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Variants at *IRF5-TNPO3*, 17q12-21 and *MMEL1* are associated with primary biliary cirrhosis

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Abstract

We genotyped individuals with primary biliary cirrhosis and unaffected controls for suggestive risk loci (genome-wide association $P < 1 \times 10^{-4}$) identified in a previous genome-wide association study. Combined analysis of the genome-wide association and replication datasets identified *IRF5-TNPO3* (combined $P = 8.66 \times 10^{-13}$), 7q12-21 (combined $P = 3.50 \times 10^{-13}$) and *MMEL1* (combined $P = 3.15 \times 10^{-8}$) as new primary biliary cirrhosis susceptibility loci. Fine-mapping studies showed that a single variant accounts for the *IRF5-TNPO3* association. As these loci are implicated in other autoimmune conditions, these findings confirm genetic overlap among such diseases.

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AUTHOR CONTRIBUTIONS

G.M.H., X.L., C.I.A. and K.A.S. designed the study. X.L., Y.H., I.P.G., Y.L., C.X., Y.L., W.C., J.Z. and C.I.A. performed the genotyping and data analysis. G.M.H., B.D.J., C.C., A.L.M., P.M., R.P.M., J.A.O., V.A.L., D.S., C.V., C.L., P.K.G., E.J.H., K.N.L. and K.A.S. developed the clinical network and sample collection and processing framework required for case and control accrual. G.M.H., C.I.A. and K.A.S. wrote the manuscript. G.M.H., X.L., C.I.A. and K.A.S. vouch for the data and its analysis. G.M.H., C.I.A. and K.A.S. (the senior and corresponding author) decided in agreement with all other authors to publish this paper.

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Primary biliary cirrhosis (PBC) is the most common autoimmune liver disease, characterized by chronic nonsuppurative destructive cholangitis, circulating anti-mitochondrial antibodies and the frequent presence of other autoimmune disorders in affected individuals and their family members[1]. We previously reported a genome-wide association study (GWAS) for PBC that identified three susceptibility loci, *HLA*, *IL12A* and *IL12RB2*, as being strongly associated with PBC, as well as a number of other loci showing suggestive association with this disease[2]. To replicate these findings and evaluate relevance of these latter loci to PBC susceptibility, we have now tested an additional independent cohort of 857 individuals with PBC (cases) of European descent and 3,198 controls of European descent (including 1,743 historic control subjects from the New York Cancer Project[3]) for PBC associations with 36 SNPs across 24 loci (Supplementary Fig. 1); for each locus, association signals at $P < 1 \times 10^{-4}$ were detected for more than one SNP found in the initial GWAS, with the exception of *IRF5-TNPO3*, a well-recognized autoimmune disease risk locus. The combination of these replication results and our prior genome-wide association data yielded a genetic dataset derived from 1,351 PBC cases and 4,700 controls (Supplementary Methods).

Fifteen SNPs replicated ($P < 1.39 \times 10^{-3}$) after Bonferroni adjustment (Table 1 and Supplementary Table 1). In addition to *HLA*, *IL12A* and *IL12RB2*, genes at the *IRF5-TNPO3* locus, at chromosome 17q12-21 (containing *IKZF3*, *ZPBP2*, *GSDMB* and *ORMDL3*) and at *MMEL1* loci showed significant association with PBC in both the replication analysis and in an analysis combining the replication and initial GWAS datasets (Fig. 1 and Supplementary Fig. 2).

Among the PBC loci confirmed by this analysis, the locus at *IRF5-TNPO3* (encoding interferon regulatory factor 5 and transportin 3) is of interest because of the integral immunoregulatory roles for *IRF5*, the prior association of this locus with systemic lupus erythematosus (SLE)[4, 5], systemic sclerosis[6] and Sjögrens syndrome[7], and the strong effect of the disease-associated SNP at this locus on disease risk (rs10488631) in the replication ($P = 1.13 \times 10^{-8}$, odds ratio (OR) = 1.58) and combined datasets ($P = 8.66 \times 10^{-13}$, OR = 1.57). Association of this locus was thus explored, initially by resequencing the *IRF5* locus intronic and exonic regions and the 5′ and 3′ flanking regions of *IRF5* in genomic pools of 100 subjects so as to delineate the genetic variation across the locus. This analysis confirmed prior reports of 41 polymorphisms across this region, but it failed to identify any new variants. Among these 41 SNPs, two (rs3807135 and rs3834330) failed repeatedly in genotyping assays and four (rs10275092, rs12537192, rs1727172 and rs754280) showed negligible polymorphism. Thus, for fine-mapping studies, 1,330 cases and 1,833 controls were geno-typed for 35 SNPs across this region. Among the associations identified (Supplementary Table 2), the strongest signals were from the rs12539741 and rs2070197 alleles. These variants map to just 3′ of the *IRF5* coding region and are in tight linkage disequilibrium with one another ($r^2 > 0.95$), and their associations with PBC reach fine-mapping *P* values of 1.65×10^{-10} (OR = 1.63) and 3.74×10^{-10} (OR = 1.62), respectively. Conditional logistic regression analysis did not demonstrate any evidence for multiple genetic effects at the *IRF5-TNPO3* locus, with the association at this locus with PBC appearing to be accounted for by a single variant, either rs12539741 or rs2070197 (Supplementary Table 3). Although unidentified variants outside the sequenced region may also be relevant to disease association or may even be disease causal, the latter two SNPs are among the SNPs at this locus that are highly associated with SLE, and both are correlated with changes in *IRF5* expression in transformed B cells4. By contrast, an insertion-deletion polymorphism 64 bp upstream of *IRF5* exon 1a, representing a putative disease-causal variant for SLE, showed modest association with PBC (fine-mapping $P = 1.00 \times 10^{-3}$). No associations were detected between PBC and another SLE-associated SNP, rs2004640, or a 3′ UTR variant, rs10954213, that has previously been identified as a predictor of *IRF5* expression level[4].

A second region of interest is the 17q12-21 locus, as all eight of the tested SNPs across this region achieved significance in the replication analysis (*P* values between 1.78 × 10−9 and 1.88 × 10−5). This chromosomal region has also been associated with asthma[8], Crohn's disease[9] and type 1 diabetes[10] and contains four genes, *ZPBP2* (encoding zona pellucida–binding protein 2), *IKZF3* (encoding IKAROS family zinc finger 3 protein, involved in leukocyte development and IgE production), *GSDMB* (encoding gasdermin-B, involved in epithelial barrier function) and *ORMDL3* (encoding ORM1-like protein 3). All eight of the SNPs tested here were in linkage disequilibrium (pairwise r^2 values ranged from 0.66 to 0.96), but the strongest association signal came from a *ZPBP2* SNP, rs11557467 (replication $P = 1.78 \times 10^{-9}$, OR = 0.71; combined $P = 3.50 \times 10{-}13$, OR = 0.72). Although further studies are needed to pinpoint the relevant disease-causative allele(s), results of additive conditional logistic regression analysis suggest that this SNP fully accounts for the association signal across this region (Supplementary Table 4).

The replication and combined association data also identified a strong association of PBC with two SNPs (rs3890745 and rs3748816) at the *MMEL1* (encoding membrane metallo-endopeptidase–like 1) locus on chromosome 1p36 (replication $P = 3.31 \times 10{-}6$, OR = 1.31, combined $P = 2.28 \times 10{-}9$, OR = 1.32 and replication $P = 8.14 \times 10{-}5$, OR = 1.31, combined $P = 3.15 \times 10{-}8$, OR = 1.33, respectively) (Table 1 and Supplementary Table 1). These SNPs are in linkage disequilibrium with one another ($r^2 > 0.88$) and one of them (rs3748816) is a nonsynonymous SNP in exon 16 that encodes a potentially functional methionine-to-threonine substitution, whereas the other (rs3890745) maps within intron 2 of *MMEL1* and has been associated with risk for rheumatoid arthritis and for celiac disease[11,12].

A suggestive association signal (combined $P = 9.12 \times 10^{-7}$, OR = 1.27) was observed in the combined case-control cohort for rs3745516, an intronic SNP in *SPIB*, which is a gene encoding the Spi-B transcription factor. This SNP did not achieve significance in the replication cohort after Bonferroni correction ($P = 6.21 \times 10^{-3}$, OR = 1.18), but the strength of association in the combined analysis as well as the role of Spi-B in dendritic cell development[13] and B-cell receptor signaling are in keeping with potential relevance of this locus to PBC pathogenesis.

Anti-mitochondrial antibodies (AMA) are found in most individuals with PBC, but no correlation of specific PBC genotypes with AMA status was observed in our prior[2] or current association studies (data not shown). PBC is also associated with specific nuclear antibodies, with some 20% of individuals with PBC manifesting glycoprotein-210 antibodies (anti-gp210) directed against the human nuclear pore complex and/or sp100 antibodies that recognize a 53-kDa nuclear antigen[14]. Evaluation of genotype status in the subset of 462 cases typed for nuclear antibodies revealed a strong association of the *HLA* locus (rs9277535 at *HLA-DPB1*) with disease ($P = 4.25 \times 10^{-8}$, OR = 2.25) in anti-sp100–positive individuals (Supplementary Table 5a) in contrast to anti-sp100–negative individuals ($P = 3.52 \times 10^{-4}$, OR = 1.36). A further analysis incorporating GWAS data available for 412 of the individuals with known anti-sp100 status also revealed that among 13 MHC-region SNPs tested, three SNPs at the *HLA-DPB1* locus were the most strongly associated with disease in anti-sp100–positive individuals but not in anti-sp100–negative individuals (Supplementary Table 5b). In particular, for these SNPs, the OR associations with PBC risk were much higher in anti-sp100– positive cases compared to anti-sp100–negative cases. By contrast, no genetic distinctions were apparent for the anti-gp210–positive subgroup (Supplementary Table 6). These findings need to be interpreted cautiously in view of the relatively small sample size used here, but they suggest a relevance of the *HLA* locus to anti-sp100 status, which parallels previously reported correlations of anti-CCP and *HLA-DRB1* status in rheumatoid arthritis[15].

In conclusion, we identify *IRF5-TNPO3*, 17q12-21 and *MMEL1* as three new risk loci for PBC. Our data also suggest some genetic sub-structure may exist in PBC in relation to anti-sp100 status. Notably, all the PBC risk loci replicated or identified here have been implicated in other autoimmune diseases. Our data provide further evidence for the existence of shared autoimmunity susceptibility loci that contribute to the frequent appearance of additional autoimmune diseases in individuals with PBC and their families.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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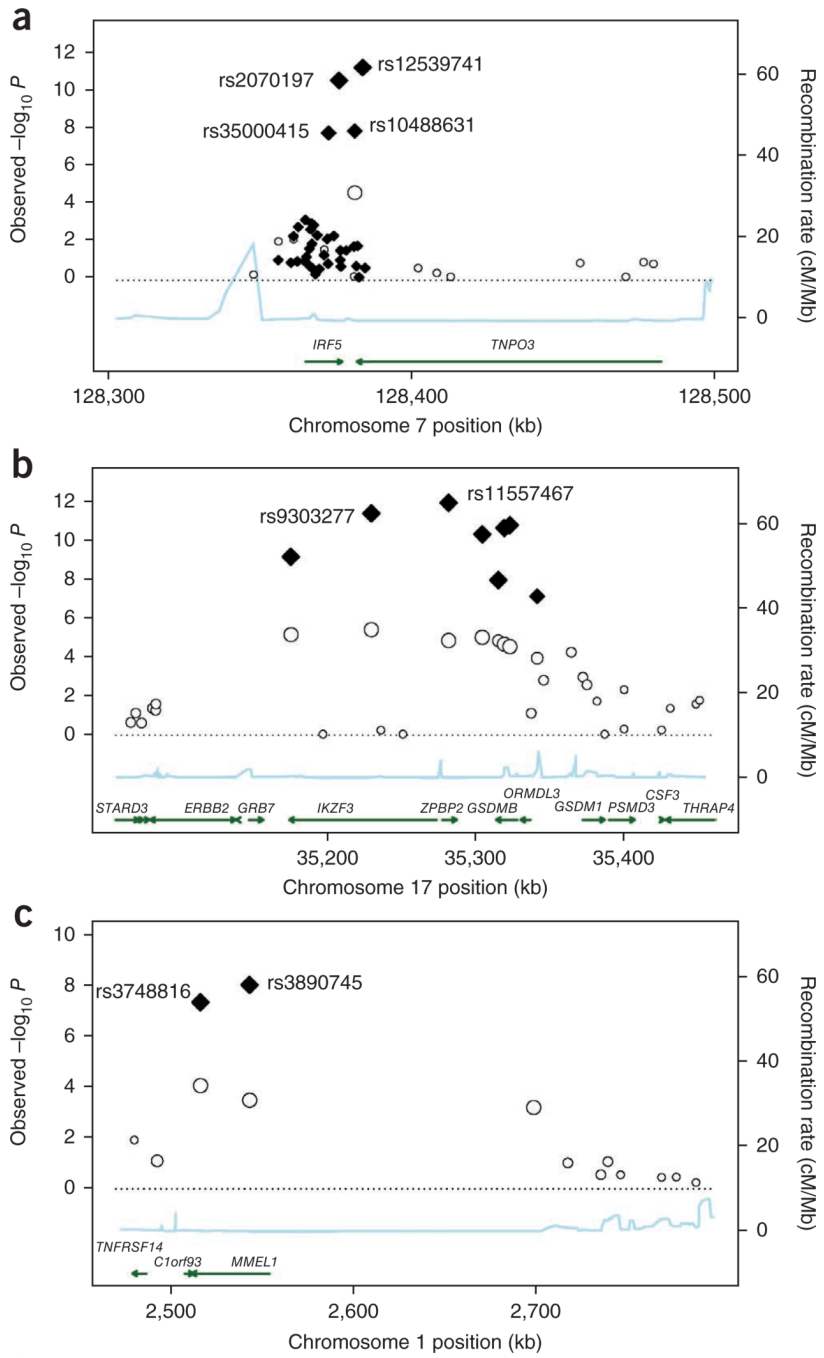

figure 1.

Association plots for the *IRF5-TNPO3*, 17q12-21 and *MMEL1* loci. (**a–c**) Strength of the associations and recombination rates estimated from HapMap data for genotyped SNPs are shown for the (**a**) *IRF5-TNPO3*, (**b**) 17q12-21 and (**c**) *MMEL1* loci. Both genome-wide association (circles) and combined fine-mapping (diamonds) data are shown where available. The extent of linkage disequilibrium with the most significant polymorphisms are indicated by the size of each data point; larger data points indicate stronger linkage disequilibrium. Gene positions for each gene region are indicated by the arrows, with the arrow direction representing the orientation of translation. Linkage disequilibrium was calculated using observed data in PLINK.

Table 1

PBC GWAS, replication and combined association analyses

			GWAS		Replication								Combined stage			
					European		North American		Total							
SNP	**Gene loci**	**Risk allele**	**RAF case/control**	**P_{GWA}**	**RAF case/control**	***P***	**RAF case/control**	***P***	**RAF case/control**	***P*[a]**	**OR[b] (95% CI)**	***P*[c]**	**RAF case/control**	***P*[d]**	**OR (95% CI)**	***P*[c]**
rs10488631	*IRF5-TNPO3*	C	0.168/0.118	4.23×10^{-5}	0.193/0.113	1.43×10^{-2}	0.158/0.108	2.20×10^{-7}	0.164/0.108	1.13×10^{-8}	1.58 (1.35–1.85)	0.48	0.165/0.111	8.66×10^{-13}	1.57 (1.38–1.77)	0.85
rs11557467	*ZPBP2*	G	0.434/0.511	3.00×10^{-5}	0.478/0.551	9.39×10^{-2}	0.425/0.510	7.25×10^{-9}	0.433/0.512	1.78×10^{-9}	0.71 (0.64–0.80)	0.79	0.434/0.511	3.50×10^{-13}	0.72 (0.66–0.79)	0.9
rs3748816	*MMEL1*	C	0.410/0.339	8.50×10^{-5}	0.355/0.283	1.30×10^{-1}	0.395/0.334	2.63×10^{-4}	0.389/0.332	8.14×10^{-5}	1.31 (1.15–1.50)	0.76	0.399/0.334	3.15×10^{-8}	1.33 (1.20–1.47)	0.93

RAF, risk allele frequency; OR, odds ratio. The GWAS included 494 cases and 1,502 controls, the replication included 857 cases and 3,198 controls, and the combined collection included 1,351 cases and 4,700 controls.

[a] Replication *P*.

[b] Odds ratios are given for the risk allele.

[c] Breslow-Day *P*.

[d] Combined *P*. This refers to analyses incorporating the combined GWAS and replication datasets.