

# Association of CCR6 functional polymorphisms with Primary Biliary Cholangitis

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## ABSTRACT

The biliary epithelial cells release CC chemokine receptor 6 (*CCR6*) ligand 20 (*CCL20*), leading to recruitment of *CCR6*<sup>+</sup> T cells and subsequent infiltration into the biliary epithelium in primary biliary cholangitis patients. Previous genome-wide multi-national meta-analysis, including our Han Chinese cohort, showed significant association of *CCR6* and *CCL20* single nucleotide polymorphisms (SNP) with PBC. We report here that significantly associated SNPs, identified in the *CCR6* locus based on our Han Chinese genome-wide association study, can be separated into "protective" and "risk" groups, but only "risk" SNPs were confirmed using a separate Han Chinese PBC cohort. Only weak association of *CCL20* SNPs was observed in Han Chinese PBC cohorts. Fine-mapping and logistical analysis identified a previously defined functional variant that, leads to increased *CCR6* expression, which contributed to increased genetic susceptibility to PBC in Han Chinese cohort.

## 1. Introduction

Primary biliary cholangitis (PBC) is a chronic progressive cholestatic autoimmune liver disease that tendentially injures small intrahepatic bile ducts, and may lead to cirrhosis without effective treatment [1]. The pathogenesis of PBC is complicated and multifactorial. A large number of studies confirmed the strong genetic predisposition to PBC [2]. Previous genome-wide association studies (GWAS) in European, Japanese, and Han Chinese population identified numbers of PBC susceptibility

loci, including human leukocyte antigen (HLA) and non-HLA loci [3–7]. The Han Chinese GWAS analysis of 1126 PBC cases and 4036 healthy controls conducted by our group identified multiple SNPs in C–C chemokine receptor type 6 (*CCR6*) locus with *P* value between  $1 \times 10^{-4}$  and  $2.04 \times 10^{-7}$ , indicating *CCR6* as a potential risk locus for PBC. A subsequent meta-analysis combining European and Japanese data and our data showed several SNPs in *CCR6* locus reaching genome-wide significance [8].

*CCR6* gene is a transmembrane G-protein coupled receptor and

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expressed mainly on T cells, B cells and immature DC cells [9,10]. *CCR6* serves as the receptor for *CCL20*, a C–C type chemokine. The genome-wide meta-analysis using multiple European cohorts identified a SNP (rs4973341) in *CCL20* locus associated with PBC [11], but rs4973341 and SNPs in its shared haplotype, were not included in the Human OmniZhongHua-8 Beadchip (v1.1), used in the Han Chinese GWAS study.

In this report, we carried out a confirmational analysis of *CCR6* and *CCL20* loci with newly collected PBC cohorts and performed linkage disequilibrium and conditional regression analyses to pinpoint potential SNPs in *CCR6* locus directly associated with PBC. We also performed genotyping analysis of rs10933215, which is in complete linkage disequilibrium with rs4973341 in Chinese cohorts. and we further analyzed the interaction among significant SNPs observed in *CCR6* and *CCL20*.

## 2. Materials and methods

### 2.1. Study subjects

The study used a two-stage approach involving GWAS-based discovery and replication using TaqMan SNP genotyping in an independent population. All patients with PBC were diagnosed in accordance with the criteria recommended by the American Association for the Study of Liver Diseases [12]. All PBC serum samples were tested for AMA-M2, anti-gp210 and anti-sp100 antibodies using in-house developed ELISA tests. The detailed diagnosis and patient inclusion have been described in our previous publication [13]. Among them, 1126 PBC patients and 4036 healthy controls were reported in our previous GWAS [7,14,15]. A total of 2093 PBC patients, 1129 healthy controls were from the Jiangsu Provincial PBC Collaborative Groups, and 2207 extra controls from the Colorectal Cancer Research Project were included in the replication cohort [16].

### 2.2. SNP genotyping

Genomic DNA is extracted from blood samples. SNP genotyping in the replication study was performed by TaqMan assay for rs12529876, rs4710181 and rs6905911. The primer and probe sequences are listed in Table S1. The TaqMan assay was performed using a Bio-Rad CFX Manager Real-Time PCR system in 10  $\mu$ L of reaction mixtures containing 1  $\times$  PCR buffer, 2 mM deoxyribonucleotide triphosphate mix, 2  $\mu$ M of each primer, 3  $\mu$ M of labeled probe, 0.25 U Taq HS polymerase (Takara), 5 ng of genomic DNA, and 6.15  $\mu$ L deionized water. PCR conditions were as follows: 95  $^{\circ}$ C for 10 min, followed by 40 cycles of 95  $^{\circ}$ C for 30 s and 60  $^{\circ}$ C or 62  $^{\circ}$ C for 30 s. To sequence for *CCR6*DNP polymorphism, PCR reaction samples were sent to the company for Sanger sequencing. Primer sequences for sequencing are as follows: 5'-CGCTTTTGACTAGCACTCGA-3', 5'-TTGTTTCATCCCAACCTCCCT-3'. Product length: 264bp.

### 2.3. Immunohistochemistry

Paraffin-embedded liver tissues, derived from ultrasound-guided needle liver biopsies of 30 PBC patients, 30 autoimmune liver hepatitis (AIH) patients, 14 non-alcoholic fatty liver disease (NAFLD) patients, 14 chronic hepatitis B (CHB) patients, and 6 healthy controls (HC), were studied. Sections were stained with either hematoxylin and eosin (H&E) or Masson and were independently reviewed “blindly”. Inflammatory degrees and fibrotic stages were graded according to the Scheuer scoring system. The six healthy liver tissues were collected from donors whose livers were subsequently used for liver transplantation. Immunohistochemistry imaging of *CCR6* staining of human liver tissues was performed on a Leica Bond system (Leica, Germany) using the standard protocol. The liver sections were pre-treated using heat-mediated antigen retrieval with sodium citrate buffer (pH 6, epitope retrieval solution 1) for 20 min followed by incubation with an anti-*CCR6* antibody (1:200

dilution, ab93086, Abcam) for 20 min at room temperature and then detected using a horse radish peroxidase-conjugated compact polymer-system. 3,3'-diaminobenzidine was used as the chromogen. The liver sections were then counterstained with hematoxylin. All the sections were visualized using light microscopy (Olympus, Japan), and five fields were randomly selected for each section. The numbers of *CCR6*-positive cells were quantified at 40  $\times$  10 magnification. All the samples were examined by a hepatic pathologist, and all data are reported as mean  $\pm$  standard error (s.e.).

### 2.4. Statistical analysis

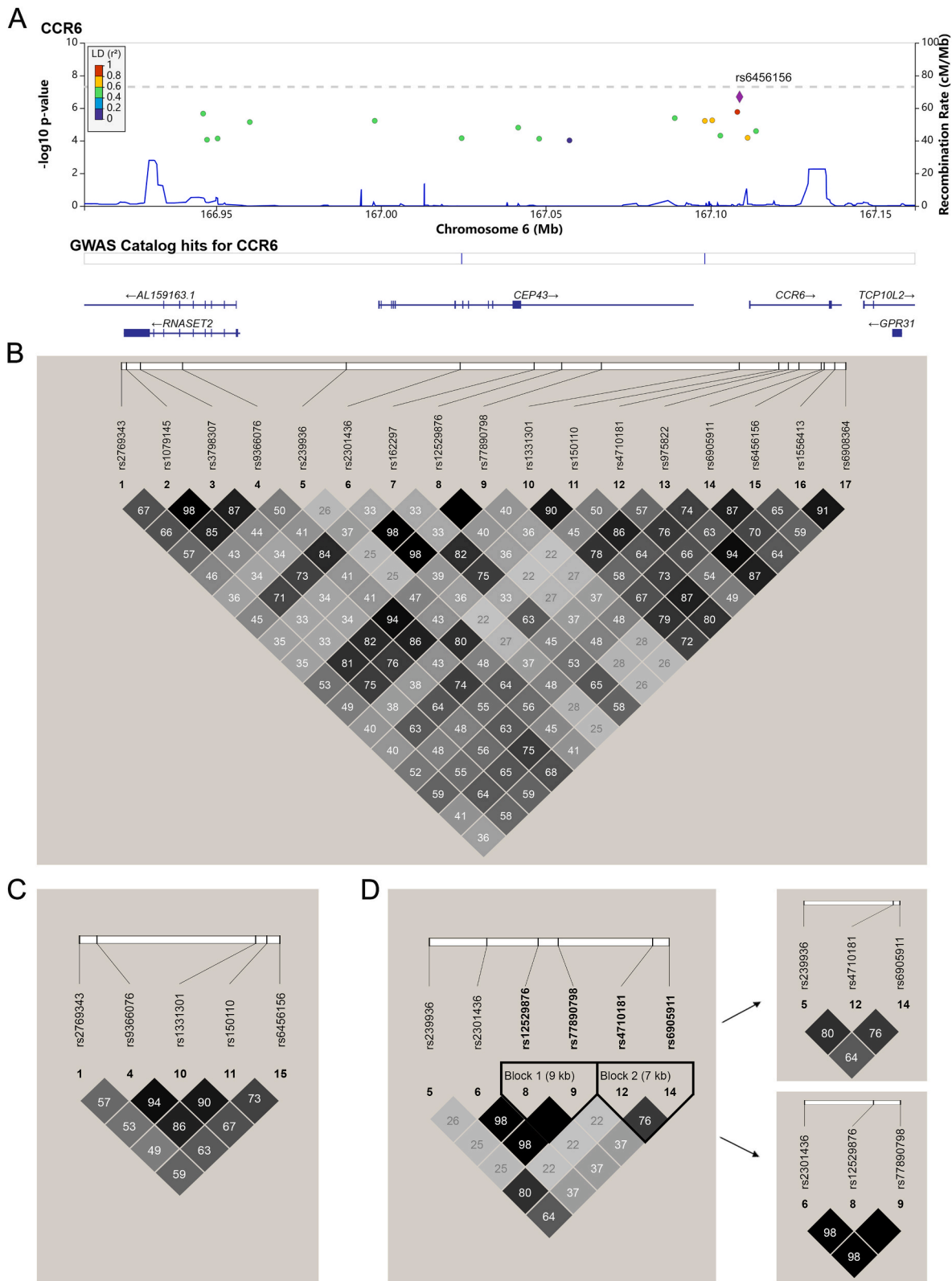
Binary logistic regression analysis with SPSS 23.0 (SPSS, Inc., Chicago, IL) was used to determine *P* values, odds ratios (OR), and confidence intervals (CI) for the association between SNPs for the validation analysis. Stata12.0 (Stata Corporation, College Station, TX) software was used to conduct a meta-analysis using a random effect model for the two distinct case-control cohorts. Conditional logistical regression analysis was performed by PLINK 1.9 (<http://pngu.mgh.harvard.edu/purcell/plink/>) [17]. The Mann–Whitney *U* test was used to evaluate differences in continuous variables, a *P* value of <0.05 was considered statistically significant, correlations were determined using the Spearman's correlation coefficient, all analyses were two-tailed and performed using Prism software Version 6.0 (Graphpad Software, La Jolla, CA, USA). Linkage disequilibrium analysis was performed using Haploview 4.2 [18]. Gene and protein expression data in cells and tissues were obtained from the Human Protein Atlas (<https://www.proteinatlas.org/>). Both SNP information and linkage disequilibrium data were downloaded from the Ensembl Database (<https://grch37.ensembl.org/index.html>). Functional annotation of SNPs was carried out using HaploReg v4.2 database (<https://pubs.broadinstitute.org/mammals/haploreg/haploreg.php>) [19] and the Regulome DB v2.2 database (<https://regulomedb.org/regulome-search/>) [20,21].

## 3. Results

### 3.1. Verification of genetic association of polymorphisms in *CCR6* locus with PBC

In previous study, we performed a genome-wide analysis using the Human OmniZhongHua-8 Beadchip (v1.1) on 1126 PBC cases and 4036 healthy controls [7]. There were seventeen SNPs in the *CCR6* locus (chr:6q27) with *P* values  $\leq 1 \times 10^{-4}$  (Fig. 1A and Fig. S1). These seventeen SNPs were divided into two groups, which played a “risk” and “protective” roles, respectively (Table S2). There is no linkage association between these two types of SNPs (Fig. 1B, C, 1D). Among the protective SNPs, the most significant SNP is rs6456156, with a *P* value of  $2.04 \times 10^{-7}$ . In the replication study, we genotyped rs6456156 in additional 907 PBC cases and 2127 controls, but the *P* value did not reach statistical significance, *P* = 0.128 [7].

Further linkage disequilibrium (LD) analysis among the “risk” SNPs revealed two sets of LD blocks represented by rs6905911 and rs12529876, respectively (Fig. 1D). We performed replication study for rs12529876 and rs4710181 and rs6905911 in a separated 1885 PBC cases and 3127 normal controls. The observed allelic associations were *P* =  $2.76 \times 10^{-5}$  (OR = 1.18, 95% CI = 1.10–1.27) for rs12529876, *P* =  $6.99 \times 10^{-4}$  (OR = 1.14, 95% CI = 1.06–1.23) for rs4710181, and *P* =  $5.54 \times 10^{-5}$  (OR = 1.17, 95% CI = 1.08–1.26) for rs6905911, respectively (Table S3). Subsequent meta-analysis combining GWAS and validation data for rs12529876, rs4710181, and rs6905911 showed *P* value of  $5.59 \times 10^{-9}$  (OR = 1.22, 95% CI = 1.14–1.30),  $2.02 \times 10^{-7}$  (OR = 1.19, 95% CI = 1.11–1.27), and  $2.68 \times 10^{-9}$  (OR = 1.22, 95% CI = 1.14–1.30), respectively, reaching GWAS significant levels (Table 1). These results confirmed that the *CCR6* locus is indeed associated with PBC susceptibility in Chinese Han cohorts.



**Fig. 1.** Locus specific plots and LD structure for SNPs in *CCR6* locus based on the GWAS data. (A) The LocusZoom plots of GWAS  $P$  value and LD ( $r^2$ ) of 17 significant SNPs with the most significant SNP are shown by the color codes (see legend) depending on their expected degree of correlation ( $r^2$ ) with the top SNP. (B) The LD plot of 17 significant SNPs in *CCR6* locus which  $P$  value less than  $1 \times 10^{-4}$  in Han Chinese. (C) The LD plot of 5 protective SNPs in *CCR6* locus. (D) The LD plot of 6 risk SNPs in *CCR6* locus. Risk SNPs were grouped into two blocks based on linkage disequilibrium. All SNPs were analyzed by Haploview v4.2. Darker color denotes a higher correlation between markers ( $r^2$ ). The plot of pair-wise LD analysis and haplotype block of SNPs of *CCR6* was drawn by using 1126 Han Chinese PBC samples from GWAS data. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

**Table 1**  
Meta-analysis of SNPs in the *CCR6* locus associated with susceptibility to PBC.

SNP	Minor Allele	GWAS cohort				Replication cohort				Meta-analysis	
		Case (n = 1126)	Control (n = 4036)	P-value	OR	Case (n = 1960)	Control (n = 3316)	P-value	OR	P-value	OR
		MAF	MAF		(95% CI)	MAF	MAF		(95% CI)		(95% CI)
rs12529876	A	0.429	0.382	$7.51 \times 10^{-5}$	1.23 (1.12–1.35)	0.416	0.377	$2.76 \times 10^{-5}$	1.18 (1.10–1.27)	$5.59 \times 10^{-9}$	1.22 (1.14–1.30)
rs4710181	C	0.478	0.424	$5.62 \times 10^{-6}$	1.24 (1.13–1.37)	0.452	0.419	$6.99 \times 10^{-4}$	1.14 (1.06–1.23)	$2.02 \times 10^{-7}$	1.19 (1.11–1.27)
rs6905911	T	0.542	0.485	$1.72 \times 10^{-6}$	1.26 (1.15–1.38)	0.518	0.481	$5.54 \times 10^{-5}$	1.17 (1.08–1.26)	$2.68 \times 10^{-9}$	1.22 (1.14–1.30)

MAF, minor allele frequency; GWAS, genome-wide association study; OR, Odds ratio; 95% CI, 95% Confidence interval.

**3.2. The functional *CCR6*DNP variant is associated with PBC susceptibility**

To further investigate potential causal variation, we sought to determine whether the identified significant SNPs were in linkage disequilibrium with the recently discovered functional dinucleotide polymorphism *CCR6*DNP variant [22–24]. The *CCR6*DNP variant was genotyped successfully in 918 PBC cases and 1297 healthy controls using Sanger sequencing method. With the allele CG as the reference genotype, allele TG ( $P = 3.72 \times 10^{-4}$ , OR = 1.26, 95% CI = 1.11–1.42) was significantly associated with the occurrence of PBC, and allele CA ( $P = 0.607$ , OR = 1.06, 95% CI = 0.84–1.35) was not significantly different between the two groups (Table 2). The *CCR6*DNP loci included rs968334 and rs117912866. To identify specific PBC susceptibility loci, PBC association analysis was performed for these two SNPs separately. Rs968334 with allele C as the reference genotype and allele T ( $P = 4.04 \times 10^{-4}$ , OR = 1.25, 95% CI = 1.10–1.41) was significantly associated with the occurrence of PBC. Rs117912866 with allele G as the reference genotype and allele A ( $P = 0.753$ , OR = 0.96, 95% CI = 0.77–1.21) was not significantly different between the two groups. It is apparent that the association of *CCR6*DNP with PBC was mainly due to the significant difference of rs968334 between PBC patients and controls (Table 2). This conclusion was further confirmed by conditional logistical regression analysis among four significant SNPs (Table 3).

**3.3. Functional annotations of susceptible variants in *CCR6* locus**

Based on Chinese Han South (CHS) and Chinese Han Beijing (CHB) 1000 genomes HapMap data, we summarized SNPs which exhibited strong LD ( $r^2 > 0.8$ ) with rs12529876, rs4710181, rs6905911 and rs968334, respectively. RegulomeDB v2.2 and HaploReg v4.2 databases were used for functional annotation of these SNPs (Table S4, Table S5,

**Table 2**  
Genotypic and allelic association analysis of *CCR6*DNP, rs968334 and rs117912866 in the PBC and control cohort.

SNP	Alleles	PBC (MAF)	Controls (MAF)	OR (95% CI)	P-value
<i>CCR6</i> DNP	CG	914 (0.498)	1422 (0.548)	1.0 (ref.)	
	TG	790 (0.430)	979 (0.377)	1.26 (1.11–1.42)	$3.72 \times 10^{-4}$
	CA	132 (0.072)	193 (0.074)	1.06 (0.84–1.35)	0.607
rs968334	C	1046 (0.570)	1615 (0.623)	1.0 (ref.)	
	T	132 (0.430)	979 (0.377)	1.25 (1.10–1.41)	$4.04 \times 10^{-4}$
rs117912866	G	1704 (0.928)	2401 (0.926)	1.0 (ref.)	
	A	132 (0.072)	193 (0.074)	0.96 (0.77–1.21)	0.753

MAF, minor allele frequency; OR, Odds ratio; 95% CI, 95% Confidence interval.

Table S6 and Table S7). According to the functional annotations, selected SNPs in *CCR6* locus might affect the regulation of transcription. All four SNPs show high scores, with rs12529876, rs4710181 and rs6905911 at “1f”, and rs968334 at “1b”, indicating a higher likelihood of functionality (Table 4).

**3.4. Elevated expression of *CCR6* in PBC liver lesions**

To further investigate the potential contribution of deregulated *CCR6* to PBC pathogenesis, we conducted immunohistochemical analysis on liver biopsy samples. Compared with AIH patients, NAFLD patients, CHB patients or HC, the expression intensity of *CCR6* was significantly elevated in the livers of PBC patients. In particular, *CCR6*<sup>+</sup> cells were found to be clustered in inflamed portal tracts and distributed near injured interlobular bile ducts (Fig. 2A and B). Notably, the number of *CCR6*<sup>+</sup> cells correlated positively with both inflammation severity ( $r = 0.682$ ,  $P < 0.001$ ) and hepatic fibrosis stage ( $r = 0.515$ ,  $P = 0.005$ ) in PBC (Fig. 2C and D). These findings suggest that upregulated expression of *CCR6* is closely associated with the pathogenesis of PBC.

**3.5. Association of SNPs in *CCL20* locus with PBC in Han Chinese cohort**

Previous GWA studies identified a SNP (rs4973341) in the *CCL20* locus was significantly associated with PBC ( $P = 2.34 \times 10^{-10}$ ) in a meta-analysis using European and North American PBC cohorts [11], but none of rs4973341 and SNPs in the shared haplotype with rs4973341 were included in the Human OmniZhongHua-8 Beadchip used in Han Chinese GWAS study. We also noticed that, in the CHB and CHS populations, rs4973341 is in complete linkage disequilibrium with rs10933215, a predicted transcription factor-binding site polymorphism. To further analyze the potential interaction of SNPs in the *CCR6*-*CCL20* axis, we performed association analysis by genotyping rs10933215 in order to replicate the observed association in European cohorts.

In 1832 PBC cases and 3158 normal controls genotyped for rs10933215, the calculated allelic association is weak, with the  $P$  value of 0.001 (OR = 0.84, 95% CI = 0.76–0.93), with the C allele as the protective allele (Table 5). This result indicated that the *CCL20* locus has a weak association with the PBC susceptibility in Han Chinese cohorts. The multiplicative interaction effect of 2-, 3-, 4- and 5-SNPs combinations were tested by binary logistic regression. For 6 interaction combination models, all combinations showed a significant difference between PBC patients and controls ( $P < 0.05$ , Table S8).

**4. Discussion**

In our previous Han Chinese GWAS analysis of 1126 PBC cases and 4036 healthy controls, seventeen SNPs in *CCR6* locus showed variable significance with the calculated  $P$  value between  $1 \times 10^{-4}$  and  $2.04 \times 10^{-7}$ . These SNPs can be divided as “risk” SNPs and protective SNPs. The most significant SNPs were observed among the “protective” SNPs,



**Table 3**  
The conditional logistic analysis of SNPs associated with PBC.

SNP	Chr	Position	Allele <sup>a</sup>	<i>P</i> <sup>b</sup> -value	OR (95% CI)	Cond. <i>P</i> -value	Cond. <i>P</i> -value	Cond. <i>P</i> -value	Cond. <i>P</i> -value
						rs12529876	rs4710181	rs6905911	rs968334
rs12529876	6	167048013	A/G	$2.76 \times 10^{-5}$	1.18 (1.10–1.27)	NA	0.004	0.035	0.415
rs4710181	6	167100510	C/T	$6.99 \times 10^{-4}$	1.14 (1.06–1.23)	0.817	NA	0.234	0.774
rs6905911	6	167108136	T/C	$5.54 \times 10^{-5}$	1.17 (1.08–1.26)	0.364	0.013	NA	0.8
rs968334	6	167112608	T/C	$4.04 \times 10^{-4}$	1.25 (1.10–1.41)	0.13	0.001	0.021	NA

<sup>a</sup> Minor/major allele.  
<sup>b</sup> Association *P*-value.

**Table 4**  
Summary of functional annotation of *CCR6* SNPs from RegulomeDB and HaploReg.

chr	pos (hg38)	LD ( <i>r</i> <sup>2</sup> )	variant	Promoter <sup>a</sup> histone marks	Enhancer <sup>b</sup> histone marks	DNase <sup>c</sup>	Proteins bound <sup>d</sup>	Motifs changed <sup>e</sup>	Selected eQTL hits	GENCODE genes	dbSNP func annot	RegulomeDB rank <sup>f</sup>
6	167048013	1	rs12529876	BLD, GI	BLD, THYM	4 tissues		Sox	11 hits	5.6 kb 3' of FGFR1OP		1f
6	167100510	1	rs4710181		BLD	BLD	5 bound proteins		5 hits	11 kb 5' of CCR6		1f
6	167108136	1	rs6905911		BLD			Cdx2, Hsf	3 hits	3.7 kb 5' of CCR6		1f
6	167112608	1	rs968334	BLD, GI		BLD, BLD		NRSF, Zfp740	7 hits	CCR6	intronic	1b

<sup>a</sup> Histone modifications of H3K4me1 and H3K27ac.  
<sup>b</sup> Histone modification of H3K4me3.  
<sup>c</sup> The levels of DNase I hypersensitivity.  
<sup>d</sup> The binding of transcription factor.  
<sup>e</sup> The alteration in regulatory motif.  
<sup>f</sup> Functional prediction scores of each SNP by the RegulomeDB database.

which were in close linkage. Subsequent replication of the most significant SNP (rs6456156) in 907 PBC and 2127 controls was not able to replicate the calculated significance. We took a close look at the haplotype structure of those “risk” SNPs and noticed that they can be separated by two LD blocks represented by rs6905911 and rs12529876, respectively. In this report, we were able to replicate their significant association with PBC susceptibility.

In order to further identify the functional variant associated with PBC susceptibility, we noticed a recently identified functional variant (CCR6DNP) in the *CCR6* locus. CCR6DNP is a dinucleotide polymorphism (rs968334 and rs117912866) and associated with genetic susceptibility with rheumatoid arthritis in Japanese cohorts and systemic sclerosis in European cohorts [22–24]. Our results indicated that the TG allele in CCR6DNP is associated with PBC, which correlated with increased *CCR6* expression [22]. The TG allele preferentially binds to poly ADP ribose polymerase 1 (PARP-1) to increase *CCR6* expression, compared to other two alleles.

*CCR6* is mainly expressed in Th17, Treg, and dendritic cells and plays a role in cell recruitment during inflammation. In the inflamed liver of PBC patients, there is increased levels of functional *CCR6* expression on liver infiltrating Th17 cells. *CCL20* is the only ligand for *CCR6* and expressed in inflamed choanocytes of bile ducts [25]. Immunohistochemical examinations found that increased number of *CCR6* expressing intrahepatic CD4<sup>+</sup> lymphocytes resided in the portal tracts of the inflamed bile ducts in PBC patients, compared to normal livers.

Recently Hitomi and coworkers reported two functional SNPs (rs9459874 and rs1012656) in the *CCR6* locus associated with PBC, based on international *meta*-GWAS results, which included our Han Chinese GWA results [26]. For these two SNPs, rs1012656 is in complete linkage with the “protective” SNPs in our GWA results, which were not replicated in our subsequent validation study. The reported rs9459874 is also in complete LD with our significant “risk” SNP rs77890798, therefore its association with PBC can be represented by functional CCR6DNP.

5. Conclusions

In conclusion, our study confirmed the *CCR6* locus is associated with genetic risk with PBC in Han Chinese cohorts. The associated CCR6DNP variant correlated with increased *CCR6* expression, which may lead to increased inflammation in intra-hepatic biliary ducts.

Funding

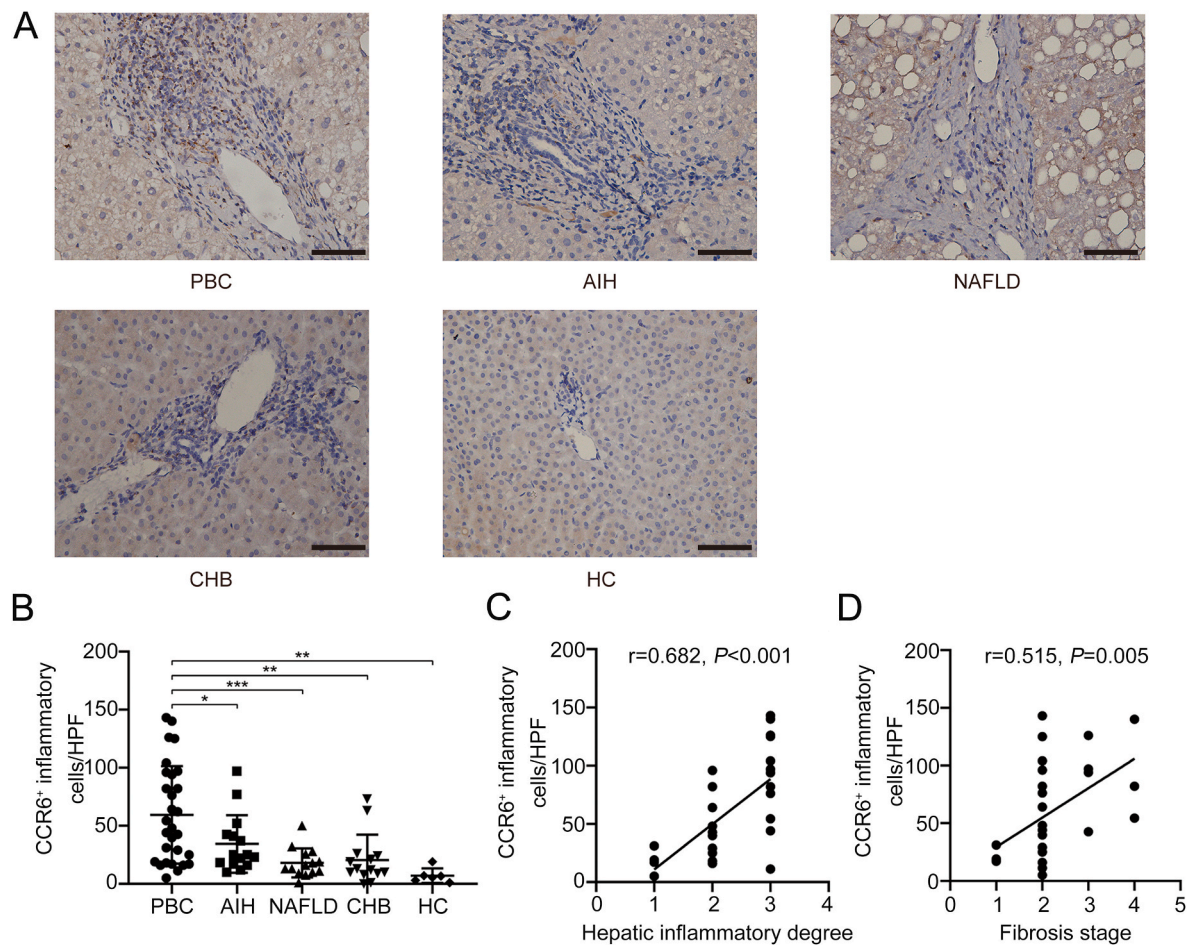
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CRedit authorship contribution statement

**Mingming Zhang:** Writing – original draft, Investigation, Formal analysis. **Zhuye Qin:** Resources. **Yexi Huang:** Investigation. **Wenyan Tian:** Resources. **You Li:** Investigation. **Chan Wang:** Formal analysis. **Weifeng Zhao:** Resources. **Yaping Dai:** Resources. **Xingjuan Shi:** Supervision. **M. Eric Gershwin:** Supervision. **Xiong Ma:** Resources. **Meilin Wang:** Formal analysis. **Xiangdong Liu:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Weichang Chen:** Funding acquisition. **Fang Qiu:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.



**Fig. 2.** Liver immunohistochemical staining of CCR6. (A) Representative staining images from patients with PBC, AIH, NAFLD, CHB and HC. (B) Quantification of hepatic CCR6<sup>+</sup> cells in PBC, AIH, NAFLD, CHB and HC, mean ± s. e.m. The frequency of hepatic CCR6<sup>+</sup> cells is positively correlated with hepatic inflammation degrees (C) and fibrosis stages (D) in PBC (\**P* < 0.05, \*\**P* < 0.01). Scale bars, 20 μm.

**Table 5**  
The genotype and allele frequencies of *CCL20* polymorphisms rs10933215 in PBC patients and controls.

	PBC (MAF)	Control (MAF)	<i>P</i> -value	OR (95%CI)
Genotypes				
TT	1244 (0.679)	2023 (0.641)	0.035	1.0 (ref.)
TC	534 (0.292)	995 (0.315)		0.87 (0.77–0.99)
CC	54 (0.029)	140 (0.044)	0.005	0.63 (0.46–0.87)
Alleles				
T	3022 (0.825)	5041 (0.798)	0.001	1.0 (ref.)
C	642 (0.175)	1275 (0.202)		0.84 (0.76–0.93)

MAF, minor allele frequency; OR, Odds ratio; 95% CI, 95% Confidence interval.

MAF, minor allele frequency; OR, Odds ratio; 95% CI, 95% Confidence interval.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jtauto.2024.100234>.

Abbreviations

AMA	anti-mitochondrial antibody
CCR6	CC chemokine receptor 6
CCL20	CC chemokine ligand 20
CHB	Chinese Han Beijing
CHS	Chinese Han South
CI	confidence interval
DC	dendritic cells
GWA	genome-wide association
GWAS	genome-wide association study
HLA	human leukocyte antigen
LD	linkage disequilibrium
MAF	minor allele frequency
OR	odds ratio
PBC	primary biliary cholangitis
SNP	single-nucleotide polymorphism
Th17	T helper 17

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