁵
1	rs1802778	158570040	COPA	0.484	0.445	8.96X10 ⁻⁴	4.63X10 ⁻⁴
1	rs912584	158612458	NHLH1	0.474	0.440	4.24X10 ⁻⁴	8.49X10 ⁻⁵
1	rs10800546	169074210	C1orf129	0.428	0.480	5.94X10 ⁻⁴	7.75X10 ⁻⁴
1	rs946263	201432004	CHI3L1	0.209	0.173	8.81X10 ⁻⁴	4.29X10 ⁻⁴
1	rs1856746	205210045	FCAMR	0.481	0.448	6.25X10 ⁻⁴	5.48X10 ⁻⁴
1	rs2353550	205213460	FCAMR	0.484	0.420	8.29X10 ⁻⁴	6.91X10 ⁻⁴
1	rs946468	214903199	ESRRG	0.225	0.182	5.15X10 ⁻⁵	2.88X10 ⁻⁴
1	rs2813711	214946534	ESRRG	0.196	0.159	2.12X10 ⁻⁴	7.88X10 ⁻⁴
1	rs2046614	228502361	GALNT2	0.389	0.456	4.06X10 ⁻⁴	3.53X10 ⁻⁴
2	rs4668535	7341604	RNF144	0.215	0.175	8.13X10 ⁻⁴	1.04X10 ⁻³
2	rs312046	21327992	APOB	0.266	0.317	6.13X10 ⁻⁴	5.45X10 ⁻³
2	rs3845790	38332007	ARL6IP2	0.094	0.061	9.01X10 ⁻⁴	4.93X10 ⁻³
2	rs12994401	51925750	NRXN1	0.244	0.184	2.27X10 ⁻⁴	2.84X10 ⁻⁴
2	rs1232	53935909	GPR75	0.290	0.237	3.20X10 ⁻⁴	1.69X10 ⁻³
2	rs10864955	117896829	DDX18	0.138	0.191	6.81X10 ⁻⁴	1.35X10 ⁻⁴
2	rs2140778	120942028	FLJ14816	0.121	0.084	9.13X10 ⁻⁴	1.96X10 ⁻³
2	rs6541772	121693024	TFCP2L1	0.186	0.139	6.02X10 ⁻⁴	6.98X10 ⁻⁴
2	rs1435591	142356238	LRP1B	0.176	0.131	6.36X10 ⁻⁴	2.10X10 ⁻⁴
2	rs351678	143434244	KYNU	0.423	0.352	3.73X10 ⁻⁴	1.64X10 ⁻⁴
2	rs768072	159941629	BAZ2B	0.222	0.169	7.16X10 ⁻⁴	1.30X10 ⁻³
2	rs2176241	164593953	FIGN	0.143	0.102	6.10X10 ⁻⁴	9.16X10 ⁻⁴
2	rs4893912	177232140	MTX2	0.391	0.452	4.18X10 ⁻⁴	7.87X10 ⁻⁵
2	rs2710024	177240458	MTX2	0.210	0.271	1.36X10 ⁻⁴	2.88X10 ⁻⁵

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2	rs2362866	177246512	MTX2	0.290	0.358	1.37X10 ⁻⁴	1.92X10 ⁻⁵
2	rs10931468	191246807	NAB1	0.174	0.121	8.70X10 ⁻⁵	4.47X10 ⁻⁵
2	rs714616	191369094	GLS	0.277	0.224	4.88X10 ⁻⁴	1.43X10 ⁻³
2	rs16833239	191648505	STAT4	0.038	0.079	1.55X10 ⁻⁵	2.60X10 ⁻⁵
2	rs7601754	191648696	STAT4	0.138	0.177	7.40X10 ⁻⁴	2.25X10 ⁻³
2	rs231735	204402121	CTLA4	0.421	0.493	1.36X10 ⁻⁴	6.79X10 ⁻⁵
2	rs231804	204416891	CTLA4	0.364	0.436	1.02X10 ⁻⁴	2.47X10 ⁻⁵
2	rs6748358	204465150	CTLA4	0.426	0.497	4.91X10 ⁻⁵	1.41X10 ⁻⁵
2	rs6861	233868852	ATG16L1	0.247	0.311	9.59X10 ⁻⁴	2.65X10 ⁻⁴
2	rs7607671	238163387	RAB17	0.048	0.080	4.05X10 ⁻⁴	1.30X10 ⁻³
3	rs923359	3916390	LRRN1	0.122	0.169	8.41X10 ⁻⁵	1.72X10 ⁻⁴
3	rs167601	4024724	LRRN1	0.235	0.280	4.27X10 ⁻⁴	8.70X10 ⁻⁴
3	rs317530	4069293	LRRN1	0.326	0.281	9.43X10 ⁻⁴	1.30X10 ⁻³
3	rs4685976	5716702	EDEM1	0.494	0.436	5.88X10 ⁻⁵	1.06X10 ⁻⁵
3	rs1869241	5729732	EDEM1	0.377	0.306	9.52X10 ⁻⁵	5.44X10 ⁻⁶
3	rs9311642	5748145	EDEM1	0.455	0.390	3.31X10 ⁻⁴	1.43X10 ⁻⁴
3	rs619481	8182706	LMCD1	0.122	0.169	2.40X10 ⁻⁴	1.38X10 ⁻⁴
3	rs1372072	16930263	PLCL2	0.427	0.358	5.38X10 ⁻⁴	2.36X10 ⁻⁴
3	rs9876137	16936269	PLCL2	0.427	0.359	5.61X10 ⁻⁴	2.64X10 ⁻⁴
3	rs1025818	16940025	PLCL2	0.408	0.339	4.17X10 ⁻⁴	2.23X10 ⁻⁴
3	rs1868575	24305287	THRB	0.325	0.397	2.26X10 ⁻⁴	2.37X10 ⁻⁴
3	rs1158265	24311166	THRB	0.242	0.309	2.08X10 ⁻⁴	1.28X10 ⁻⁴
3	rs7622481	24326875	THRB	0.227	0.284	6.79X10 ⁻⁴	7.57X10 ⁻⁴
3	rs4796	32498781	CMTM6	0.400	0.346	9.21X10 ⁻⁴	6.13X10 ⁻⁴
3	rs4683017	45009415	EXOSC7	0.408	0.463	6.99X10 ⁻⁴	8.43X10 ⁻⁴
3	rs11130040	45024190	EXOSC7	0.425	0.479	7.81X10 ⁻⁴	1.14X10 ⁻³
3	rs3197999	49696536	MST1	0.348	0.285	6.64X10 ⁻⁴	1.00X10 ⁻³
3	rs4459877	77556151	ROBO2	0.094	0.064	8.55X10 ⁻⁴	1.33X10 ⁻³
3	rs1404100	100273457	DCBLD2	0.257	0.205	9.97X10 ⁻⁴	3.79X10 ⁻⁴
3	rs2293370	120702624	C3orf1	0.142	0.189	8.86X10 ⁻⁴	8.31X10 ⁻⁴
3	rs6806898	133145979	CPNE4	0.153	0.117	7.72X10 ⁻⁴	9.93X10 ⁻⁴
3	rs6440041	143034571	GRK7	0.254	0.316	7.82X10 ⁻⁴	4.41X10 ⁻⁴
3	rs11917011	155711698	DHX36	0.032	0.064	1.25X10 ⁻⁴	1.84X10 ⁻⁴
3	rs2128535	158271886	FLJ16641	0.297	0.353	9.38X10 ⁻⁴	1.52X10 ⁻³
3	rs4680514	160944150	SCHIP1	0.187	0.237	3.80X10 ⁻⁴	5.98X10 ⁻⁴
3	rs6441276	161034716	SCHIP1	0.183	0.244	5.63X10 ⁻⁴	4.46X10 ⁻⁴
3	rs17810546	161147744	IL12A	0.068	0.114	2.05X10 ⁻⁴	8.77X10 ⁻⁵
3	rs17811014	161170723	IL12A	0.212	0.159	3.89X10 ⁻⁴	2.08X10 ⁻⁴
3	rs583911	161193084	IL12A	0.490	0.438	1.67X10 ⁻⁴	1.35X10 ⁻⁴
3	rs485497	161201826	IL12A	0.428	0.499	4.48X10 ⁻⁴	3.38X10 ⁻⁴
3	rs6441286	161211572	IL12A	0.497	0.390	1.20X10 ⁻⁸	3.25X10 ⁻⁸
3	rs574808	161215677	IL12A	0.322	0.426	3.81X10 ⁻⁷	5.34X10 ⁻⁷
3	rs6762377	161323322	IL12A	0.078	0.128	3.06X10 ⁻⁴	1.74X10 ⁻⁴
3	rs1920649	161816712	KPNA4	0.343	0.408	7.60X10 ⁻⁴	4.89X10 ⁻⁴
3	rs4679904	161823590	ARL14	0.202	0.281	2.79X10 ⁻⁵	5.38X10 ⁻⁶

3	rs1460925	193376749	FGF12	0.112	0.075	4.70X10 ⁻⁴	8.50X10 ⁻⁴
4	rs1501145	16160509	LDB2	0.143	0.105	7.91X10 ⁻⁴	1.40X10 ⁻³
4	rs7678820	22901080	GBA3	0.128	0.176	2.43X10 ⁻⁴	7.14X10 ⁻⁴
4	rs10222962	32036553	PCDH7	0.111	0.070	1.55X10 ⁻⁵	1.67X10 ⁻⁵
4	rs11133215	32081629	PCDH7	0.422	0.354	5.14X10 ⁻⁴	3.51X10 ⁻⁴
4	rs2166096	36580530	CENTD1	0.459	0.393	4.92X10 ⁻⁴	4.56X10 ⁻⁴
4	rs6838613	48171296	SLC10A4	0.478	0.453	8.38X10 ⁻⁴	2.02X10 ⁻⁴
4	rs2046107	71156441	CSN3	0.158	0.128	7.81X10 ⁻⁴	9.37X10 ⁻⁴
4	rs722113	71197092	NYD-SP26	0.198	0.250	8.22X10 ⁻⁴	3.07X10 ⁻⁴
4	rs2869442	87640302	MAPK10	0.078	0.049	1.51X10 ⁻⁴	4.34X10 ⁻⁴
4	rs10516775	87840497	PTPN13	0.074	0.047	3.96X10 ⁻⁴	7.70X10 ⁻⁴
4	rs4446314	97988643	MGC46496	0.150	0.195	2.75X10 ⁻⁴	4.38X10 ⁻⁴
4	rs7655049	106271913	FLJ20032	0.181	0.143	8.48X10 ⁻⁴	5.71X10 ⁻⁴
4	rs1358229	123116184	TRPC3	0.479	0.442	3.45X10 ⁻⁵	6.34X10 ⁻⁶
4	rs6838639	123118615	TRPC3	0.196	0.271	1.36X10 ⁻⁶	3.45X10 ⁻⁷
4	rs17009825	126628036	FAT4	0.046	0.079	5.37X10 ⁻⁴	3.83X10 ⁻⁴
4	rs2291713	141764847	TBC1D9	0.184	0.135	4.68X10 ⁻⁵	7.53X10 ⁻⁵
4	rs4835362	148189967	NYD-SP14	0.200	0.158	9.48X10 ⁻⁴	1.75X10 ⁻³
4	rs1434412	158659335	GRIA2	0.152	0.110	5.27X10 ⁻⁴	3.33X10 ⁻⁴
4	rs725037	158663072	GRIA2	0.153	0.109	3.15X10 ⁻⁴	1.90X10 ⁻⁴
4	rs4690488	178289408	NEIL3	0.337	0.279	8.70X10 ⁻⁴	5.13X10 ⁻⁴
5	rs931283	2440550	IRX2	0.414	0.485	1.18X10 ⁻⁴	2.10X10 ⁻⁴
5	rs1908976	2842932	CEI	0.363	0.423	3.23X10 ⁻⁴	3.83X10 ⁻⁴
5	rs2136101	20440037	CDH18	0.357	0.420	4.90X10 ⁻⁴	8.23X10 ⁻⁴
5	rs12520102	54071686	SNAG1	0.482	0.453	9.92X10 ⁻⁴	7.74X10 ⁻⁴
5	rs2301079	83355369	EDIL3	0.187	0.250	9.47X10 ⁻⁴	1.15X10 ⁻⁴
5	rs3846608	101372656	SLCO4C1	0.348	0.293	3.25X10 ⁻⁴	6.96X10 ⁻⁴
5	rs397655	101594674	SLCO4C1	0.312	0.375	8.24X10 ⁻⁴	1.73X10 ⁻⁴
5	rs7716454	107310687	FBXL17	0.175	0.228	3.36X10 ⁻⁴	1.74X10 ⁻⁴
5	rs12651994	147036387	JAKMIP2	0.150	0.102	3.51X10 ⁻⁴	2.05X10 ⁻⁴
5	rs1422850	159035655	ADRA1B	0.490	0.420	8.50X10 ⁻⁴	4.58X10 ⁻⁴
5	rs2287745	169551421	LCP2	0.112	0.077	5.22X10 ⁻⁴	2.55X10 ⁻⁴
6	rs10484984	54568605	TINAG	0.493	0.426	5.74X10 ⁻⁴	1.45X10 ⁻⁴
6	rs3003684	64829301	PHF3	0.030	0.052	6.10X10 ⁻⁴	2.77X10 ⁻³
6	rs12196601	65351159	EGFL11	0.294	0.237	1.89X10 ⁻⁴	1.47X10 ⁻³
6	rs9363107	65463971	EGFL11	0.188	0.245	5.30X10 ⁻⁴	1.21X10 ⁻⁴
6	rs9361785	82064105	FAM46A	0.074	0.096	7.46X10 ⁻⁴	4.59X10 ⁻³
6	rs9443958	82885557	IBTK	0.118	0.170	9.58X10 ⁻⁴	3.39X10 ⁻⁴
6	rs7750307	102742534	GRIK2	0.231	0.289	3.57X10 ⁻⁴	6.89X10 ⁻⁴
6	rs2858829	116875610	SART2	0.361	0.417	9.73X10 ⁻⁴	2.25X10 ⁻³
6	rs4945684	121759194	GJA1	0.287	0.343	8.91X10 ⁻⁴	1.84X10 ⁻³
6	rs3799488	137561473	IFNGR1	0.096	0.131	3.92X10 ⁻⁴	1.34X10 ⁻³
6	rs13216266	140059450	CITED2	0.397	0.321	5.56X10 ⁻⁵	7.55X10 ⁻⁶
6	rs1883447	144429391	SF3B5	0.164	0.116	4.97X10 ⁻⁴	6.66X10 ⁻⁴
6	rs6929952	158571383	GTF2H5	0.353	0.420	1.31X10 ⁻⁴	1.43X10 ⁻⁴

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6	rs262825	158598619	TULP4	0.430	0.498	1.92X10 ⁻⁵	3.53X10 ⁻⁵
6	rs9356571	158624723	TULP4	0.368	0.437	1.72X10 ⁻⁴	1.70X10 ⁻⁴
6	rs827987	158719128	TULP4	0.475	0.415	8.43X10 ⁻⁴	1.65X10 ⁻³
6	rs2197296	160488987	SLC22A1	0.268	0.315	9.85X10 ⁻⁴	2.08X10 ⁻³
7	rs1859586	9960922	NDUFA4	0.364	0.303	7.06X10 ⁻⁴	6.87X10 ⁻⁴
7	rs1990365	23082491	KLHL7	0.450	0.398	4.18X10 ⁻⁴	1.20X10 ⁻³
7	rs156425	23275644	GPNMB	0.431	0.379	5.57X10 ⁻⁴	1.72X10 ⁻³
7	rs10250643	24502175	MPP6	0.208	0.282	1.50X10 ⁻⁴	5.72X10 ⁻⁵
7	rs1012775	24815354	OSBPL3	0.408	0.346	3.53X10 ⁻⁴	3.81X10 ⁻⁴
7	rs7785415	25877852	NFE2L3	0.136	0.092	4.10X10 ⁻⁴	1.13X10 ⁻⁴
7	rs2717952	37352623	ELMO1	0.457	0.402	8.63X10 ⁻⁴	1.13X10 ⁻³
7	rs6971502	40033546	CDC2L5	0.230	0.289	6.99X10 ⁻⁴	1.28X10 ⁻⁴
7	rs740214	40578548	C7orf10	0.101	0.136	9.94X10 ⁻⁴	1.45X10 ⁻³
7	rs7792186	82257077	CACNA2D1	0.315	0.373	5.41X10 ⁻⁴	1.05X10 ⁻³
7	rs2254595	120794485	FAM3C	0.424	0.494	3.42X10 ⁻⁴	2.55X10 ⁻⁴
7	rs2237780	126461428	GRM8	0.232	0.191	5.00X10 ⁻⁴	1.96X10 ⁻³
7	rs10954144	126522030	GRM8	0.231	0.193	4.11X10 ⁻⁴	1.93X10 ⁻³
7	rs11563719	126537558	GRM8	0.242	0.207	7.67X10 ⁻⁴	5.11X10 ⁻³
7	rs2237791	126545431	GRM8	0.242	0.207	8.40X10 ⁻⁴	5.49X10 ⁻³
7	rs2299541	126573040	GRM8	0.242	0.207	9.39X10 ⁻⁴	6.12X10 ⁻³
7	rs2299546	126615107	GRM8	0.213	0.263	9.95X10 ⁻⁴	1.05X10 ⁻³
7	rs10487484	126892650	LOC168850	0.071	0.118	3.79X10 ⁻⁴	5.11X10 ⁻⁵
7	rs10488631	128381419	IRF5/TNPO3	0.168	0.117	4.57X10 ⁻⁵	2.14X10 ⁻⁵
7	rs12537284	128505142	LOC286016	0.179	0.133	2.79X10 ⁻⁴	2.71X10 ⁻⁴
7	rs10273156	137315489	CREB3L2	0.092	0.061	4.82X10 ⁻⁴	2.01X10 ⁻⁴
8	rs1760	432079	C8orf42	0.191	0.144	2.18X10 ⁻⁴	1.08X10 ⁻⁴
8	rs2126259	9222556	PPP1R3B	0.124	0.082	3.17X10 ⁻⁴	1.81X10 ⁻⁴
8	rs1841907	13982707	SGCZ	0.116	0.077	3.52X10 ⁻⁴	2.52X10 ⁻⁴
8	rs10503543	15304728	TUSC3	0.228	0.276	5.13X10 ⁻⁴	1.92X10 ⁻³
8	rs17698981	23452046	SLC25A37	0.123	0.179	3.99X10 ⁻⁴	2.10X10 ⁻⁴
8	rs2320350	24538029	ADAM7	0.037	0.071	2.64X10 ⁻⁴	2.02X10 ⁻⁴
8	rs7821268	29520356	DUSP4	0.272	0.222	8.07X10 ⁻⁵	6.96X10 ⁻⁵
8	rs4732980	29529350	DUSP4	0.375	0.317	1.11X10 ⁻⁴	1.78X10 ⁻⁴
8	rs10866875	29532541	DUSP4	0.241	0.197	4.90X10 ⁻⁴	5.76X10 ⁻⁴
8	rs13279569	38460773	FGFR1	0.097	0.066	5.85X10 ⁻⁴	1.16X10 ⁻³
8	rs7827569	62048489	CHD7	0.447	0.479	3.39X10 ⁻⁴	9.20X10 ⁻⁵
8	rs10113311	62051023	CHD7	0.367	0.424	8.86X10 ⁻⁴	4.67X10 ⁻⁴
8	rs7842384	62053366	CHD7	0.428	0.498	1.63X10 ⁻⁴	4.82X10 ⁻⁵
8	rs2555561	67594660	C8orf46	0.169	0.227	3.03X10 ⁻⁴	1.11X10 ⁻⁴
8	rs1491558	85324539	LOC138046	0.042	0.022	2.11X10 ⁻⁴	3.77X10 ⁻⁴
8	rs2444889	98977374	MATN2	0.449	0.488	9.85X10 ⁻⁴	1.78X10 ⁻⁴
8	rs7834444	99455892	KCNS2	0.266	0.319	8.16X10 ⁻⁵	1.69X10 ⁻⁴
8	rs7838130	99456091	KCNS2	0.266	0.319	7.45X10 ⁻⁵	1.59X10 ⁻⁴
8	rs4259391	99460461	KCNS2	0.258	0.310	1.02X10 ⁻⁴	2.53X10 ⁻⁴
8	rs10504989	100501854	VPS13B	0.429	0.357	9.16X10 ⁻⁴	4.72X10 ⁻⁵

8	rs1529335	129085529	MYC	0.133	0.177	9.29X10 ⁻⁴	1.57X10 ⁻³
8	rs4907323	142689368	FLJ43860	0.063	0.097	2.23X10 ⁻⁴	1.16X10 ⁻⁴
8	rs7835431	142718373	FLJ43860	0.045	0.075	3.43X10 ⁻⁴	5.66X10 ⁻⁴
8	rs7819659	143182478	TSNARE1	0.228	0.279	2.28X10 ⁻⁴	1.38X10 ⁻³
9	rs10814589	3762674	GLIS3	0.369	0.308	8.56X10 ⁻⁴	7.22X10 ⁻⁴
9	rs12001295	5508260	PDCD1LG2	0.086	0.057	6.09X10 ⁻⁴	2.57X10 ⁻⁴
9	rs2381904	8835177	PTPRD	0.205	0.251	5.01X10 ⁻⁴	9.79X10 ⁻⁴
9	rs2150696	12454876	TYRP1	0.186	0.237	4.18X10 ⁻⁴	6.37X10 ⁻⁴
9	rs10491888	28432343	LRRN6C	0.148	0.111	6.56X10 ⁻⁴	4.94X10 ⁻⁴
9	rs2182317	37631851	FRMPD1	0.098	0.131	8.45X10 ⁻⁴	3.03X10 ⁻³
9	rs7863144	71028859	TJP2	0.315	0.371	9.49X10 ⁻⁴	4.94X10 ⁻⁴
9	rs1930543	79831364	GNAQ	0.308	0.259	6.13X10 ⁻⁴	2.67X10 ⁻³
9	rs7039360	81668608	TLE4	0.418	0.467	8.10X10 ⁻⁴	9.22X10 ⁻⁴
9	rs10992619	94863568	SUSD3	0.098	0.069	5.90X10 ⁻⁴	6.52X10 ⁻⁴
9	rs1018691	133993386	NTNG2	0.442	0.358	3.36X10 ⁻⁵	6.70X10 ⁻⁶
9	rs9411446	134005748	NTNG2	0.466	0.392	2.31X10 ⁻⁴	6.78X10 ⁻⁵
9	rs3124607	138534660	NOTCH1	0.270	0.335	9.85X10 ⁻⁶	6.45X10 ⁻⁶
10	rs2458686	2548037	PFKP	0.123	0.168	7.37X10 ⁻⁴	1.80X10 ⁻³
10	rs1327860	10772118	CUGBP2	0.311	0.262	9.30X10 ⁻⁴	9.88X10 ⁻⁴
10	rs12764425	11783817	ECHDC3	0.206	0.260	7.96X10 ⁻⁴	1.96X10 ⁻³
10	rs1330581	26568841	GAD2	0.310	0.244	6.18X10 ⁻⁵	5.08X10 ⁻⁵
10	rs2368160	26580777	GAD2	0.310	0.250	3.03X10 ⁻⁴	2.38X10 ⁻⁴
10	rs12414701	26585409	GAD2	0.308	0.250	3.11X10 ⁻⁴	3.12X10 ⁻⁴
10	rs7906725	26602903	GAD2	0.312	0.251	3.14X10 ⁻⁴	2.50X10 ⁻⁴
10	rs4749107	26612848	GAD2	0.313	0.250	1.79X10 ⁻⁴	1.29X10 ⁻⁴
10	rs11015040	26664893	GAD2	0.333	0.271	4.87X10 ⁻⁴	2.51X10 ⁻⁴
10	rs4749114	26670036	GAD2	0.451	0.388	7.39X10 ⁻⁴	8.53X10 ⁻⁴
10	rs7477689	30055806	SVIL	0.135	0.092	1.14X10 ⁻⁴	1.14X10 ⁻⁴
10	rs1927461	30084320	SVIL	0.198	0.149	4.21X10 ⁻⁴	5.30X10 ⁻⁴
10	rs10764019	35295871	CUL2	0.100	0.149	3.28X10 ⁻⁴	1.47X10 ⁻⁴
10	rs2505647	43528257	ZNF32	0.248	0.299	9.40X10 ⁻⁴	2.75X10 ⁻³
10	rs920964	61250973	CCDC6	0.079	0.050	7.73X10 ⁻⁴	2.76X10 ⁻⁴
10	rs7076156	64085190	ZNF365	0.314	0.257	5.51X10 ⁻⁴	1.37X10 ⁻⁴
10	rs551674	100322286	HPSE2	0.045	0.081	1.59X10 ⁻⁴	2.52X10 ⁻⁴
10	rs11190842	102885447	TLX1	0.082	0.131	1.95X10 ⁻⁴	1.69X10 ⁻⁴
10	rs9420843	103254177	BTRC	0.418	0.480	2.32X10 ⁻⁴	2.49X10 ⁻⁴
10	rs808338	116484534	ABLM1	0.051	0.094	5.40X10 ⁻⁵	3.49X10 ⁻⁵
10	rs9804335	130137163	MKI67	0.168	0.220	6.84X10 ⁻⁴	5.83X10 ⁻⁴
10	rs7069785	133331878	FLJ46300	0.411	0.361	9.18X10 ⁻⁴	5.12X10 ⁻⁴
11	rs1044281	27105823	BBOX1	0.093	0.134	9.64X10 ⁻⁴	2.74X10 ⁻³
11	rs198464	61278197	C11orf9	0.469	0.466	3.08X10 ⁻⁴	1.94X10 ⁻⁴
11	rs198462	61280695	C11orf9	0.466	0.472	5.45X10 ⁻⁴	4.89X10 ⁻⁴
11	rs1535	61354548	FADS2	0.311	0.362	6.41X10 ⁻⁴	6.83X10 ⁻⁴
11	rs541207	63881718	FLJ37970	0.385	0.451	2.45X10 ⁻⁴	4.96X10 ⁻⁴
11	rs670345	75123917	MOGAT2	0.098	0.063	2.50X10 ⁻⁴	2.33X10 ⁻⁴

11	rs3858359	91148975	MTNR1B	0.072	0.119	2.73X10 ⁻⁴	3.22X10 ⁻⁵
11	rs9667295	94623763	SESN3	0.052	0.082	9.92X10 ⁻⁴	8.71X10 ⁻⁴
11	rs674637	119530453	TRIM29	0.457	0.406	8.32X10 ⁻⁴	3.02X10 ⁻⁴
11	rs11827471	119627604	POU2F3	0.101	0.074	6.75X10 ⁻⁴	4.92X10 ⁻⁴
11	rs1020777	129409707	C11orf37	0.243	0.191	5.79X10 ⁻⁴	1.02X10 ⁻³
12	rs3782578	12941686	GPRC5A	0.271	0.315	6.67X10 ⁻⁴	1.51X10 ⁻³
12	rs2728851	24269390	SOX5	0.465	0.476	6.83X10 ⁻⁴	4.94X10 ⁻⁴
12	rs10880346	41360328	PRICKLE1	0.317	0.373	6.84X10 ⁻⁴	5.90X10 ⁻⁴
12	rs12580965	74562859	PHLDA1	0.038	0.072	8.23X10 ⁻⁴	7.10X10 ⁻⁵
12	rs1435969	80078696	FLJ21963	0.308	0.257	5.36X10 ⁻⁴	3.63X10 ⁻⁴
12	rs790722	91200002	BTG1	0.110	0.077	8.25X10 ⁻⁴	4.54X10 ⁻⁴
12	rs7137346	129162349	FZD10	0.431	0.496	4.59X10 ⁻⁴	4.74X10 ⁻⁵
13	rs1159752	89977669	GPC5	0.375	0.311	6.48X10 ⁻⁴	1.50X10 ⁻³
13	rs731908	102365664	ERCC5	0.241	0.184	2.54X10 ⁻⁴	2.02X10 ⁻⁴
13	rs2211312	107203928	LIG4	0.075	0.122	1.78X10 ⁻⁵	5.08X10 ⁻⁵
14	rs226999	22048417	GeneID:28723	0.092	0.140	5.00X10 ⁻⁵	2.89X10 ⁻⁵
14	rs1956621	25531905	NOVA1	0.241	0.296	7.48X10 ⁻⁴	2.19X10 ⁻⁴
14	rs12589992	29457271	PRKD1	0.060	0.094	9.97X10 ⁻⁴	1.36X10 ⁻³
14	rs1187751	94964331	C14orf49	0.476	0.459	6.10X10 ⁻⁴	4.78X10 ⁻⁴
14	rs7143963	102374178	TRAF3	0.225	0.172	6.82X10 ⁻⁴	1.78X10 ⁻³
15	rs7497973	21499664	NDN	0.465	0.456	1.98X10 ⁻⁴	7.87X10 ⁻⁵
15	rs2676066	31379891	RYR3	0.318	0.367	8.86X10 ⁻⁴	2.30X10 ⁻³
15	rs10520077	36170627	TMCO5	0.203	0.156	5.06X10 ⁻⁴	2.51X10 ⁻⁴
15	rs8024749	36173868	TMCO5	0.151	0.102	1.16X10 ⁻⁵	5.64X10 ⁻⁶
15	rs2278166	48284646	SLC27A2	0.371	0.426	7.90X10 ⁻⁴	5.27X10 ⁻⁴
15	rs12442008	51512404	WDR72	0.317	0.248	4.14X10 ⁻⁴	3.53X10 ⁻⁴
15	rs10520743	92469600	MCTP2	0.060	0.031	5.75X10 ⁻⁵	7.19X10 ⁻⁵
16	rs2158962	3678028	TRAP1	0.448	0.499	9.78X10 ⁻⁴	5.86X10 ⁻⁴
16	rs243324	11262471	SOCS1	0.446	0.498	3.07X10 ⁻⁴	8.73X10 ⁻⁴
16	rs11640138	11270230	TNP2	0.435	0.479	9.78X10 ⁻⁴	2.75X10 ⁻³
16	rs12928822	11311394	PRM1	0.141	0.184	7.80X10 ⁻⁴	7.38X10 ⁻³
16	rs7199670	22782739	HS3ST2	0.075	0.112	2.75X10 ⁻⁴	1.05X10 ⁻⁴
16	rs2073917	30423993	ITGAL	0.281	0.343	3.52X10 ⁻⁵	5.57X10 ⁻⁵
16	rs3853178	34455044	UBE2MP1	0.128	0.092	3.51X10 ⁻⁴	2.58X10 ⁻⁴
16	rs4785408	48919240	BRD7	0.241	0.194	6.73X10 ⁻⁴	3.12X10 ⁻⁴
16	rs2380340	72172192	C16orf47	0.197	0.152	8.46X10 ⁻⁴	1.43X10 ⁻³
16	rs1922604	76649679	CLEC3A	0.422	0.368	6.22X10 ⁻⁴	3.25X10 ⁻⁴
16	rs2222896	76649999	CLEC3A	0.423	0.369	6.28X10 ⁻⁴	3.04X10 ⁻⁴
16	rs2344493	76653845	CLEC3A	0.425	0.369	3.51X10 ⁻⁴	1.98X10 ⁻⁴
16	rs1922617	76669764	WWOX	0.432	0.487	7.35X10 ⁻⁴	3.69X10 ⁻⁴
16	rs4889384	80387976	PLCG2	0.461	0.397	3.50X10 ⁻⁴	2.81X10 ⁻⁴
16	rs3924409	84585738	IRF8	0.352	0.412	2.32X10 ⁻⁴	1.09X10 ⁻⁴
17	rs907092	35175785	ZNFN1A3	0.478	0.449	2.05X10 ⁻⁵	7.04X10 ⁻⁶
17	rs9303277	35229995	ZNFN1A3	0.429	0.495	2.75X10 ⁻⁵	3.93X10 ⁻⁶
17	rs11557467	35282160	ZBPB2	0.440	0.490	8.03X10 ⁻⁵	1.41X10 ⁻⁵

17	rs8067378	35304874	GSDML	0.436	0.493	6.33X10 ⁻⁵	9.63X10 ⁻⁶
17	rs2305480	35315722	GSDML	0.491	0.438	3.66X10 ⁻⁵	1.41X10 ⁻⁵
17	rs2290400	35319766	GSDML	0.443	0.489	1.43X10 ⁻⁴	2.19X10 ⁻⁵
17	rs7216389	35323475	GSDML	0.442	0.490	1.97X10 ⁻⁴	2.91X10 ⁻⁵
17	rs4795405	35341943	ORMDL3	0.488	0.426	2.83X10 ⁻⁴	1.12X10 ⁻⁴
17	rs1007654	35364880	GSDM1	0.419	0.355	1.75X10 ⁻⁴	5.45X10 ⁻⁵
17	rs6503750	52067138	NOG	0.037	0.064	7.78X10 ⁻⁴	6.11X10 ⁻⁴
18	rs2186831	369644	COLEC12	0.252	0.193	2.44X10 ⁻⁴	5.38X10 ⁻⁵
18	rs1539790	391573	COLEC12	0.277	0.212	1.08X10 ⁻⁴	1.12X10 ⁻⁵
18	rs9303778	12978783	SEH1L	0.099	0.067	3.02X10 ⁻⁴	7.23X10 ⁻⁴
18	rs10775517	22856288	CHST9	0.473	0.416	7.91X10 ⁻⁴	2.49X10 ⁻³
18	rs10502510	24479614	CDH2	0.128	0.161	7.21X10 ⁻⁴	2.13X10 ⁻³
18	rs1426679	34339087	BRUNOL4	0.338	0.397	2.53X10 ⁻⁴	7.38X10 ⁻⁴
18	rs2000835	50741329	CCDC68	0.269	0.207	8.47X10 ⁻⁵	4.98X10 ⁻⁴
18	rs9964104	50751695	CCDC68	0.313	0.394	8.37X10 ⁻⁶	2.77X10 ⁻⁵
18	rs2282585	50776945	CCDC68	0.481	0.434	1.97X10 ⁻⁵	5.36X10 ⁻⁵
18	rs949031	50784263	CCDC68	0.480	0.434	1.30X10 ⁻⁵	3.74X10 ⁻⁵
18	rs10502992	50794597	CCDC68	0.313	0.251	4.84X10 ⁻⁴	9.02X10 ⁻⁴
18	rs1944353	50802939	CCDC68	0.315	0.392	1.55X10 ⁻⁵	4.12X10 ⁻⁵
18	rs10502993	50816269	CCDC68	0.302	0.245	7.13X10 ⁻⁴	1.23X10 ⁻³
18	rs11152150	50851961	CCDC68	0.412	0.480	1.64X10 ⁻⁴	3.71X10 ⁻⁴
18	rs8085797	52967581	WDR7	0.306	0.250	2.16X10 ⁻⁴	2.27X10 ⁻⁴
18	rs4077090	52992869	WDR7	0.328	0.273	2.49X10 ⁻⁴	1.83X10 ⁻⁴
18	rs1943386	53014869	ST8SIA3	0.317	0.257	5.41X10 ⁻⁴	4.50X10 ⁻⁴
18	rs2111286	53040512	ST8SIA3	0.277	0.225	9.01X10 ⁻⁴	8.59X10 ⁻⁴
18	rs1009081	53073827	ST8SIA3	0.284	0.225	1.16X10 ⁻⁴	7.43X10 ⁻⁵
18	rs515551	58803732	PHLPP	0.405	0.348	1.26X10 ⁻⁴	1.12X10 ⁻⁴
18	rs7232459	58853985	PHLPP	0.120	0.089	6.57X10 ⁻⁴	1.61X10 ⁻³
18	rs6567366	59316049	SERPINB5	0.306	0.369	8.38X10 ⁻⁴	1.95X10 ⁻⁴
18	rs1472870	65036963	C18orf14	0.426	0.356	8.25X10 ⁻⁴	1.10X10 ⁻⁴
19	rs4808419	15961918	OR10H4	0.448	0.480	5.47X10 ⁻⁵	4.85X10 ⁻⁵
19	rs2435039	21828007	ZNF43	0.321	0.368	7.21X10 ⁻⁴	4.45X10 ⁻³
19	rs3745516	55618554	SPIB	0.312	0.240	3.36X10 ⁻⁴	3.47X10 ⁻⁵
19	rs10418743	60245180	RDH13	0.066	0.111	1.31X10 ⁻⁴	1.65X10 ⁻⁴
19	rs1189	60247966	RDH13	0.065	0.109	1.62X10 ⁻⁴	1.87X10 ⁻⁴
19	rs775821	60250380	RDH13	0.243	0.296	7.68X10 ⁻⁴	6.79X10 ⁻⁴
19	rs6509916	60254214	RDH13	0.464	0.470	8.82X10 ⁻⁴	7.71X10 ⁻⁴
19	rs897799	60564003	IL11	0.180	0.218	8.38X10 ⁻⁴	1.06X10 ⁻³
20	rs6140113	691770	C20orf54	0.109	0.165	3.81X10 ⁻⁵	3.75X10 ⁻⁵
20	rs4810899	35643566	BLCAP	0.452	0.488	7.32X10 ⁻⁴	5.69X10 ⁻³
20	rs6021318	49662158	ATP9A	0.338	0.284	6.33X10 ⁻⁴	2.57X10 ⁻⁴
20	rs6067846	49663483	ATP9A	0.129	0.096	3.72X10 ⁻⁴	6.00X10 ⁻⁴
20	rs6021332	49681156	ATP9A	0.385	0.317	1.37X10 ⁻⁴	4.89X10 ⁻⁵
20	rs6021380	49737973	ATP9A	0.320	0.270	4.05X10 ⁻⁴	2.29X10 ⁻⁴
20	rs6015914	59086748	CDH4	0.416	0.350	9.69X10 ⁻⁴	9.78X10 ⁻⁴

21	rs242372	27792231	C21orf94	0.120	0.176	9.60X10 ⁻⁴	1.65X10 ⁻⁴
22	rs2241049	15967680	IL17R	0.323	0.383	6.49X10 ⁻⁴	1.63X10 ⁻³
22	rs2401125	16390945	SLC25A18	0.250	0.207	9.78X10 ⁻⁴	3.08X10 ⁻⁴
22	rs1296793	16398940	SLC25A18	0.235	0.191	6.99X10 ⁻⁴	1.90X10 ⁻⁴
22	rs1296794	16401604	SLC25A18	0.235	0.191	5.89X10 ⁻⁴	1.69X10 ⁻⁴
22	rs2401158	16476378	ATP6V1E1	0.199	0.154	3.38X10 ⁻⁴	9.47X10 ⁻⁵
22	rs4821797	37346128	PGEA1	0.386	0.331	7.64X10 ⁻⁴	1.68X10 ⁻³
22	rs715001	42075141	SCUBE1	0.194	0.248	3.10X10 ⁻⁴	4.02X10 ⁻⁴
22	rs5770418	48198080	FLJ44385	0.121	0.166	9.11X10 ⁻⁴	8.15X10 ⁻⁴

Figure 6 - Table 1 Results of Stage I genomewide association study and stage II replication *

Stage I – GWA				Stage II Replication				Joint Replication Analysis		Combined analysis						
n=505 cases and 1507 controls (383 Canadian and 1124 M.D. Anderson “historic”)				Ila – US-based study cohort n=410 cases and 310 controls				Iib – Canada-based study cohort n=116 cases and 896 controls				n=1031 cases and 2713 controls				
SNP ID	Chr.	Location	Risk Allele	Risk allele frequency		EIGENSTRAT P	PLINK P	Odds Ratio (95% CI)	Risk allele frequency		P Bonf **	P ***	Odds Ratio (95% CI)			
				Cases	Controls				Cases	Controls						
A. HLA regions																
rs2856683	6p21.3	32763196	HLA-DQB1	0.360	0.222	1.34 × 10 ⁻⁴⁴	8.58 × 10 ⁻¹⁷	1.99 (1.69-2.34)	0.334	0.228	1.44 × 10 ⁻⁵	0.298	0.224	1.30 × 10 ⁻²	1.78 × 10 ⁻¹⁹	1.75 (1.55-1.98)
rs9275312	6p21.3	32773706	HLA-DQB1	0.227	0.128	1.99 × 10 ⁻⁴¹	3.84 × 10 ⁻¹³	2.01 (1.66-2.42)								
rs9275390	6p21.3	32777134	HLA-DQB1	0.373	0.246	5.73 × 10 ⁻⁴¹	1.13 × 10 ⁻¹³	1.81 (1.55-2.11)								
rs7775228	6p21.3	32766057	HLA-DQB1	0.207	0.122	1.90 × 10 ⁻⁴⁰	1.70 × 10 ⁻¹⁰	1.87 (1.54-2.27)								
rs2395148	6p21.3	32429532	Ccrp10	0.055	0.019	1.50 × 10 ⁻⁹	5.62 × 10 ⁻¹⁰	3.24 (2.20-4.77)	0.065	0.026	8.19 × 10 ⁻⁴	0.068	0.025	2.77 × 10 ⁻⁴	3.62 × 10 ⁻¹⁴	2.87 (2.16-3.82)
rs9277553	6p21.3	33162839	HLA-DPB1	0.326	0.236	7.26 × 10 ⁻⁹	8.28 × 10 ⁻⁹	1.60 (1.36-1.87)	0.318	0.248	3.85 × 10 ⁻³	0.331	0.250	7.83 × 10 ⁻³	3.92 × 10 ⁻¹¹	1.50 (1.33-1.70)
rs3806156	6p21.3	32481676	RTN1.2	0.457	0.354	1.31 × 10 ⁻⁸	1.27 × 10 ⁻⁹	1.58 (1.37-1.84)	0.428	0.361	0.011	0.42	0.360	0.070	1.11 × 10 ⁻⁹	1.42 (1.27-1.58)
rs9357152	6p21.3	32772938	HLA-DQB1	0.826	0.741	3.88 × 10 ⁻⁸	7.25 × 10 ⁻⁸	1.65 (1.37-1.97)								
rs3135363	6p21.3	32497626	RTN1.2	0.784	0.718	4.46 × 10 ⁻⁸	3.72 × 10 ⁻⁷	1.56 (1.30-1.85)								
rs9277565	6p21.3	33164875	HLA-DPB1	0.276	0.198	5.94 × 10 ⁻⁸	8.32 × 10 ⁻⁸	1.58 (1.34-1.87)								
rs2281389	6p21.3	33167774	HLA-DPB1	0.236	0.168	1.58 × 10 ⁻⁷	2.38 × 10 ⁻⁷	1.59 (1.33-1.90)								
rs660895	6p21.3	32685358	HLA-DRB1	0.277	0.195	3.41 × 10 ⁻⁷	4.68 × 10 ⁻⁸	1.60 (1.35-1.90)								
rs901626	6p21.3	32508322	HLA-DRA	0.173	0.110	4.43 × 10 ⁻⁷	6.83 × 10 ⁻⁷	1.68 (1.37-2.07)								
B. Non-HLA regions																
rs6441286	3q25.33-q26	161211572	IL12A	0.497	0.390	1.20 × 10 ⁻⁸	3.25 × 10 ⁻⁸	1.51 (1.30-1.75)	0.488	0.371	1.11 × 10 ⁻³	0.471	0.395	2.55 × 10 ⁻²	2.42 × 10 ⁻¹⁴	1.54 (1.38-1.72)
rs3790567	1p31.2	67594965	IL12RB2	0.341	0.240	4.51 × 10 ⁻⁸	8.60 × 10 ⁻⁸	1.54 (1.32-1.81)	0.311	0.241	5.19 × 10 ⁻³	0.340	0.246	1.72 × 10 ⁻³	2.76 × 10 ⁻¹¹	1.51 (1.33-1.70)
rs574808	3q25.33-q26	161215677	IL12A	0.678	0.574	3.81 × 10 ⁻⁷	5.34 × 10 ⁻⁷	1.47 (1.27-1.72)	0.668	0.564	6.97 × 10 ⁻⁵	0.677	0.588	9.26 × 10 ⁻³	1.88 × 10 ⁻¹³	1.54 (1.37-1.73)
rs6818639	4q27	123118615	TRPC3	0.804	0.729	1.36 × 10 ⁻⁶	3.45 × 10 ⁻⁷	1.59 (1.33-1.92)	0.764	0.780	0.477	0.727	0.735	0.799	2.35 × 10 ⁻³	1.23 (1.08-1.40)
rs3790565	1p31.2	67583944	IL12RB2	0.258	0.183	2.50 × 10 ⁻⁶	1.41 × 10 ⁻⁶	1.53 (1.29-1.82)	0.232	0.195	0.097	0.269	0.184	1.91 × 10 ⁻³	1.24 × 10 ⁻⁴	1.46 (1.28-1.67)
rs9864104	18q21	50751695	CCDC68	0.687	0.606	8.37 × 10 ⁻⁶	2.77 × 10 ⁻⁵	1.39 (1.19-1.61)	0.642	0.643	0.973	0.639	0.626	0.708	2.13 × 10 ⁻⁴	1.25 (1.11-1.40)
rs3124607	9q34.3	138534660	NOTCH1	0.730	0.665	9.85 × 10 ⁻⁶	6.45 × 10 ⁻⁶	1.45 (1.24-1.70)	0.934	0.924	0.482	0.933	0.922	0.544	4.67 × 10 ⁻⁵	1.65 (1.30-2.10)
rs16833239	2q32	191648505	STAT4	0.962	0.921	1.55 × 10 ⁻⁵	2.60 × 10 ⁻⁵	2.13 (1.47-3.03)	0.879	0.887	0.633	0.865	0.878	0.589	0.608	1.05 (0.88-1.24)
rs10222962	4p15	32036553	PCDH7	0.111	0.070	1.55 × 10 ⁻⁵	1.67 × 10 ⁻⁵	1.70 (1.33-2.18)	0.536	0.487	0.068	0.492	0.456	0.179	7.61 × 10 ⁻⁶	1.29 (1.15-1.44)
rs2211312	13q33.3	107203928	FAM155A	0.925	0.878	1.78 × 10 ⁻⁵	5.08 × 10 ⁻⁵	1.75 (1.33-2.33)								
rs907092	17q21	35175785	IKZF3	0.522	0.449	2.05 × 10 ⁻⁵	7.04 × 10 ⁻⁶	1.40 (1.21-1.62)								
rs9303277	17q21	35229995	IKZF3	0.571	0.495	2.75 × 10 ⁻⁵	3.95 × 10 ⁻⁵	1.41 (1.22-1.66)								
rs4679904	3q26.1	161823590	ARF7	0.798	0.719	2.79 × 10 ⁻⁵	5.38 × 10 ⁻⁶	1.52 (1.27-1.79)	0.767	0.710	0.016	0.748	0.730	0.549	1.13 × 10 ⁻⁶	1.38(1.21-1.57)
rs2305480	17q12	35315722	GSDML	0.509	0.438	3.66 × 10 ⁻⁵	1.41 × 10 ⁻⁵	1.38 (1.19-1.60)								
rs6140113	20p13	691770	C20orf54	0.891	0.835	3.81 × 10 ⁻⁵	3.75 × 10 ⁻⁵	1.61 (1.30-2.04)	0.849	0.831	0.361	0.765	0.813	0.053	0.338	1.08 (0.93-1.25)
rs10486631	7q32.1	128381419	IRF5-TNPO3	0.168	0.117	4.57 × 10 ⁻⁵	2.14 × 10 ⁻⁵	1.55 (1.26-1.90)	0.167	0.113	4.72 × 10 ⁻³	0.130	0.090	0.142	1.52 × 10 ⁻⁷	1.52 (1.30-1.78)
rs6748358	2q33	204465150	CTLA4	0.574	0.497	4.91 × 10 ⁻⁵	1.41 × 10 ⁻⁵	1.39 (1.19-1.62)								

Figure 7 - Table 2 Fine mapping results from the *IL12A* and *IL12RB2* loci *

SNP ID (Location)	Physical Location	Minor/Major Alleles	RAF		P	Odds Ratio (95% CI)
			Cases (N=621)	Controls (N=1279)		
IL12A (Chr. 3q25.33-q26)						
rs17810546 (5' Flanking)	161147744	G/A	0.93	0.88	9.77 x 10 ⁻⁶	1.78 (1.38-2.31)
rs583911 (Intron 2)	161193084	G/A	0.50	0.42	4.34 x 10 ⁻⁶	1.39 (1.21-1.60)
rs668998 (3' Flanking)	161198245	G/A	0.50	0.42	1.12 x 10 ⁻⁵	1.37 (1.19-1.58)
rs6441284 (3' Flanking)	161200962	G/A	0.66	0.59	4.47x 10 ⁻⁵	1.36 (1.17-1.58)
rs485497 (3' Flanking)	161201826	G/A	0.56	0.48	1.09 x 10 ⁻⁵	1.37 (1.19-1.58)
rs4680536 (3' Flanking)	161202965	G/A	0.66	0.58	4.08 x 10 ⁻⁶	1.41 (1.22-1.63)
rs9852519 (3' Flanking)	161203322	T/C	0.47	0.37	2.11 x 10 ⁻⁸	1.50 (1.30-1.73)
rs4679867 (3' Flanking)	161206597	A/T	0.69	0.60	4.52 x 10 ⁻⁷	1.46 (1.26-1.70)
rs4679868 (3'Flanking)	161206848	A/G	0.49	0.39	1.58 x 10 ⁻⁹	1.55 (1.34-1.78)
rs6441286 (3' Flanking)	161211572	G/T	0.49	0.39	4.83 x 10 ⁻⁹	1.52 (1.32-1.76)
rs574808 (3' Flanking)	161215677	C/T	0.68	0.59	1.15 x 10 ⁻⁷	1.49 (1.29-1.73)
rs589545 (3' Flanking)	161216294	A/G	0.68	0.59	1.34 x 10 ⁻⁷	1.49 (1.28-1.72)
IL12RB2 (Chr. 1p31.2)						
rs11209050 (Intron 3)	67564324	A/C	0.28	0.20	2.76 x 10 ⁻⁷	1.53 (1.30-1.79)
rs1908632 (Intron 8)	67578394	G/T	0.33	0.25	1.22 x 10 ⁻⁷	1.52 (1.30-1.77)
rs3790565 (Intron 8)	67583944	C/T	0.25	0.18	2.44 x 10 ⁻⁶	1.50 (1.27-1.79)
rs946685 (Intron 8)	67588303	A/G	0.25	0.18	1.93 x 10 ⁻⁶	1.49 (1.26-1.76)
rs6679356 (Intron 9)	67592782	C/T	0.27	0.19	7.02 x 10 ⁻⁸	1.57 (1.33-1.86)
rs10749775 (Intron 9)	67594675	C/A	0.19	0.13	1.88 x 10 ⁻⁷	1.66 (1.37-2.03)
rs3790567 (Intron 9)	67594965	A/G	0.34	0.25	8.32 x 10 ⁻⁸	1.52 (1.30-1.77)
rs6695348 (Intron 9)	67599604	T/C	0.33	0.25	9.87 x 10 ⁻⁸	1.52 (1.30-1.77)

* SNPs across the *IL12A* and the *IL12RB2* loci were tested on the extended Canada-based case/control collection and marked associations ($P < 5.0 \times 10^{-5}$) with PBC are shown. The complete dataset is provided in Supplementary Table S8. P values and odds ratios are for the comparison in allele frequency between case subjects and control subjects and were calculated using PLINK. The risk allele for each SNP is underlined and the highest association is shown in bold.

Evaluation of IL12A (p35) gene expression levels in healthy subjects

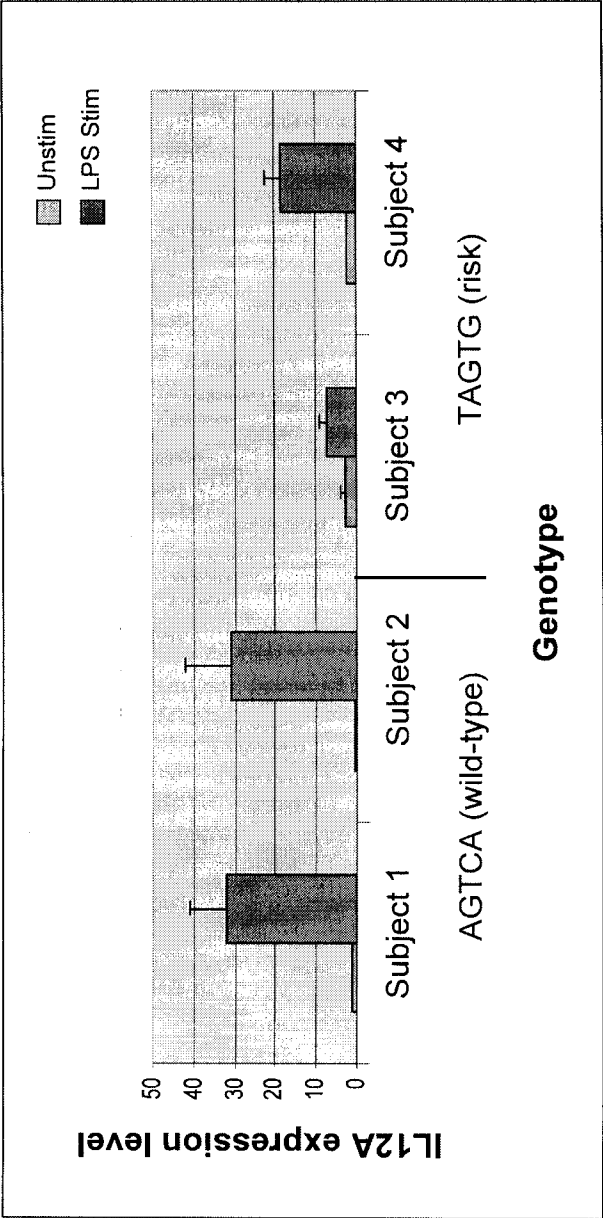


Figure legend: Human peripheral blood mononuclear cells were purified from healthy donors and genotyped so as to identify individuals homozygous for the primary biliary cirrhosis risk (TAGTG) and non-risk (AGTCA) IL12A haplotypes. Representative data from two subjects with non-risk genotype (1 and 2) and from 2 with the risk genotype (3 and 4) are shown. Cells were stimulated with lipopolysaccharide (LPS) and interferon- γ for 8 hours and harvested for total RNA purification using Qiagen kit. A two-step qPCR were performed to detect the expression of IL12A (p35) and the housekeeping gene, glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Primers for IL12A: 5'TATTGATGAGCTGATGCAGGC3', 5'GTCACTGCCCGAATTCTGAA3'. Primers for GAPDH: 5'ACCTCAACTACATGGTTTAC3', 5'GAAGATGGTGATGGGATTTC3'. The results are presented as IL12A relative to GAPDH expression levels.

Figure 8

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2010/000324

A. CLASSIFICATION OF SUBJECT MATTER IPC: A61K 38/20 (2006.01) , A61P 1/16 (2006.01) , C12Q 1/68 (2006.01) , C30B 30/04 (2006.01) , G01N 33/50 (2006.01) , G01N 33/53 (2006.01) G01N 33/68 (2006.01) According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) IPC: A61K 38/20 (2006.01) , A61P 1/16 (2006.01) , C12Q 1/68 (2006.01) , C30B 30/04 (2006.01) , G01N 33/50 (2006.01) , G01N 33/53 (2006.01) G01N 33/68 (2006.01) Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used) Canadian Patent Database, Medline, Epodoc, TotalPatent (search terms used: interleukin-12/IL-12, biliary/liver cirrhosis, polymorphism)		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Pulickal et al., "Biliary Cirrhosis in a child with inherited interleukin-12 deficiency", J TROPICAL PEDIATRICS, Aug 2008, Vol. 54, No. 4, pg. 269-271. ISSN 0142-6338. whole document.	1, 12-17 and 22-23
X	Caspi, "IL-12 in autoimmunity", CLIN. IMMUNOL. IMMUNOPATHOL., July 1998, Vol. 88, No. 1, pg. 4-13. ISSN 0090-1229. pg. 6-7.	19-21
X	Donaldson et al., "HLA class II alleles, genotypes, haplotypes, and amino acids in primary biliary cirrhosis: a large-scale study", HEPATOLOGY, Sept 2006, Vol. 44, No. 3, pg. 667-674. ISSN 0270-9139. whole document.	24
P, X	Hirschfield et al., "Primary biliary cirrhosis associated with HLA, IL12A, and IL12RB2 variants", N ENGL J MED, June 11, 2009, Vol. 360, No. 24, pg. 2544-2555	1-24
A	Juran and Lazaridis, "Genetics and genomics of PBC", CLINICS IN LIVER DISEASE, May 2008, Vol. 12, No. 2, pg. 349-365. ISSN 1089-3261. whole document.	
☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.		
* Special categories of cited documents :	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family	
"A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed		
Date of the actual completion of the international search 30 June 2010 (30-06-2010)	Date of mailing of the international search report 15 July 2010 (15-07-2010)	
Name and mailing address of the ISA/CA Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 001-819-953-2476	Authorized officer Micheal O'Hare (819) 994-4161	

INTERNATIONAL SEARCH REPORTInternational application No.
PCT/CA2010/000324**Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)**

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing filed or furnished:
 - a. (means)
☐ on paper
☒ in electronic form
 - b. (time)
☐ in the international application as filed
☐ together with the international application in electronic form
☒ subsequently to this Authority for the purposes of search
2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments :

INTERNATIONAL SEARCH REPORTInternational application No.
PCT/CA2010/000324**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons :

1. ☒ Claim Nos. : 23

because they relate to subject matter not required to be searched by this Authority, namely :

This claim is directed to a method for the treatment of the human or animal body by therapy which the International Search Authority is not required to search under Rule 39.1(iv). However, this Authority has carried out an examination based on the alleged therapeutic effect or purpose/use of the product defined in said claims.

2. ☐ Claim Nos. :

because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically :

3. ☐ Claim Nos. :

because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows :

Group A - Claims 1-23 (completely), and claim 24 (partially), directed to methods of identifying a subject at risk of developing primary biliary cirrhosis (PBC) by screening for polymorphisms within the gene for one or both of IL-12A and IL-12RB. The use of an agent to stimulate the IL-12/IL-12R axis in the treatment of PBC is also claimed.

Groups B-M - Claim 24 (partially) directed to methods of identifying a subject at risk for PBC by screening for polymorphism within one of the following genes: HLA-DQB1 (Group B), C6orf10 (Group C), HLA-DPB1 (Group D), BTNL-2 (Group E), STAT4 (Group F), IKZF3 (Group G), ZPBP2 (Group H), GSDMB (Group I), ORMDL3 (Group J), MMEL1 (Group K), IRF5-TNPO3 (Group L), and IRF5 (Group M).

Groups A-M are directed to the screening of distinct genes for polymorphisms predisposing to PBC and as such are not linked by a common inventive concept.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☒ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos. :

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos. :

Remark on Protest ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.