Original Article

Association of Estrogen Receptor Gene Polymorphisms and Primary Biliary Cirrhosis in a Chinese Population: A Case–Control Study

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Abstract

Background: Primary biliary cirrhosis (PBC) is a chronic and slowly progressive cholestatic liver disease characterized by destruction of the interlobular bile ducts and a striking female predominance. The aim of this study was to identify associations between estrogen receptor (ESR) gene polymorphisms with the risk of developing PBC and abnormal serum liver tests in a Chinese population.
Methods: Thirty-six patients with PBC (case group) and 35 healthy individuals (control group) from the First Hospital of Jilin University were studied. Whole genomic DNA was extracted from all the participants. Three single-nucleotide polymorphisms (rs2234693, rs2228480, and rs3798577) from ESR1 and two (rs1256030 and rs1048315) from ESR2 were analyzed by a pyrosequencing method. Demographic data and liver biochemical data were collected.
Results: Subjects with the T allele at ESR2 rs1256030 had 1.5 times higher risk of developing PBC than those with the C allele (odds ratio [*OR*] = 2.1277, 95% confidence interval [*CI*] = 1.1872–4.5517). Haplotypes TGC of ESR1 rs2234693, rs2228480, and rs3798577 were risk factors for having PBC. The C allele at ESR1 rs2234693 was associated with abnormal alkaline phosphatase (*OR* = 5.2469, 95% *CI* = 1.3704–20.0895) and gamma-glutamyl transferase (*OR* = 3.4286, 95% *CI* = 1.0083–13.6578) levels in PBC patients.
Conclusions: ESR2 rs1256030 T allele may be a significant risk factor for the development of PBC. Screening for patients with gene polymorphisms may help to make early diagnoses in patients with PBC.

Key words: Estrogen Receptor; Gene Polymorphism; Primary Biliary Cirrhosis

Introduction

Primary biliary cirrhosis (PBC) is a chronic and slowly progressive cholestatic liver disease characterized by destruction of the interlobular bile ducts and a striking female predominance. If untreated, it frequently leads to fibrosis, cirrhosis, and liver failure. Because liver transplantation remains the only curative option for PBC, the goals of treatment are to slow the rate of progression, to alleviate related symptoms, and to prevent complications. Ursodeoxycholic acid is the only US Food and Drug Administration-approved medical treatment of PBC. The presence of positive anti-mitochondrial antibody (AMA) is the main serological criterion for making the diagnosis.[1,2]

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Although details of the pathogenesis are unknown, genetic factors have been implicated to be involved in the development of PBC.[3] In previous studies, the genetic typing of HLA class II and III alleles revealed a highly significant increase in HLA DR8, DPB1 * 0501, and 0301, C4A*B2 and C4A*Q0 complement alleles in patients with PBC compared with controls, and the HLA

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DRB1 * 0801-DQA1 * 0401-DQB1 * 0402 haplotype was considered to represent a marker of disease progression.[3-5] A number of genes outside the MHC locus may play a role in susceptibility to autoimmune liver diseases.[6,7] Recently, polymorphisms of cytotoxic T lymphocyte-associated antigen-4 and vitamin D receptor genes have been associated with autoimmune hepatitis (AIH) and PBC in Chinese patients. However, there was no association between polymorphisms of the tumor necrosis factor-alpha promoter and the same group patients with AIH and PBC.[8] The polymorphisms of interleukin (IL)-1RN and IL-6-174G/C appear to be associated with PBC in Chinese patients.[9] No statistically significant difference was found in the distribution of the IL-10 promoter genotype in AIH and PBC patients compared with controls.[8]

Estrogens are not only essential for the female reproductive system, but they also control fundamental functions in other tissues including the cardiovascular system, bone, brain, and liver. Recently, estrogens have been shown to target the biliary tree, where they modulate the proliferative and secretory activities of cholangiocytes, the epithelial cells lining bile ducts. By acting on both estrogen receptor (ESR) 1 and ESR2 subtypes and by activating either genomic or nongenomic pathways, estrogens play a key role in the complex loop of growth factors and cytokines, which modulates the proliferative response of cholangiocytes to damage.[10] PBC, in fact, predominantly affects females (10:1 female/male ratio) with a typical clinical presentation occurring during the peri- and post-menopausal period. It is thought that estrogens can influence the clinical course of PBC, as for most autoimmune disorders.[10-13]

The aim of this study was to identify associations of ESR gene polymorphisms with the risk of having PBC and abnormal serum liver tests in a Chinese population.

Methods

Study population

Between January 2000 and March 2010, 36 patients with PBC were selected at the First Hospital of Jilin University. This study included 5 males and 31 females aged from 37 to 75 years with an average age of 57.2 years. A healthy control group was selected from an epidemiological survey by the First Hospital of Jilin University. Data on routine blood, urine, liver, kidney tests, abdominal ultrasound, anti-nuclear antibody, and AMA antibody were obtained and all found to be normal. After written informed consent was provided, 5 ml of fasting venous blood samples were collected in EDTA tubes and stored at −80°C until analyzed. Liver biochemical data were obtained at the clinical laboratory of the First Hospital of Jilin University. The study protocol was approved by the Ethical Committee of the First Hospital, Jilin University.

The diagnosis of PBC was made according to the diagnostic criteria for PBC in the American Association for the Study of Liver Diseases Practice Guidelines:[10,13] (1) Presence of cholestatic liver disease including jaundice, fatigue, and pruritus with abnormally high levels of cholestatic parameters such as serum alkaline phosphatase (ALP), serum bilirubin, and gamma-glutamyl transpeptidase (GGT); (2) absence of biliary obstruction as assessed by ultrasound or endoscopic retrograde cholangiopancreatography; (3) serum positive AMA titer 1:1000 and type M2 AMA positive; (4) absence of serological markers for hepatitis B and C virus and HIV infection by immunoassays.

AMA was positive in all the 36 PBC subjects. Five subjects underwent liver biopsy and were proved to have PBC. Serum bilirubin, ALP, and GGT levels are shown in Table 1. ALP and GGT levels of most PBC subjects were abnormal, but the total bilirubin (TBIL) levels of most PBC subjects were normal.

Single-nucleotide polymorphism genotyping

Single-nucleotide polymorphism (SNP) loci of the human ESR1 gene were found in PubMed. The features of all studies focusing on SNP loci according to the published literature associated with SNP and disease are summarized. We selected SNP sites in which the minor allele frequency (MAF) threshold was more than 5%, and pairwise squared threshold was more than 80% using SNPbrowser application software (Thermo Fisher Scientific, USA). We identified three sites: rs223469 (3 t > C, MAF = 0.4, CRH *P* = 152163335, Intron1), rs2228480 (CRH A > G, MAF = 0.144, *P* = 152420095, CDS - synon), and rs3798577 (T > C, MAF = 0.40, CRH *P* = 152421130, UTR - 3) for the study. We determined that two study sites were located in the human ESR2 gene: rs1256030 (CRH C > T, MAF = 0.318, *P* = 64747170, exon) and rs1048315 (T > C, MAF = 0.375, CRH *P* = 64692465, UTR - 3).

Pyrosequencing

Human genomic DNA was extracted from the blood samples and isolated using a genomic DNA purification kit (Promega, San Luis Obispo, CA, USA). ESR1 (rs2234693, rs2228480, and rs3798577) and ESR2 (rs1256030 and rs1048315) SNPs were typed using pyrosequencing technology, according to the protocol of the manufacturer (PyroMark ID Pyrosequencing Machine, Qiagen, Valencia, CA, USA). Primers for PCR amplification are shown in Table 2.

Statistical analysis

The Hardy–Weinberg equilibrium test was used for each

Table 1: Serum bilirubin, ALP, and GGT level of the 36 PBC subjects

Parameter	ALP (U/L)	GGT (U/L)	TBIL (μmol/L)
Mean	210.38	191.98	47.29
SD	166.99	230.32	94.25
Median	164.00	97.50	16.05
Minimum	41.00	13.00	5.40
Maximum	816.00	970.00	401.70

SD: Standard deviation; ALP: Alkaline phosphatase; GGT: Gamma-glutamyl transpeptidase; TBIL: Total bilirubin; PBC: Primary biliary cirrhosis.

SNP from the patient and control groups. The differences of genotype and allele distributions between PBC patients and healthy controls were assessed using the Chi-square test or Fisher's exact probability test. Odds ratio (*OR*) and 95% confidence intervals (95% *CI*s) were used to analyze the relationship between alleles and serum liver tests by using logistic regression. $P < 0.05$ was considered statistically significant. The SAS (SAS Institute Inc., Cary, NC, USA) statistical package 9.1 was used.

Results

There were no significant differences between the mean ages of PBC and the control groups, 56.17 ± 11.53 and 55.49 ± 12.35 years, respectively, $P > 0.05$.

Association of estrogen receptor single-nucleotide polymorphisms with primary biliary cirrhosis susceptibility

All the five SNPs for 36 PBC cases and 35 controls were successfully genotyped. No significant differences of genotype frequencies from the Hardy–Weinberg equilibrium test were noted in the target SNPs of the PBC patients or of the normal controls. As shown in Table 3, the distribution of genotypes and alleles of rs1256030 was significantly different between the PBC patients and the normal controls. The frequencies of T allele at rs1256030 were significantly increased in PBC patients when compared with normal controls ($P = 0.0495$, $OR = 2.1277$, 95% $CI = 1.1872–4.5517$). As for rs2234693, rs2228480, rs3798577, and rs1048315, we found no significant differences between the two groups in terms of the distribution of either alleles or genotypes [Table 3].

The linkage disequilibrium coefficient D' between ESR rs2228480 and rs3798577 was 0.855, $P < 0.05$. The linkage disequilibrium coefficient D' between ESR rs1048315 and rs1256030 was 1, $P < 0.05$. The coefficient D' was not significant between the other SNP sites.

The TGC genotype frequency in the PBC group was significantly higher than the control group ($P = 0.0103$). The TAC haplotype frequency in the PBC group was lower than that in the control group ($P = 0.0032$) [Table 4].

We analyzed a sub-group analysis between males and females focusing on genetic association of ESR gene polymorphism and gender in PBC patients. There was no difference in polymorphism distribution between males and females, indicating that gender was not associated with differences in the distribution of ESR polymorphism in PBC (data not shown). The incidence of PBC in males was lower than that in females. This small sample size limited the power of analysis for gender difference. We performed a sub-group analysis among Child–Pugh A, B, and C patients focusing on possible genetic associations of ESR gene polymorphism in PBC patients. There was no difference in the polymorphism distribution among Child–Pugh scores indicating that the stage of cirrhosis was not associated with the distribution of ESR gene polymorphisms in PBC (data not shown).

Association of estrogen receptor single-nucleotide polymorphisms with abnormal alkaline phosphatase, gamma-glutamyl transpeptidase, and TBIL in primary biliary cirrhosis patients

PBC patients were divided into two groups according to the levels of liver tests, normal and abnormal. The value for normal ALP was <112 U/L and abnormal was ALP ≥112 U/L. For ESR1 rs2234693 genotypes, CC, CT, and TT frequencies were significantly different. Frequency of alleles C and T was also significantly different ($P = 0.0025$ and $P = 0.0099$, respectively) between the two groups.

The genotype and allele distribution between the two groups were not obviously different at other sites. The TGT haplotype frequency in the normal ALP group was significantly higher than that in the abnormal ALP group ($P = 0.0107$) [Tables 5 and 6].

Table 2: PCR primers

Site	Primer	Sequencing section
rs2234693	AGGGTTATGTGGCAATGACGTA	CYGTTTTATGC
	Bio-GGGAAACAGAGACAAAGCATAAA	
	TTCCAAATGTCCCAG	
rs2228480	AAAGTATTACATCACGGGGGAGG	CRGTCTG
	Bio-AGGGATTATCTGAACCGTGTGG	
	GAGGGTTTCCCTGCCA	
rs3798577	TGGTGATGCATGATGAGGGTAAAT	ACYTGTGCAGGA
	Bio-CTGCCCTACTTTCCCTCTTGTTCT	
	GCATGGAGCTGAACAGT	
rs1256030	CTGGCCACTCCTTTCATTACA	YCCCACCCCATGGC
	Bio-TGTTTGAAAGTGGGTAGGTGAGTT	
	ATTACACTTAGAGATGTAGC	
rs1048315	AGCAGGGAACCTGTGTGG	AYTTTGGCAG
	Bio-TGGCTCTTCAGGAAACTGACAT	
	GCAGGTGCCCCGGGT	

PCR: Polymerase chain reaction.

Table 3: ESR gene polymorphism distributions and their associations with PBC

SNP rs ID	Genotype/allelotype	PBC group (n = 36), n (%)	Control group (n = 35), n (%)	χ^2	P	OR (95% CI)
rs2234693	CC	5 (13.9)	6 (17.1)			
	CT	18 (50.0)	13 (37.2)			
	TT	13 (36.1)	16 (45.7)	1.1938	0.5505	
	C	28 (38.9)	25 (35.7)			
	T	44 (61.1)	45 (64.3)	0.1529	0.6958	1.1455 (0.5798–2.2628)
rs2228480	AA	1 (2.8)	2 (5.7)			
	AG	15 (41.7)	16 (45.7)			
	GG	20 (55.6)	17 (48.6)	0.5949	0.7427	
	A	17 (23.6)	20 (28.6)			
	G	55 (76.4)	50 (71.4)	0.4533	0.5008	0.7727 (0.3645–1.6383)
rs3798577	CC	9 (25.0)	9 (25.7)			
	CT	18 (50.0)	17 (48.6)			
	TT	9 (25.0)	8 (25.7)	0.0047	0.9976	
	C	36 (50.0)	35 (50.0)			
	T	36 (50.0)	35 (50.0)	0.0000	1.0000	1.0000 (0.5179–1.9309)
rs1256030	CC	16 (44.4)	23 (65.7)			
	CT	15 (41.7)	10 (28.6)			
	TT	5 (13.9)	2 (5.7)	3.5287	0.1714	
	C	47 (65.3)	56 (80.0)			
	T	25 (34.7)	14 (20.0)	3.8616	0.0495	2.1277 (1.1872–4.5517)
rs1048315	CC	5 (13.9)	8 (22.9)			
	CT	13 (36.1)	17 (48.6)			
	TT	18 (50.0)	10 (28.6)	3.4980	0.1741	
	C	23 (31.9)	33 (47.1)			
	T	49 (68.1)	37 (52.9)	3.4326	0.0640	0.5263 (0.2660–1.0413)

SNP: Single-nucleotide polymorphisms; PBC: Primary biliary cirrhosis; *OR*: Odds ratio; *CI*: Confidence interval; ESR: Estrogen receptor.

Table 4: Haplotype analysis of ESR1 SNPs

Items	PBC group, n (%)	Control group, n (%)	χ^2	P	OR	95% CI
CAC	11.29 (15.7)	6.68 (9.5)	1.210	0.2714	1.763	0.636–4.887
CGT	12.91 (17.9)	9.78 (14.0)	0.414	0.5199	1.345	0.544–3.326
TAC	2.48 (3.4)	13.32 (19.0)	8.712	0.0032	0.152	0.038–0.616
TGC	18.43 (25.6)	6.46 (9.2)	6.582	0.0103	3.386	1.287–8.907
TGT	19.86 (27.6)	25.22 (36.0)	1.167	0.2800	0.676	0.332–1.377

OR: Odds ratio; *CI*: Confidence interval; SNP: Single-nucleotide polymorphisms; ESR1: Estrogen receptor 1; PBC: Primary biliary cirrhosis.

Table 5: rs2234693 gene polymorphism distributions and their associations with ALP in PBC patients

Groups	Genotype frequency, n (%)				Allele frequency, n (%)	
	n	CC	CT	TT	C	T
Abnormal ALP group	26	4 (15.4)	17 (65.4)	5 (19.2)	25 (48.1)	27 (51.9)
Normal ALP group	10	1 (10.0)	1 (10.0)	8 (80.0)	3 (15.0)	17 (85.0)
χ^2		11.9673			6.6498	
P		0.0025			0.0099	
OR (95% CI)					5.2469 (1.3704–20.0895)	

OR: Odds ratio; *CI*: Confidence interval; ALP: Alkaline phosphatase; PBC: Primary biliary cirrhosis.

Table 6: Haplotype analysis of ESR1 SNPs with ALP in PBC patients

Items	Abnormal ALP group, n (%)	Normal ALP group, n (%)	χ^2	P	OR	95% CI
TAC	1.46 (0.028)	1.50 (0.075)	0.807	0.3690	0.356	0.034–3.702
TGC	14.28 (0.275)	3.50 (0.175)	0.770	0.3802	1.784	0.484–6.574
TGT	11.26 (0.216)	10.50 (0.525)	6.518	0.0107	0.250	0.083–0.750

SNP: Single-nucleotide polymorphisms; ESR1: Estrogen receptor 1; ALP: Alkaline phosphatase; PBC: Primary biliary cirrhosis; *OR*: Odds ratio; *CI*: Confidence interval.

The value for normal GGT was <54 U/L and for abnormal GGT level, it was ≥54 U/L. In ESR1 rs2234693 genotype, CC, CT, and TT frequencies were significantly different.

The frequencies of alleles C and T were also significantly different ($P = 0.0296$ and $P = 0.0415$, respectively) between the two groups. There was no obvious difference between the two groups in genotype and allele distribution at other sites. The TAT haplotype frequency in the normal ALP group was significantly higher than that in the abnormal ALP group ($P = 0.0415$) [Tables 7 and 8].

The value for normal TBIL levels was <60 μmol/L and for abnormal, it was TBIL ≥60 μmol/L. There were no obvious differences between the two groups in genotype and allele distribution at these ESR SNP sites (data not shown). The TAC haplotype frequency in the abnormal TBIL group was significantly higher than that in the normal TBIL group ($P = 0.0268$) [Table 9].

Discussion

The intrahepatic biliary tree is a complex three-dimensional network of interconnected ducts, which starts at the level of the Canals of Hering, continues to the intrahepatic ducts of increasing diameter, and ends at the level of main extrahepatic bile ducts.[14-17] Recent studies have demonstrated that the intrahepatic biliary tree plays a critical role in many liver functions including bile formation, regeneration, injury repair, fibrosis, angiogenesis, and regulation of blood flow.[17] We have learned that estrogens and their receptors influence the pathophysiology of cholangiocytes and that this mainly occurs during experimental and human conditions characterized by cholangiocyte injury and proliferation.[18] Estrogens have been considered for many years to play a role in the development and progression of pathologies involving the biliary tree.[11,19]

PBC, the most prevalent acquired cholangiopathy, specifically affects females with a clinical presentation typically occurring during the peri- and post-menopausal period. Endocrine dysfunction is frequent in patients with PBC including an increased incidence of menstrual disturbance and hysterectomy.[18] This has been associated with a high incidence of postmenopausal osteoporosis due to estrogenic deficiency, which can be corrected by estrogen replacement therapy.[20] The progression of polycystic liver disease is significantly influenced by female gender, pregnancies, and estrogen replacement treatment, which are associated with changes in serum estrogen levels.[18] In addition, marked alterations of estrogen hepatic metabolism

Table 7: rs2234693 gene polymorphism distributions and their associations with GGT in PBC patients

Groups	Genotype frequency, *n* (%)				Allele frequency, *n* (%)	
	n	CC	CT	TT	C	T
Abnormal GGT group	26	4 (15.4)	16 (61.5)	6 (23.1)	24 (46.2)	28 (53.8)
Normal GGT group	10	1 (10.0)	2 (20.0)	7 (70.0)	4 (20)	16 (80)
χ^2			7.0466		4.1574	
P			0.0296		0.0415	
OR (95% *CI*)					3.4286 (1.0083–13.6578)	

GGT: Gamma-glutamyl transpeptidase; PBC: Primary biliary cirrhosis; *OR*: Odds ratio; *CI*: Confidence interval.

Table 8: Haplotype analysis of ESR1 SNPs with GGT in PBC patients

Items	Normal GGT group, *n* (%)	Abnormal GGT group, *n* (%)	χ^2	*P*	*OR*	95% *CI*
CGT	8.66 (16.7)	3.00 (15.0)	0.046	0.8300	1.169	0.280–4.880
TAC	2.93 (5.6)	0.00 (0.0)	0.529	0.4669	917.974	45.338–18,586.377
TAT	0.01 (0.0)	2.00 (0.1)	4.476	0.0344	0.002	0.000–0.039
TGC	12.08 (23.2)	6.00 (30.0)	0.284	0.5938	0.731	0.230–2.318
TGT	12.98 (25.0)	8.00 (40.0)	1.422	0.2331	0.517	0.173–1.543

SNP: Single-nucleotide polymorphisms; ESR1: Estrogen receptor 1; GGT: Gamma-glutamyl transpeptidase; PBC: Primary biliary cirrhosis; *OR*: Odds ratio; *CI*: Confidence interval.

Table 9: Haplotype analysis of ESR1 SNPs with TBIL in PBC patients

Items	Significantly increased group, *n* (%)	Control group (TBIL <60 μmol/L), *n* (%)	χ^2	*P*	*OR*	95% *CI*
CGC	2.00 (12.5)	3.45 (6.2)	0.716	0.3976	2.177	0.346–13.697
CGT	3.00 (18.7)	9.91 (17.7)	0.009	0.9234	1.073	0.257–4.486
TAC	3.00 (18.7)	1.76 (3.1)	4.907	0.0268	7.109	1.004–50.308
TGC	2.00 (12.5)	14.16 (25.3)	1.169	0.2795	0.422	0.085–2.089
TGT	3.00 (18.7)	17.48 (31.2)	0.950	0.3296	0.508	0.128–2.015

SNP: Single-nucleotide polymorphisms; ESR1: Estrogen receptor 1; TBIL: Total bilirubin; PBC: Primary biliary cirrhosis; *OR*: Odds ratio; *CI*: Confidence interval.

occur in cholestasis, which is one of the hallmarks of cholangiopathies, leading to enhanced estradiol serum levels, which could influence disease progression.[21] In spite of all these clinical considerations, the role and mechanism by which estrogens modulate PBC have been explored in only a few studies at the clinical level.[20-22]

By switching the immunological response toward the Th2 profile, estrogens stimulate the production of Th2 anti-inflammatory cytokines (such as IL-10, IL-4, and transforming growth factor-beta) thus potentiating the anti-inflammatory response. In addition, estrogens can prevent oxidative stress in hepatocytes, which are injured by cholestasis.[23,24] ESR1 activation, in fact, inhibits inflammatory gene expression by preventing nuclear factor-kappa B nuclear translocation. Interestingly, through the ESR1, estrogens can positively modulate the growth hormone/insulin-like growth factor 1 (GH/IGF-1) axis. This further supports a possible therapeutic role of ESR1 positive modulators in cholangiopathies, since IGF-1 and estrogens play additive effect on cholangiocyte proliferation and that the GH/IGF-1 axis plays a pivotal role in liver injury repair. Consistently, IGF-1 replacement therapy has shown clinical benefit in PBC and alcoholic cirrhotic patients.[25]

ESRs are involved in the first step along the path of signaling cell growth and development upon stimulation with estrogens. Gene polymorphisms may cause gene dysregulation at transcriptional and translation levels. The ESR intron 1 in the AF-1 region contains important transcriptional regulatory sequences. Base substitutions can lead to novel splice sites or disappearance of original shear sites. Both can lead to messenger RNA (mRNA) shear errors, abnormal formation of mRNAs, and proteins.[26]

The ESR1 PvuII and XbaI Pp and Xx genotypes were more frequent in 33 female Hungarian patients with PBC patients compared to healthy controls. This indicated that there is an association between the polymorphisms and PBC.[12] There has been no large-scale research on ESR gene polymorphism and genetic susceptibility to PBC in China. Preliminary exploration of ESR gene polymorphism may be useful for early screening and prevention of PBC.

This study used a case–control method focusing on the PBC patients (except lost to follow-up, seriously ill, and death patients). These SNP loci met Hardy–Weinberg equilibrium, indicating as a representative group.

The ESR2 rs1256030 allele C and T frequencies were different between PBC group and health control group ($P = 0.0495$, $OR = 2.1277$, 95% $CI = 1.1872–4.5517$). According to an analysis of relative risk allele frequency, T allele risk of PBC was 1.5 times than that of the C allele. Alvaro found that 50–65% of liver tissue samples from patient with various stages of PBC expressed ESR2.[18] The current results confirm the relationship between the ESR2 gene mutation and ESR2 expression in hyperplastic bile duct cells. The Rs1048315 SNP of ESR2 is located at the 3'-noncoding region. Its allele C/T ratio was found to be about 0.469 in the PBC group and about 0.892 in the health control group, suggesting that the C allele may be a PBC protection factor, while type T allele may be a risk factor.

A population study on ESR gene polymorphisms, conducted in Shanghai, suggested that the ESR1 rs1801132G allele was associated with bile duct disease ($OR = 1.7$, 95% $CI = 1.1–2.8$) and ESR2 rs4986938 GG genotype was associated with a high risk of cholangiocarcinoma ($OR = 3.3$, 95% $CI = 1.3–8.7$). However, ESR rs2234693, rs2228480, and rs1256049 did not have obvious associations with these diseases.[27]

Yang *et al*.[28] found that ESR2 and anti-mitochondrial markers can coexist. Exogenous estrogen application did not cause the ESR2 to move into the cell nucleus, suggesting that estrogen can affect mitochondria in the absence of nuclear effects. In PBC patients, autoantibodies are mainly aimed at the pyruvate dehydrogenase complex on the mitochondrial membrane.[29] This study showed that the ESR may play a role in the development of PBC in patients in China. There are limitations to the current study. The lack of sample size estimation and small sample size limited the power of analysis for the association of ESR gene polymorphisms with PBC and gender differences. In the future, a larger sample size will be needed to study the relationship between ESR gene polymorphisms with PBC and gender differences.

In conclusion, the SNP of ESR2 was found to be associated with PBC. The ESR2 rs1256030 T allele may be an important risk factor for the development of PBC. ESR1 rs2234693 was associated with abnormal serum liver tests in patients with PBC. The C allele may be a potential risk factor for PBC. Among the haplotypes of ESR1 rs2234693, rs2228480, rs3798577, genotypes TGC conferred risk for PBC. However, genotype TAC may have been protective. The genotype TAC was an important risk factor for cholestasis.

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Conflicts of interest

There are no conflicts of interest.

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