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Association of *IL12A* Expression Quantitative Trait Loci (eQTL) With Primary Biliary Cirrhosis in a Chinese Han Population

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Abstract: Genome-wide association studies in European individuals have revealed that *IL12A* is strongly associated with primary biliary cirrhosis (PBC). However, this association was not detected in replicative studies conducted in Chinese Han and Japanese populations.

To verify contributions of genetic variants of *IL12A* to the pathogenesis of PBC in Chinese populations, a replicative study of 22 single nucleotide polymorphisms (SNPs) around the *IL12A* gene locus was performed in a cohort of 586 PBC cases and 726 healthy controls. Three out of the 22 SNPs were significantly associated with PBC. The 2 SNPs with the most significant association signal were rs4679868 ($P = 6.59E{-}05$, odds ratio [OR] = 1.554 [1.253–1.927]) and rs6441286 ($P = 8.00E{-}05$, OR = 1.551 [1.250–1.924]). These 2 SNPs were strongly linked to each other ($r^2 = 0.981$), and both were found to be significantly associated with PBC in European populations.

An expression quantitative trait loci (eQTL) analysis was performed based on the observation that these 2 SNPs were located in proximity to 2 enhancers verified by luciferase reporter systems in the HEK293 cell line. The results of eQTL analysis, conducted using the publically accessible data, showed that the risk alleles of rs4679868 and rs6441286 were significantly associated with decreased expression of *IL12A* in lymphoblastoid cell lines derived from individuals of Chinese Han ancestry ($P = 0.0031$ for rs4679868 and $P = 0.0073$ for rs6441286). In addition, the risk alleles of the 2 SNPs were significantly associated with down-regulation of *SCHIP1*, a celiac disease susceptible gene, 91.5 kb upstream of *IL12A*.

These results not only demonstrated that *IL12A* is associated with PBC in the Chinese Han population but also identified a potential mechanism for its involvement in the pathogenesis of PBC.

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Abbreviations: APC = antigen-presenting cells, CEU = Utah residents of Northern and Western European ancestry, CHB = Han Chinese in Beijing, China, eQTL = expression quantitative trait loci, GWAS = genome-wide association studies, HWE = Hardy–Weinberg equilibrium, JPT = Japanese in Tokyo, Japan, LD = linkage disequilibrium, MAF = minor allele frequency, OR = odds ratio, GenevarGene Expression Variation, PBC = primary biliary cirrhosis, UTR = untranslated region.

INTRODUCTION

Primary biliary cirrhosis (PBC) is a common autoimmune liver disease, characterized by the production of antimitochondrial antibodies, and affects up to 1 in 1000 women over the age of 40.[1] In the past decade, dozens of PBC susceptibility genes have been identified using genome-wide association studies (GWAS) in European and Japanese populations.[2–5] The association signal was the strongest in the *HLA* region in almost all of these studies. However, the association of non-*HLA* genes with PBC was inconsistent for different populations.[6] For example, *TNFSF15* was specifically associated with PBC in Japanese and Chinese cohorts,[5,7,8] but not in European populations. In contrast, *IL12A* was found to be significantly associated with PBC in Canadian, Italian, and British populations,[2–4] but the association signal was not detected in the Japanese population.[5,9,10] A reasonable interpretation of this inconsistency is that the results could be because of the differences in genetic background of European and Asian individuals, or to the biased single nucleotide polymorphism (SNP) selection in the candidate gene-based association studies.[11]

IL12A encodes a subunit (p35) of interleukin-12 (IL-12), a heterodimeric cytokine produced by antigen-presenting cells, phagocytic cells, and B-cells during infection.[12] When initially discovered, IL-12 was reported to activate cytotoxic lymphocytes and natural killer cells.[13,14] Subsequently, it was revealed that IL12 could also regulate the differentiation of naive $CD4^+$ T cells into mature Th1 and Th2 effector cells,[15–17] and was required for the T-cell-independent induction of interferon-γ.[18]

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PL and GL made an equal contribution to this study.
GL and YL conceived the project. GL designed the assays and experiments and wrote the paper. PL and GL performed the experiments and supervised this project and revised the manuscript. PL, ZW, SC, JL, SL, and YC collected the samples and extracted genomic DNA. FZ provided valuable discussion.
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TABLE 1. Demographics and Clinical Features of Samples

	Cases	Controls
Gender, male/female	41/545	46/680
Ages range	16–80	11–79
Mean ± SD	35.27 ± 10.68	34.20 ± 11.25
Concomitant autoimmune diseases, %		
Sjögren syndrome	7.51	
Systemic lupus erythematosus	3.24	
Hashimoto thyroiditis	3.24	
Rheumatoid arthritis	2.90	
Autoimmune hepatitis	2.73	
Systemic sclerosis	2.39	
CREST syndrome	1.36	

Recently, IL-12 was found to be associated with a variety of autoimmune and inflammatory diseases, such as Sjögren syndrome (SS),[19] rheumatoid arthritis (RA),[20] Graves disease,[21] multiple sclerosis,[22] and asthma.[23] Furthermore, according to previous studies, evidence suggests that the IL-12/IL-23-mediated Th1/Th17 signaling pathway and T-helper lymphocytes are implicated in the pathogenesis of PBC.[2,24–29]

To study the genetic association of *IL12A* with PBC in the Chinese Han population, tag SNPs in the *IL12A* gene were selected and genotyped, and the association test was performed by comparing allele frequencies between PBC cases and controls recruited from Han ethnic groups. A cis-expression quantitative trait loci (cis-eQTL) analysis was also performed to explore the regulatory mechanism of *IL12A* expression. Consequently, it was hypothesized that the involvement of *IL12A* in the pathogenesis of PBC in the Han population could be clarified by this analysis.

METHODS

Study Populations

Samples from 586 patients with PBC and 726 age-matched healthy controls were collected by the Rheumatology Department of Peking Union Medical College Hospital, and 201 PBC samples were collected from multiple medical centers in China (Table 1). Of the 586 PBC patients, 545 (93%) were female and 41 (7%) were male. The recruited PBC case and control subjects were unrelated individuals of Han Chinese ethnicity, determined by self-report; informed consent was obtained from all participants of the study. All PBC patients were diagnosed according to the criteria of the American Association for the Study of Liver Diseases for PBC.[30] The demographic and clinical features of samples were listed in Table 1. This study was approved by the ethics committee of Peking Union Medical College Hospital.

SNP Selection and Genotyping

Based on GWAS results of the European and Japanese PBC cohorts, we selected the most relevant 22 tag SNPs, at the chromosomal region (chr3:159619322–159800889) surrounding *IL12A*, from the 1000 Genomes Project for Asian populations with minor allele frequency >2% by Haploview v4.2.[31] The selected 22 SNPs around the genomic region of *IL12A* were genotyped in a cohort of Chinese Han individuals (586 cases/726 controls) by MassArray iPLEX.

The genomic DNA of each sample was extracted using a QIAamp DNA mini kit (Qiagen, Hilden, Germany), and SNP genotyping was performed by the MassArray iPLEX system (Sequenom, San Diego, CA, USA) at Beijing DNALead Co. LTD. All procedures were performed according to the manufacturer's instructions. Approximately 10 ng of genomic DNA was amplified by multiplex polymerase chain reaction (PCR) and the amplicons were subjected to locus-specific single-base extension reactions. The extended products were desalted and transferred to a 384-element SpectroCHIP array. Allele detection was performed using MALDI-TOF mass spectrometry, and the mass spectrograms were analyzed by the MassArray TYPER software v4.0 (Sequenom).

The procedures for SNP genotyping and Hardy–Weinberg equilibrium analysis were performed as previously reported.[32]

Statistical Test for Association

A PLINK tool set was primarily used to conduct the association analysis.[33] The χ^2 test was applied to compare allele frequencies between case and control groups. The gender and age of subjects were taken as covariates because of their correlation with the incidence of PBC. The odds ratio and 95% confidence interval (95% CI) were also calculated. The SNPs with a P value <0.05, after Bonferroni correction, for multiple testing were regarded as significantly associated with PBC.

Cis-eQTL Analysis

Cis-eQTL analysis was performed with publically accessible data using Genevar (Gene Expression Variation) v3.2.0 software.[34] The gene expression data of lymphoblastoid cell lines isolated from Utah residents of Northern and Western European ancestry (CEU), Han Chinese in Beijing, China (CHB), and Japanese in Tokyo, Japan (JPT) samples in the HapMap Phase 3 were downloaded.[35] For the 3 SNPs significantly associated with PBC, genotypes of the corresponding individuals were retrieved from the 1000 Genomes Project Integrated Phase 1 Release.[36] Spearman rank correlation coefficient (ρ) was calculated to explore the association between genotype of SNPs and the expression of nearby genes (±1 Mb from the SNP); 10,000 permutations were applied to calculate the P values for the 3 SNPs, and SNPs with a P value <0.01 were regarded as potential eQTLs.

RESULTS

Significant Association Between *IL12A* and PBC in the Chinese Han Population

Samples from 586 PBC patients (16–80 years old, median age = 35), as well as 726 age-matched healthy controls, were collected (Table 1). Of the 586 PBC patients, 93% (n = 545) were female, which is indicative of the significantly higher incidence of PBC in the female Chinese Han population compared to that in the male population. To investigate the association of *IL12A* with PBC in the Han Chinese population, 22 tag SNPs were genotyped in 586 PBC patients and 726 healthy controls (see ''Methods'' section).

After the association test, 3 out of the 22 SNPs were found to be strongly associated with PBC (rs4679868, $P = 6.59E{-}05$; rs6441286, $P = 8.00E{-}05$; rs666251, $P = 1.22E{-}03$) and 2 were marginally linked to the disease (rs4680534, $P = 0.01$; rs6441282, $P = 0.017$) (Table 2). Among these 3 SNPs, the most significant association signals were detected at rs4679868 and rs6441286, 10 and 15 kb downstream of *IL12A*, respectively.

TABLE 2. Association of *IL12A* SNPs With PBC in Chinese Han

SNP ID	Risk Allele	Chromosomal Location	Relative to *IL12A*	Minor Allele Frequency		*P* Value	Odds Ratio (95% CI)
				Case	Control		
rs1075498	A	chr3:159619322	5′-Flanking region	0.224	0.225	0.962	0.994 (0.825–1.197)
rs12494630	C	chr3:159623558	5′-Flanking region	0.113	0.138	0.059	0.797 (0.630–1.008)
rs1353248	A	chr3:159625796	5′-Flanking region	0.213	0.219	0.736	0.964 (0.798–1.165)
rs17810546	G	chr3:159665049	5′-Flanking region	0.001	0.003	0.390	0.312 (0.035–2.798)
rs2243123	C	chr3:159678735	5′-Flanking region	0.077	0.091	0.231	0.840 (0.635–1.111)
rs2243143	A	chr3:159689399	5′-Flanking region	0.150	0.167	0.263	0.879 (0.704–1.097)
rs248919	G	chr3:159698944	5′-Flanking region	0.305	0.322	0.571	0.924 (0.722–1.184)
rs2647938	T	chr3:159699077	5′-Flanking region	0.191	0.219	0.080	0.841 (0.694–1.019)
rs2936298	A	chr3:159709650	5′-Flanking region	0.455	0.416	0.069	1.171 (0.988–1.387)
rs33036	C	chr3:159714801	3′-Flanking region	0.384	0.421	0.090	0.858 (0.720–1.022)
rs4679868	A	chr3:159719132	3′-Flanking region	0.544	0.435	6.59E−05	1.554 (1.253–1.927)
rs4680534	C	chr3:159724153	3′-Flanking region	0.115	0.149	0.010	0.737 (0.583–0.931)
rs485497	G	chr3:159728877	3′-Flanking region	0.215	0.253	0.084	0.805 (0.633–1.023)
rs485499	C	chr3:159729288	3′-Flanking region	0.122	0.133	0.409	0.903 (0.716–1.139)
rs485789	T	chr3:159730147	3′-Flanking region	0.121	0.133	0.374	0.895 (0.708–1.130)
rs6441282	T	chr3:159732881	3′-Flanking region	0.202	0.248	0.017	0.767 (0.618–0.952)
rs6441286	G	chr3:159743882	3′-Flanking region	0.544	0.434	8.00E−05	1.551 (1.250–1.924)
rs6441289	C	chr3:159745862	3′-Flanking region	0.204	0.206	0.956	0.992 (0.806–1.221)
rs659596	C	chr3:159750078	3′-Flanking region	0.007	0.008	1	0.900 (0.361–2.246)
rs666251	G	chr3:159756450	3′-Flanking region	0.229	0.285	1.22E−03	0.744 (0.623–0.889)
rs6795264	C	chr3:159797189	3′-Flanking region	0.158	0.174	0.309	0.892 (0.722–1.101)
rs9847915	A	chr3:159800889	3′-Flanking region	0.298	0.300	0.896	0.987 (0.832–1.170)

CI = confidence interval, PBC = primary biliary cirrhosis, SNP = single nucleotide polymorphism.

rs6441286 and rs4679868 have been reported to be associated with PBC in European populations by the GWAS study with Illumina Human Hap370 BeadChip and by fine-mapping analysis, respectively.[2] However, in the replication analysis with rs6441286 and subsequent GWAS study by Affymetrix Axiom Genome-Wide ASI 1 Array Plate in the Japanese population, the association was negative.[5]

Putative Regulatory Regions Around the 2 SNPs

In particular, rs4679868 and rs6441286 were mapped to a 5-kb region of strong linkage disequilibrium (LD) flanked by 2 recombination hotspots. Furthermore, this region was located in proximity to 2 putative enhancers, according to the chromatin modification patterns (Figure 1B) from the ENCODE project.[37] The expression of luciferase was increased in luciferase reporter assays using these 2 putative enhancers. Therefore, it was postulated that the genetic variants in the 5-kb region harboring rs4679868 and rs6441286 could affect the expression-regulation activity of the 2 putative enhancers. Alternatively, other unknown regulation elements could be mapped to this 5-kb region.

rs4679868 and rs6441286 Are Potential eQTLs

To further investigate the possible mechanism of *IL12A* in the pathogenesis of PBC, we conducted a cis-eQTL analysis for the 3 significantly associated SNPs within CHB, JPT, and CEU populations from the HapMap Phase 3 data set (see ''Methods'' section). In the ±1 Mb genome region around the 3 SNPs, 8 genes, namely *SCHIP1*, *IL12A*, *SMC4*, *ARL14*, *PPM1L*, *KPNA4*, *TRIM59*, and *IFT80* were selected for the eQTL analysis.

In the lymphoblastoid cell lines derived from individuals of HapMap Phase 3, genotypes of rs4679868 and rs6441286 were found to be significantly associated with the expression of *IL12A* in the CHB population (Table 3). Moreover, the risk alleles of both rs4679868 and rs6441286 were significantly associated with decreased expression of *IL12A* in CHB samples (Figure 2B and D). The expressions of IL12A at mRNA level were validated in 15 healthy Chinese controls and showed that A allele of rs6441286 associated with lower expression of IL12A (data not shown). However, in CEU samples, the risk alleles of these 2 SNPs were significantly correlated with decreased expression of *SCHIP1* (Figure 2C and E), rather than the reported PBC susceptible *IL12A* gene. Furthermore, for the Japanese population, although the genotype of rs6441286 was found to be significantly ($P = 0.0039$) associated with expression of *IFT80*, only a marginal association ($P = 0.029$) was detected between the genotype of rs4679868 and expression of *IFT80* (Table 3). There was no data for rs666251 in the Genevar dataset.

DISCUSSION

To explore the association of *IL12A* with PBC in the Chinese Han population, we recruited more than 1200 PBC cases and age-matched controls for this study. Particularly, 22 SNPs, including both the known PBC-associated SNPs and tag SNPs, were selected based on the haplotype structure of CHB samples in the HapMap Project. The region covered the entire loci of *IL12A*, including untranslated portions of *IL12A*. This study was the first fine-mapping genetic analysis for *IL12A* in a Chinese Han PBC cohort. In contrast to other studies conducted

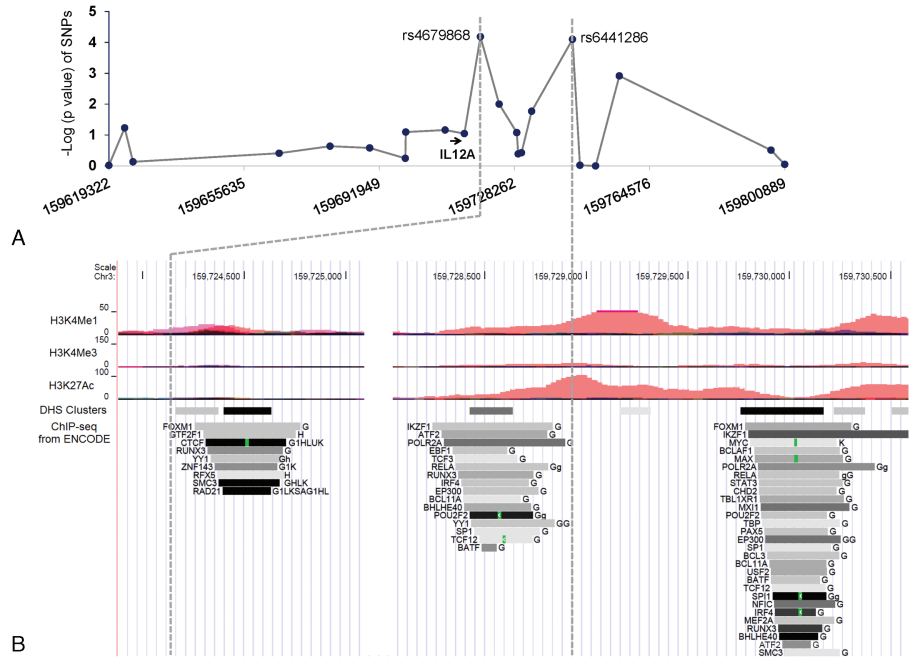

FIGURE 1. Regional plot of association results for SNPs in the region of *IL12A* at 3q25.33 and the regulatory marks at this region. (A) Data plotted within the region containing *IL12A* show the results of fine mapping of the 22 SNPs (dark blue dots). The −log10 (*P* values) of SNPs are presented according to their chromosomal positions (x-axis). The *IL12A* gene within the region is shown by a black arrow. The top 2 SNPs are labeled with their rs numbers. (B) The regulatory marks at this region were extracted from UCSC Genome Browser. The left panel shows the data for H3K4Me1 (mono methylation), H3K4Me3 (triple methylation), H3K27Ac (acetylation), DNaseI hypersensitivity sites (DHS), and transcription factor ChIP-seq around SNP rs4679868. The broken light gray lines indicate the location of rs4679868 and rs6441286 in the bottom sets. The right panel is the same data around rs6441286. For the ChIP-seq data, bar weight corresponds to stronger transcription factor binding within the region. The characters to the right of the bars are abbreviations of cell lines used in the ENCODE project. G, GM12878; H, HeLa-S3; 1, H1-hESC; A, A549; K, K562; L, HepG2; S, SK-N-SH; g, other B cell lines, such as GM06990, GM08714, GM10847, GM12801, GM12864, GM12865, GM12872, GM12873, GM12874, GM12875, GM12891, GM12892, GM15510, GM18505, GM18526, GM18951, GM19099, GM19193, GM19238, GM19239, and GM19240. SNP = single nucleotide polymorphism.

in Chinese Han[7] and Japanese populations,[5] this analysis clearly demonstrated that the genetic variants of *IL12A* were significantly associated with PBC.

Previous studies conducted in European populations demonstrated the significant association of rs4679868 and rs6441286 with PBC.[2,4] In this study of a Chinese Han population, the top 2 significant signals for association with PBC were also detected at these loci. However, the candidate gene-based assay and GWAS study in the Japanese cohort found no such association of rs6441286 with PBC.[5] As for rs4679868, it

TABLE 3. eQTL Analysis of the Top 2 PBC-Associated SNPs With Nearby Genes (<1 Mb)

	rs4679868						rs6441286					
	CEU		CHB		JPT		CEU		CHB		JPT	
Genes	rho	*P* Value	rho	*P* Value	rho	*P* Value	rho	*P* Value	rho	*P* Value	rho	*P* Value
SCHIP1	0.266	0.005	0.150	0.183	0.007	0.948	0.271	0.004	0.180	0.111	0.007	0.951
IL12A	0.187	0.051	0.327	0.003	0.071	0.525	0.191	0.048	0.298	0.007	0.097	0.389
SMC4	0.035	0.720	−0.110	0.332	0.076	0.498	0.039	0.685	−0.104	0.359	0.093	0.413
ARL14	0.024	0.803	0.118	0.297	0.072	0.523	0.032	0.744	0.098	0.388	0.081	0.475
PPM1L	−0.148	0.124	0.109	0.336	−0.020	0.857	−0.156	0.107	0.129	0.256	0.016	0.886
KPNA4	−0.048	0.621	−0.001	0.994	0.126	0.258	−0.043	0.660	−0.005	0.967	0.172	0.127
TRIM59	−0.159	0.099	0.033	0.770	0.133	0.235	−0.154	0.113	0.056	0.623	0.120	0.288
IFT80	−0.106	0.273	−0.087	0.445	0.241	0.029	−0.103	0.289	−0.098	0.387	0.319	0.004

CEU = Utah residents of Northern and Western European ancestry, CHB = Han Chinese in Beijing, China, eQTL = expression quantitative trait loci, JPT = Japanese in Tokyo, Japan, PBC = primary biliary cirrhosis, SNP = single nucleotide polymorphism.

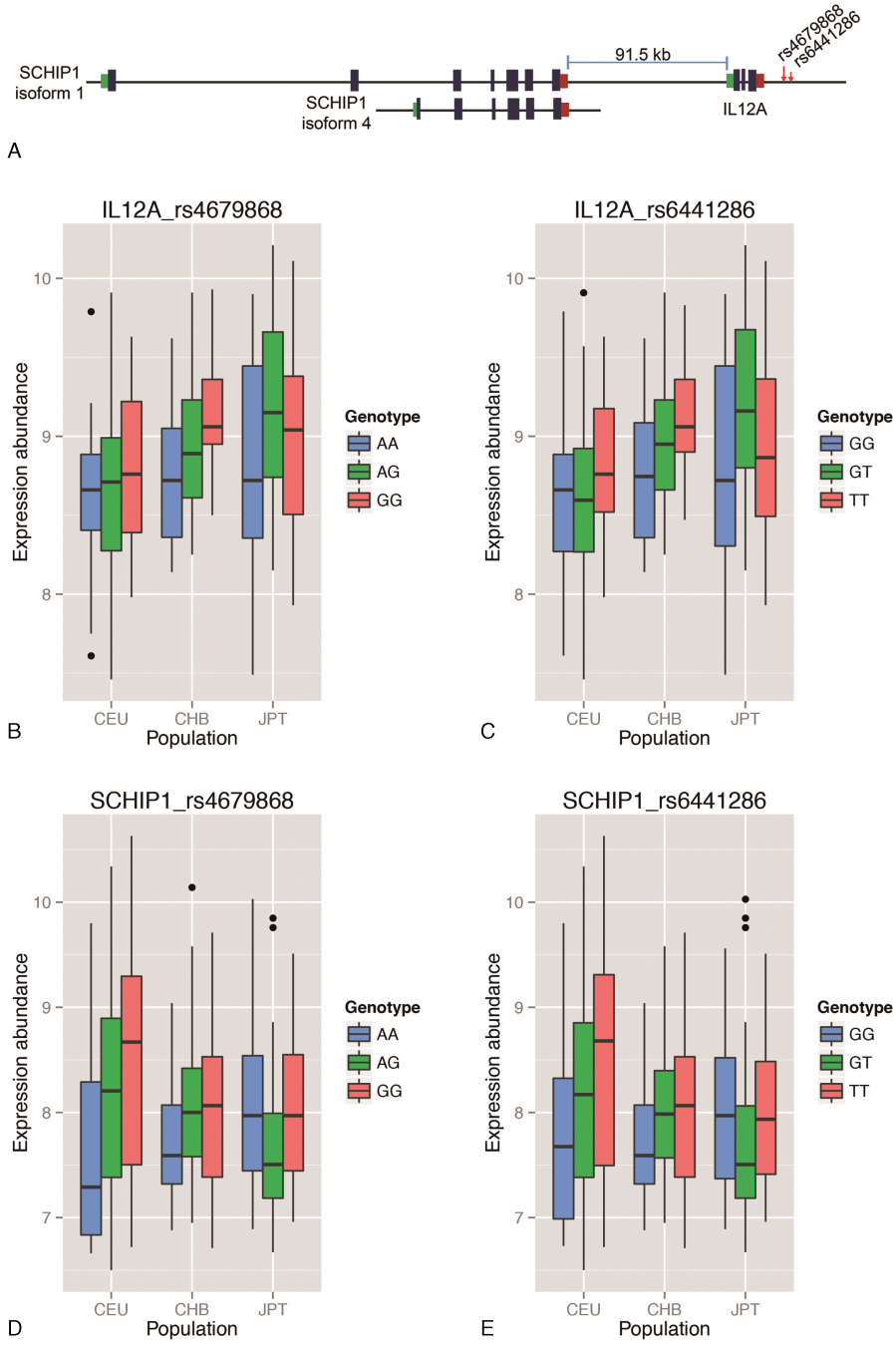

FIGURE 2. eQTL for rs4679868 and rs6441286 in CEU, CHB, and JPT populations. (A) Diagrams of *SCHIP1*, *IL12A*, and the relative location of rs4679868 and rs6441286. Dark blue horizontal lines represent genome DNA, green boxes are 5′ UTRs, dark blue vertical boxes are exons, and red boxes represent 3′ UTRs. The distance between the 3′ UTR of *SCHIP1* and the 5′ UTR of *IL12A* is 91.5 kb. (B, C) eQTL of *IL12A* by rs4679868 (B) and rs6441286 (C). (D, E) The eQTL of *SCHIP1* by rs4679868 (D) and rs6441286 (E). CEU = Utah residents of Northern and Western European ancestry, CHB = Han Chinese in Beijing, China, eQTL = expression quantitative trait loci, JPT = Japanese in Tokyo, Japan, UTR = untranslated region.

was not included in the Affymetrix Axiom Genome-Wide ASI 1 Array Plate used for the GWAS study.[5]

Some reasons could exist for this disparity. First, allele frequencies of the 2 SNPs in Chinese and European populations were different from those in Japanese populations. We extracted genotyping results of rs4679868 and rs6441286 for CHB, JPT, and CEU populations from HapMap phase 2 and phase 3 and 1000 Genomes projects (Supplementary Table 1, http://links.lww.com/MD/A963). The number of samples in these projects for CHB and JPT was increased from 45 in HapMap

phase 2 to about 100 in HapMap phase 3 and 1000 genome project. In HapMap phase 2, the minor alleles of the 2 SNPs were identical in the 3 populations. However, the frequencies of the minor alleles in JPT were slightly lower than those in CHB and CEU populations. In HapMap phase 3 and 1000 Genome project, the minor alleles in CHB and CEU were major alleles in JPT. These were consistent with the genotyping results in our study. As for the population size, the recruited samples for cases and controls in our study were 586 and 726, respectively. The cohort was larger than those of the Japanese cohort in the discovery stage (case/control = 487/476). Therefore, it seems that the divergent allele frequencies in these populations may be not caused by the reduced effect size. Therefore, we inferred that the allele frequency differences of the SNPs in CHB, CEU, and JPT might be the main reason for inconsistencies between the association signals in these populations. Recently, a replication study for only 2 SNPs (rs2366408 and rs485499) of *IL12A* was performed in another Chinese Han cohort; however, that study failed to detect association signals with PBC.[7] One SNP, rs485499, was also included in our fine mapping study. Consistent with this previous study, no association signal with PBC was detected for rs485499 in our analysis ($P = 0.518$). This made our study reliable. Second, the inconformity of gender and age distributions in the Japanese cohort might impair the detection power of positive signals. To boost the detection power of GWAS, except for the interested diseases, other traits such as gender, age, and origin should be as uniform as possible in the case and control groups. However, for the Japanese cohorts, the difference between females and males was highly significant in the discovery stage ($P = 9.47E{-}15$) and replication stage ($P = 2.71E{-}14$) (Supplementary Table 2, http://links.lww.com/MD/A963). The age distributions of Japanese cohorts were not even in the 2 stages either. As for the Chinese samples in this study, the gender and age distributions in the groups were very even ($P = 0.66$). In our study, the cohort (586 cases/726 controls) was larger than that of the Japanese one in the discovery stage (case/control = 487/476). Moreover, other traits such gender and age were evener than those of the Japanese cohort. According to the clinical information, sometimes PBC is concomitant with other autoimmune diseases (Table 1). If the ratio were higher, it might interfere with the detection power of interested diseases. As for our samples, the top 1 concomitant disease was SS, accounted for only 7.51%. Other diseases such as systemic lupus erythematosus (SLE), Hashimoto thyroiditis, RA, autoimmune hepatitis, systemic sclerosis, and CREST syndrome were <3.5%. Since the concomitant diseases were only account for a very small portion, it could only confer a very limited effect on the detection power. Third, the LD structures of the region containing rs4679868 and rs6441286 were different between CHB and JPT populations. After analyzing the genotyping data from HapMap phase 3 for CHB and JPT by HaploView, the 2 SNPs were outside of the 2 highly linked LD blocks in CHB (Supplementary Figure 1A, http://links.lww.com/MD/A963). However, in JPT, the 2 SNPs were encircled by 1 large LD block (Supplementary Figure 1B, http://links.lww.com/MD/A963). This might be other reasons for the divergent results in CHB and JPT.

It has been reported that the p35 (*IL12A*) mRNA expression in untreated and treated SLE patients was significantly lower than that in healthy controls ($P = 0.015$ and $P = 0.000$, respectively).[38] The p35 subunit was also detected to be substantially lower in the duodenal mucosa of cats with inflammatory enteropathy compared with that in the mucosa of healthy cats ($P = 0.001$).[39] These findings suggest that lower expression of *IL12A* is correlated with autoimmune diseases. To better understand the involvement of *IL12A* in PBC pathogenesis, a cis-eQTL analysis was performed using publically accessible genotype and gene expression data. Although only a limited number of genes were investigated, the result of this cis-eQTL analysis revealed the potential involvement of a 5-kb region, harboring rs4679868 and rs6441286, in the regulation of expression of nearby genes. Surprisingly, the risk alleles of rs4679868 and rs6441286 were significantly associated with decreased expression of *IL12A* and *SCHIP1* in CHB and CEU populations, respectively.

SCHIP1, 91.5 kb upstream of *IL12A*, encoded a Schwannomin-interacting protein, reported to be associated with major depressive disorder.[40] It has been reported that the ectopic expression of *SCHIP1* is associated with Celiac disease, an autoimmune disorder.[41,42] Results of the cis-eQTL analysis implied a novel phenomenon, wherein the same locus affected the pathogenesis of 1 or 2 diseases by adjusting the expression of different genes.

Additionally, in an attempt to identify the causal variant of gene expression regulation in the 5-kb region harboring rs4679868 and rs6441286, we also sequenced 98 PBC samples and 98 healthy controls, after PCR amplification. However, no novel genetic elements were discovered (data not shown). Therefore, further investigation on the function of this region is urgently needed, using appropriate cell or animal models, and more experimental evidence is required to confirm the involvement of *IL12A* in PBC.[8]

In conclusion, this analysis clearly demonstrated the association of *IL12A* with the pathogenesis of PBC in the Han Chinese population. It was also shown that the SNPs associated with PBC in this group were potential cis-eQTLs, implying a possible mechanism for their involvement in PBC pathogenesis. Although the risk alleles of these SNPs were significantly associated with decreased expression of *IL12A* in the lymphoblastoid cell lines from Chinese Han individuals, precise characterization of the causal variants and functional sequelae of the loci that confer a risk for PBC need to be elucidated.

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