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Genome-wide meta-analyses identify three loci associated with primary biliary cirrhosis

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X.L., P.I., Yue Lu and R.K. contributed to initial data analyses and manuscript preparation. P.I., R.S., C.S. and A.L. managed DNA samples. A.L., F. Macciardi, A.T.L. and P.K.G. performed genotyping. M.R. provided database management. P.I., Yan Lu, I.B., M.P., C.X., G.X., A.L.M., R.P.M., K.M.P., C.N.G., F.B., M.Z., E.J.H., S.L., F.R., E.B., A.F., R.L., G.N., A.A., L. Muratori, P.M., P.L.A., P.A., M. Margotti, M.B., B.C., D.A., M.C.B., F. Marra, A. Pisano, C.R., M.C., M. Marzioni, A.B., L.F., M.S., P.P., V.O.P., C.T., L.C., S.B., S.R., M.V., C.P., A.M., P.T., A. Picciotto, A.G., C.F., S.C., G.C., L. Morini, N.C., A.C., G.S. and R.M. contributed to experimental design, subject assessment and sample collection. P.I., G.M.H., K.A.S., C.I.A., M.E.G. and M.F.S. contributed to experimental design and interpretation, statistical analyses, and initial manuscript preparation. All authors contributed to the final paper.

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Abstract

A genome-wide association screen for primary biliary cirrhosis risk alleles was performed in an Italian cohort. The results from the Italian cohort replicated *IL12A* and *IL12RB* associations, and a combined meta-analysis using a Canadian dataset identified newly associated loci at *SPIB* (P = 7.9

× 10^{-11}, odds ratio (OR) = 1.46), *IRF5-TNPO3* ($P = 2.8 \times 10^{-10}$, OR = 1.63) and 17q12-21 ($P = 1.7 \times 10^{-10}$, OR = 1.38).

Primary biliary cirrhosis (PBC) is an uncommon condition (with a prevalence of ~5 per 10,000 people) of unknown etiology characterized by chronic cholestatic liver disease and, frequently, the presence of serum anti-mitochondrial antibodies (AMA). PBC's association with AMA, other autoantibodies, autoreactive T cells and HLA alleles implies an autoimmune etiology for this disease[1]. In addition, the association of PBC with *IL12A* and *IL12RB2* has previously been demonstrated in a genome-wide association study (GWAS) of a Canadian PBC cohort[2].

To further define the genetic factors conferring risk for PBC, we performed another GWAS in a cohort of Italian individuals with PBC (cases) and unaffected controls. We analyzed the data separately using the earlier Canadian PBC GWAS as a replication dataset and then combined the data with the Canadian GWAS in a meta-analysis. We genotyped Italian PBC cases and controls using the Illumina 610K array and Illumina 1Mb arrays, respectively. After quality control filtering and combination with additional European population controls, we performed principal components analyses, which defined a homogeneous Italian subject set encompassing 453 PBC cases and 945 controls (Supplementary Methods, Supplementary Fig. 1 and Supplementary Table 1). The Canadian PBC GWAS (which used the Illumina 370K array for genotyping) included 481 Canadian PBC cases and 3,706 population controls. We applied association methods controlling for population substructure to each dataset and used Cochran-Mantel-Haenszel adjustments when combining the separately analyzed datasets.

We obtained results for the homogeneous Italian subset and the meta-analysis of the entire sample set (Italian plus Canadian cohorts) (**Fig. 1a**). The Italian dataset showed genome-wide significant associations at the HLA region (**Fig. 1a,b**), with several other loci showing suggestive association signals. Analysis of the combined dataset showed multiple loci reaching conservative genome-wide *P* values ($P < 5 \times 10^{-8}$), including several newly associated loci (**Table 1**). For the HLA region, the SNPs showing the strongest associations nearly overlapped between the two datasets (**Fig. 1b**). For the Italian cases, the highest- and lowest-risk haplotypes generally corresponded with high- and low-risk *HLA DRB1* alleles[3] (χ^2 2 × 2 contingency, $P = 0.002$); however, there was insufficient data for fine mapping.

In the combined data, *IL12A* and *IL12RB2* were the strongest non-HLA associations, consistent with the previous Canadian GWAS ($P = 3.3 \times 10^{-10}$ and $P = 7.5 \times 10^{-12}$, respectively) (**Supplementary Table 2**). SNPs within *SCHIP1* (located ~100 kb proximal to *IL12A*) also showed suggestive association with PBC (for example, rs3863075, $P = 3.05 \times 10^{-6}$) in the combined analysis. Independent effects in the *IL12A-SCHIP1* interval were suggested by logistic regression, and risk signals from distinct haplotypes suggested that multiple and/or independently derived variations underlie the risk association in this region (**Supplementary Table 3**). For *IL12RB2*, a stronger association signal was detected in the Canadian study cohort relative to the Italian study cohort, but the *P* value observed for one SNP (rs3790567, $P = 1.5 \times 10^{-3}$) in the Italian group was among the 500 most strongly disease-associated SNPs (the most significant 0.1%) in this cohort.

Excluding the MHC region and *IL12A*, in the Italian cohort, 57 SNPs showed an association signal with a $P < 10^{-5}$. Of these, eight SNPs reached significance (using a threshold of $P < 8 \times 10^{-4}$; **Supplementary Methods**) when the Canadian cohort was used as a replication set, and most of these SNPs had significant combined *P* values (**Table 1**). The significant SNPs included several in an ~150-kb gene-rich region on chromosome 17 containing *IKZF3-ZPBP2-GSDMB-ORMDL3* that has been implicated in asthma, type 1 diabetes, Crohn's

disease and ulcerative colitis[4–6]. A stepwise multiple logistic regression model identified a single SNP (rs907902) that could account for the observed signal. Within this region, *IKZF3* (encoding IKAROS family zinc finger 3, also known as Aiolos) is a transcription factor that prevents apoptosis of IL2-deprived B cells[7], regulates B-cell activation[8] and has been implicated in autoimmunity; a lupus-like syndrome develops in *Ikzf3*-deficient mice[9]. However, although *IKZF3* is an attractive association candidate, other genes may also account for this association signal, including *ORMDL3*, which has been suggested to underlie the association with asthma[4].

A previously unidentified association resulting from the meta-analysis involved rs3745516 within *SPIB* (**Table 1**). A member of the ETS transcription factor family, Spi-B is an important mediator of both B-cell receptor signaling[10] and early T-cell lineage decisions[11]. Spi-B also induces the development of plasmacytoid dendritic cells and can mediate IL7R, c-rel and IFN-γ–induced CD40 expression[12]. Additional studies are needed to define the causal mutation at this locus and the biological pathway connecting it to PBC predisposition.

The results from the combined data also support the association at the locus comprising *IRF5* and *TNPO3* (encoding interferon regulatory factor 5 and transportin 3, respectively). Although the Italian PBC cohort showed only modest association at the *IRF5-TNPO3* locus (rs10488631, $P = 4.2 \times 10^{-3}$), the combined *P* value ($P = 2.8 \times 10^{-10}$) exceeded genome-wide significant thresholds.

Among the genes with suggestive evidence of association with PBC in the combined analysis, *STAT4* ($P = 1.3 \times 10^{-5}$) and *DENND1B* ($P = 1.8 \times 10^{-4}$) are noteworthy because of their prior associations with autoimmune disorders. *STAT4* is associated with systemic lupus erythematosus (SLE) and rheumatoid arthritis[13], and its critical role in *IL12-IL12R* signaling makes it a strong candidate for influencing PBC risk. *DENND1B* has recently been identified as a susceptibility gene in pediatric asthma[14]. Another suggestive association signal that may merit further investigation is that for *CDGAP* (combined $P = 1.6 \times 10^{-7}$). *CDGAP* is a noteworthy candidate because the Cdc42 GTPase-activating protein it encodes is important in lymphocyte development and proliferation[15]. Other SNPs showed suggestive signals, but these signals were restricted to either the Italian or Canadian subgroup (**Supplementary Table 2**). Additional studies will be needed to ascertain whether these results can be replicated and whether they are restricted to a particular population subset.

The use of Illumina 610K and Illumina 1Mb arrays enabled an exploration of PBC association with mitochondrial SNPs, a potentially relevant issue in this AMA-associated disease. Although these arrays do not interrogate all mitochondrial haplogroups, the lack of disease association seen here with these SNPs ($P > 0.05$) suggests that variants in mitochondrial genes are unlikely to account for PBC heritability or AMA positivity. Analyses using only AMA-positive PBC cases also showed odds ratios similar to those observed with the entire set, and the odds ratios from each group showed strong correlation to each other (r^2 odds ratios = 0.95), implying a similar genetic etiology regardless of AMA status (**Supplementary Table 4**).

Multiple genes implicated in PBC are apparently shared between different autoimmune diseases. This includes *IL12A*, which is shared with celiac disease; however, the SNPs and haplotypes containing the risk alleles are distinctly different for the two diseases (**Supplementary Table 5**). Similarly, PBC appears to be associated with at least one (the 17q12-21 locus, also shared with other autoimmune diseases) and possibly a second (*DENND1B*) locus implicated in pediatric asthma. For the 17q12-21 association, however, the haplotype is opposite that observed in pediatric asthma (**Supplementary Fig. 2**), and for *DENND1B*, the suggestive association also appears to be disparate from that observed with

asthma. Finally, the associated SNP for *IRF5-TNPO3* is within a haplotype shared with SLE and several other autoimmune diseases. In contrast, many of the other prominent associations observed in SLE, celiac disease and asthma are not apparent in PBC. Although the specific genes and variants responsible for the association signals discovered here remain to be defined, the current findings suggest specific molecular pathways as factors in the pathogenesis of PBC.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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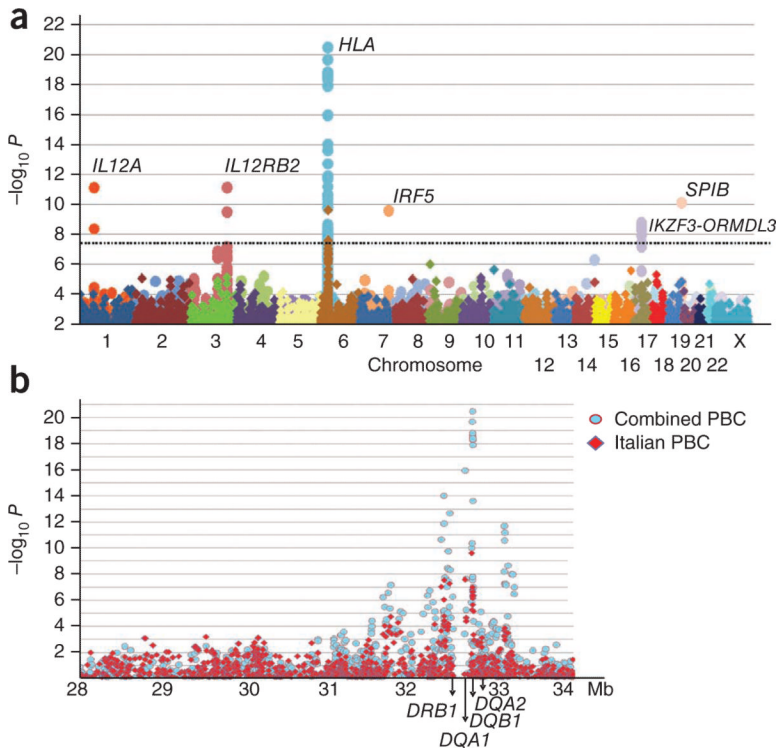

Figure 1.

Results of genome-wide association tests for PBC. (**a,b**) The ordinate shows the level of significance for each SNP along each chromosome (**a**) or the HLA region (**b**). The Italian PBC subset (diamond symbols) and combined European dataset (circle symbols) are shown. The dashed line corresponds to $P = 5 \times 10^{-8}$.

Table 1

Summary of non-MHO association tests for italian PBC and combined italian and Canadian PBC study[a]

					Italian				Canadian				Meta-analysis	
SNP	**Chr.**	**Position**	**MA**[b]	**Gene(s)**	**Ctrl AF**[c]	**Case AF**	***P***	**OR**[d]	**Ctrl AF**	**Case AF**	***P***	**OR**	***P***	**OR**
rs10488631	7	128,381,419	C	*IRF5-TNPO3*	0.08	0.12	4.24×10^{-3}	1.48	0.11	0.17	2.17×10^{-8}	1.71	2.78×10^{-10}	1.63
rs9303277	17	35,229,995	T	*IKZF3* [e]	0.45	0.53	4.01×10^{-5}	1.41	0.51	0.58	2.59×10^{-5}	1.34	1.69×10^{-9}	1.38
rs3745516	19	55,618,554	A	*SPIB*	0.27	0.36	1.41×10^{-5}	1.47	0.23	0.31	8.88×10^{-7}	1.45	7.97×10^{-11}	1.46

Chr., chromosome; MA, minor allele; AF, allele frequency.

[a] The SNP with the strongest evidence of association in the combined meta-analyses are presented for each gene and locus. Each SNP had a combined $P < 10^{-8}$. See **Supplementary Table 1** for *IL12A* and *IL12RB2* data.

[b] Minor allele base pair in forward strand.

[c] The control minor allele frequency.

[d] Odds ratio for the minor allele for each analysis.

[e] SNPs for *ZPBP2, GSDMB* and *ORMDL3* in addition to *IKZF3* all showed significant association for this 17q12-21 locus.