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Genetic Risk Factors for Autoimmune Thyroid Disease might Affect the Susceptibility to and Modulate the Progression of Primary Biliary Cholangitis

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ABSTRACT

Background & Aims: Patients with primary biliary cholangitis (PBC) frequently suffer from extrahepatic autoimmune conditions, of which autoimmune thyroid disease (AITD) is one of the most common. Previous studies identified several genetic variants increasing the odds of developing AITD. Here we investigate whether AITD-associated polymorphisms might also play a role in the development and clinical course of PBC and PBC associated with AITD (PBC-AITD).
Methods: To this end, we prospectively recruited 230 patients with PBC and 421 healthy controls. Among recruited patients, 64 (30.9%) had PBC-AITD as diagnosed by elevated serum TPO-antibodies. In all subjects we genotyped 10 variants previously associated with AITD.
Results: We detected significant associations between the *PTPN22* polymorphism and risk of developing PBC (rs2476601, OR=1.43, P=0.035) as well as PBC-AITD (OR=1.74, P=0.028). The *IL2RA* polymorphism was associated with liver cirrhosis (rs41295061, OR=1.76, P=0.033) whereas the *MMEL1* polymorphism increased the risk of requiring liver transplantation (rs2843403, OR=1.70, P=0.023). Although no significant differences in clinical or biochemical characteristics between patients with PBC and PBC-AITD were seen (all P>0.05), liver function tests and metabolic traits in PBC patients were significantly (all P<0.05) affected by the *CTLA4* (rs3087243), *MMEL1* (rs2843403), *PTPN22* (rs2476601) and *RNASET2* (rs9355610) variants.
Conclusion: Our study demonstrates the existence of a genetic overlap between PBC and AITD. Apparently, genetic variants known to increase the AITD risk might affect the clinical course of PBC. On the other hand, AITD per se does not seem to significantly influence the natural history of PBC.

Key words: autoimmune liver disease – cholestasis – liver cirrhosis – liver transplantation – single nucleotide polymorphism – thyroid peroxidase antibody.

Abbreviations: AITD: autoimmune thyroid disease; ALP: alkaline phosphatase; ALT: alanine transaminase; AMA: anti-mitochondrial autoantibodies; AST: aspartate aminotransferase; DM1: diabetes mellitus type 1; ELISA: enzyme-linked immunosorbent assay method; GGT: gamma-glutamyl transpeptidase; GD: Graves' disease; GWAS: genome-wide association studies; HT: Hashimoto thyroiditis; HWE: Hardy-Weinberg equilibrium; Lyp: lymphoid-specific protein tyrosine phosphatase non-receptor type 22; OR: odds ratio; PBC: primary biliary cholangitis; RA: rheumatoid arthritis; SLE: systemic lupus erythematosus; TPOAb: thyroid peroxidase antibody; UDCA: ursodeoxycholic acid.

INTRODUCTION

Primary biliary cholangitis (PBC) belongs to the liver diseases with an autoimmune background. It is characterized by a chronic cholestatic process which affects small to medium sized bile ducts and is caused by immune-mediated destruction of the epithelium, leading in consequence to cholestasis, and in a proportion of patients to liver impairment and cirrhosis [1]. The presence of the anti-mitochondrial autoantibodies (AMA) in almost 90% of PBC patients as well as identification of antinuclear antibodies, targeting the sp100 and gp210 antigens in as much as 30% patients [2-4], underscore the role of the autoimmune process in the development of PBC. This condition is frequently seen in middle-aged women with other extrahepatic autoimmune disease [5, 6]. Indeed, previously published reports demonstrated that patients with PBC might have a 61% [7] risk of coincidence of more than one disorder connected

with autoimmunity. Autoimmune thyroid disease (AITD) is one of the disorders with the highest evidence for association with PBC. Indeed, a recent study [8] demonstrates that 14% of patients with PBC might suffer from AITD. Prevalence of thyroid disorder is confirmed by the presence of positive thyroid antibodies, and is clinically manifested mainly as Hashimoto thyroiditis (HT) or Graves' disease (GD). An extensive number of genetic studies, including analyzes of twin pairs, family studies and genome-wide association studies (GWAS), revealed the evidence of a significant genetic component of hepatic autoimmunity [9, 10]. Given the fact that patients with PBC often develop extrahepatic autoimmune conditions one could hypothesize that genetic background might be responsible for this phenomenon, and that it also might have an impact on the natural history of PBC. Of note, the majority of genetic studies have focused on triggers of PBC, whereas the analysis of genetic variants that might modulate the clinical course of PBC has gained less attention.

In the present study we analyzed 10 variants of genes previously associated with AITD in a large group of patients with PBC. Our aim was to find out whether AITD-triggering polymorphisms might play a role in the development and in the course of PBC and PBC associated with AITD (PBC-AITD).

MATERIAL AND METHODS

Subjects

In this cross-sectional study we prospectively recruited from 2007 to 2016 a total of 230 patients with PBC. All patients were recruited in two Polish university Centres (Pomeranian Medical University, Szczecin and Medical University of Warsaw, Warsaw). The diagnosis of PBC was established according to the European Association for the Study of Liver (EASL) criteria [11]. The presence of acute and chronic liver diseases other than PBC, as well as liver tumors, was excluded in all patients. Fasting venous blood samples were drawn for routine biochemical analyses, including liver function tests, and DNA genotyping. The presence of liver cirrhosis was established either by liver biopsy or imaging screening (e.g. transient elastography [12]). Serum levels of thyroid peroxidase antibody (TPOAb) in PBC patients were quantified using QUANTA Lite® TPO assay (Inova Diagnostics, San Diego, CA, USA) based on the enzyme-linked immunosorbent assay method (ELISA) [13]. The control group consisted of 421 healthy Polish adults (330 females, 91 males) recruited from subjects requesting paternity testing who gave their consent for anonymous use of their genetic material for scientific research. The protocol of this study conforms to the ethical guidelines of the Declaration of Helsinki as reflected in a priori approval by the institution's Human Research Ethics Committee. All patients and controls gave written informed consent to participate in the study.

SNPs selection and genotyping

Genomic DNA was isolated from peripheral blood mononuclear cells using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) or salting-out method [14]. In all subjects, we analyzed 10 SNPs previously associated with AITD (Table I). All selected SNPs were genotyped using the predesigned TaqMan SNP genotyping assays provided by Life Technologies, Carlsbad, CA, USA. The genotyping procedure was performed according to the manufacturer's protocol on the QuantStudioTM 12K Flex instrument (Life Technologies).

Statistical analysis

P-value less than 0.05 was considered statistically significant. The genotype distributions of the analyzed SNPs in both patients and controls were tested for Hardy-Weinberg Equilibrium (HWE) using the chi-square test. Allele frequencies in patients and in controls as well as in subgroups of patients were compared using the chi-square test or Fisher's exact test, when appropriate and Armitage's trend test was used to confirm association in positive findings. The Shapiro-Wilk test for normality was used to examine the distribution of quantitative variables and

Table I. Genetic variants selected for the analysis.

Gene	SNP	SNP type	Chr.	Position (GRCh38.p7)	Alleles	Minor allele	MAF (%)
MMEL1	rs2843403	Intron variant	1	2597658	T/C	T	33.61
PTPN22	rs2476601	Missense variant	1	113834946	A/G	A	11.16
FCRL3	rs7528684	Upstream gene variant	1	157701026	A/G	G	35.75
CTLA4	rs3087243	Downstream gene variant	2	203874196	A/G	A	38.12
BACH2	rs10944479	Intron variant	6	90170674	A/G	A	21.14
RNASET2 [RP1-167A14.2]	rs9355610	Upstream gene variant [Downstream gene variant]	6	166969587	A/G	A	34.32
TG	rs2076740	Missense variant	8	132971813	C/T	T	37.89
IL2RA [RPL32P23]	rs41295061	Upstream gene variant [Upstream gene variant]	10	6072697	A/C	A	7.72
TSHR	rs12101255	Intron variant	14	80984708	C/T	T	28.86
CD40	rs1883832	5 prime UTR variant	20	46118343	C/T	T	26.37

SNP: single nucleotide polymorphism; Chr: chromosome; MAF: minor allele frequency in the control group; MMEL1: membrane metalloendopeptidase like 1; PTPN22: protein tyrosine phosphatase, non-receptor type 22, FCRL3: Fc receptor like 3; CTLA4: cytotoxic T-lymphocyte associated protein 4; BACH2: BTB domain and CNC homolog 2; RNASET2: ribonuclease T2; TG: thyroglobulin; IL2RA: interleukin 2 receptor subunit alpha; RPL32P23: ribosomal protein L32 pseudogene 23; TSHR: thyroid stimulating hormone receptor; CD40: CD40 molecule; UTR: untranslated region.

variables with non-normal distribution were presented as median and range. Mann–Whitney U test was used to compare quantitative traits with non-normal distribution between PBC and PBC-AITD patients and Kruskal-Wallis ANOVA test was used to compare quantitative traits with non-normal distribution in PBC patients stratified by genotype for each SNP. Statistica (StatSoft Inc., Tulsa, OK, US) and PLINK v1.07 (http://zzz.bwh.harvard.edu/plink/) software were used for the analyses. All results of association tests are presented in relation to minor alleles.

RESULTS

Characteristics of the study cohort

The clinical and laboratory characteristics of the study group are shown in Table II. A total of 230 Caucasian PBC patients (90% women) were included in the study. The median age at diagnosis was 53 years (range 22-83 years) and reached 58 years (range 23-87 years) at inclusion into the study. A positive serum level of autoantibodies significant in PBC was found in 204 (89%) patients. Eighty-two patients presented with histological, clinical and/or imaging features of liver cirrhosis (data available in 209 patients). In 64 (30.9%) patients we detected PBC-AITD concomitance as diagnosed by elevated TPOAb serum levels (data available in 207 patients). All patients who underwent liver transplantation were included in the study before the procedure.

Table II. Clinical and laboratory characteristics of the PBC cohort.

Feature	PBC (N= 230)
Age at the enrolment (median; range), years	58 (23-87)
Age at the diagnosis (median; range), years	53 (22-83)
Gender (male/female)	22/208
Cirrhosis (yes/no) (%)	82/127 (39.2%)
Liver transplantation during follow-up (yes/no) (%)	40/188 (17.5%)
TPO-antibodies positive (yes/no) (%)	64/143 (30.9%)
ALT (median; range) IU/l	68 (10-987)
AST (median; range) IU/l	71 (13-1114)
ALP (median; range) IU/l	279 (37-1620)
GGT (median; range) IU/l	256 (11-2755)
Bilirubin (median; range) mg/dL	1.0 (0.2-40.5)
Albumin (median; range) g/dL	3.8 (2.1-5.8)
INR (median; range)	1.0 (0.6-2.3)
Triglycerides (median; range) mg/dL	96 (24-448)
Total cholesterol (median; range) mg/dL	226 (65-1096)

PBC: primary biliary cholangitis; TPO-antibodies: thyroid peroxidase antibody; ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase; GGT: gamma-glutamyl transferase; INR: International Normalized Ratio.

The *PTPN22* polymorphism might increase the risk of developing PBC and PBC-AITD

All selected variants were successfully genotyped in cases and controls and their frequencies did not deviate from the frequencies deposited in the ExAc Browser (exac.broadinstitute.org) and were in line with the frequencies reported in the previous publications. None of the variants deviated from HWE (all P >0.05). Intriguingly, we found a significant association between the *PTPN22* polymorphism and risk of developing PBC (OR=1.43, P=0.035, Table III). Moreover, we observed a trend for the association between the *RNASET2* polymorphism and the presence of PBC (OR=0.79, P=0.067, Suppl. Table A). As presented in the Suppl. Table A, none of the remaining variants was significantly associated with the odds of developing PBC (all P >0.05). The analysis restricted to 64 patients with PBC-AITD demonstrated an increased frequency of the *PTPN22* allele A in this subgroup (17.97% vs. 11.16% in controls). Indeed, carriers of the *PTPN22* polymorphism had a significantly increased risk of developing concomitant PBC-AITD (OR=1.74, P=0.028, Table IV). We also detected a borderline association between the *CTLA4* polymorphism and PBC-AITD (OR=0.69, P=0.066, Suppl. Table B). On the other hand, as demonstrated in Suppl. Table B, no significant association was observed between other tested variants and the PBC-AITD overlap (all P >0.05).

Table III. Distribution of *PTPN22* rs2476601 alleles and genotypes in patients with PBC and healthy controls.

PTPN22 rs2476601	PBC patients (N = 230)	Healthy controls (N = 421)
Counts of alleles/genotypes		
[A]	70 (15.22%)	94 (11.16%)
[G]	390 (84.78 %)	748 (88.84%)
[AA]	7	5
[AG]	56	84
[GG]	167	332
Association tests	chi^2	P
Allele frequency difference test	4.44	0.035
Armitage's trend test	4.34	0.037
OR statistics	OR	95% Cl
[A] ↔ [G]	1.43	1.02 - 1.99

CI: confidence interval; PBC: primary biliary cholangitis; A: adenine; G: guanine; P: p-value; PTPN22: protein tyrosine phosphatase non-receptor type 22; OR: odds ratio.

Associations of the studied variants with liver injury and metabolic traits in the PBC patients

The *IL2RA* polymorphism was significantly associated with the odds of developing liver cirrhosis in the PBC cohort (OR=1.76, P=0.033, Table V & Suppl. Table C). As demonstrated in Table II, 40 (17.5%) PBC patients required liver transplantation. Carriers of the *MMEL1* polymorphism proved to be at increased risk of requiring liver transplantation (OR=1.70, P=0.023, Table VI). We also detected a trend of association between liver transplantation and the *RNASET2* polymorphism (OR=0.72, P=0.087, Suppl. Table D). A comprehensive comparison of laboratory features between subgroups of PBC patients with different genotypes for all studied SNPs is shown in Suppl. Table E. Although we did not detect any significant differences in clinical or biochemical characteristics between patients with PBC and PBC-AITD (all P >0.05, Suppl. Table F), investigated variants significantly

affected liver tests and metabolic traits. Indeed, as shown in Fig. 1, both *CTLA4* and *MMEL1* polymorphisms were associated with serum ALP levels (P=0.005 and P=0.042, respectively), carriers of the *PTPN22* variant had higher GGT and cholesterol (P=0.047 and P=0.028, respectively), the *CTLA4* variant affected serum triglycerides (P=0.003) whereas the *RNASET2* polymorphism was related to albumin concentrations (P=0.018).

Table IV. Distribution of *PTPN22* rs2476601 alleles and genotypes in PBC patients with concomitant AITD and healthy controls.

PTPN22 *rs2476601*	PBC-AITD patients (N = 64)	Healthy controls (N = 421)
Counts of alleles/genotypes		
[A]	23 (17.97%)	94 (1.16 %)
[G]	105 (82.03 %)	748 (88.84 %)
[AA]	0	5
[AG]	23	84
[GG]	41	332
Association tests	chi^2	P
Allele frequency difference test	4.85	0.027
Armitage's trend test	5.05	0.024
OR statistics	OR	95% Cl
[A] ←→[G]	1.74	1.06 - 2.87

CI: confidence interval; PBC: primary biliary cholangitis; A: adenine; G: guanine; P: p-value; PTPN22: protein tyrosine phosphatase non-receptor type 22; OR: odds ratio.

Table V. Distribution of *IL2RA* rs41295061 alleles and genotypes in PBC patients with liver cirrhosis and healthy controls.

IL2RA *rs41295061*	PBC-CIRR patients (N = 82)	Healthy controls (N = 421)
Counts of alleles/genotypes		
[A]	21 (12.80 %)	65 (7.72%)
[C]	143 (87.20 %)	777 (92.28 %)
[AA]	1	3
[AC]	19	59
[CC]	62	359
Association tests	chi^2	P
Allele frequency difference test	4.54	0.033
Armitage's trend test	4.50	0.033
OR statistics	OR	95% Cl
[A] ←→ [C]	1.76	1.04 - 2.96

CI: confidence interval; PBC: primary biliary cholangitis; CIRR: liver cirrhosis; A: adenine; C: cytosine; P: p-value; IL2RA: interleukin 2 receptor subunit alpha; OR: odds ratio.

DISCUSSION

The pathogenesis of PBC remains unclear. Modifiers of PBC progression have also not been fully elucidated [15-17]. Nevertheless, as much as 26% of patients with PBC are expected to develop liver cirrhosis within 5 years from diagnosis despite the ursodeoxycholic acid (UDCA) treatment [18]. Numerous

Table VI. Distribution of *MMEL1* rs2843403 alleles and genotypes in PBC patients requiring liver transplantation and healthy controls.

MMEL1 rs2843403	PBC-LTx patients (N = 40)	Healthy controls (N = 421)
Counts of alleles/genotypes		
[T]	37 (46.25 %)	283 (33.61 %)
[C]	43 (53.75 %)	559 (66.39 %)
[TT]	9	57
[TC]	19	169
[CC]	12	195
Association tests	chi2	P
Allele frequency difference test	5.15	0.023
Armitage's trend test	4.68	0.030
OR statistics	OR	95% Cl
[T]←→ [C]	1.70	1.07 - 2.70

CI: confidence interval; PBC: primary biliary cholangitis; LTx: liver transplantation; T: thymine; C: cytosine; P: p-value; MMEL1: membrane metalloendopeptidase like 1; OR: odds ratio.

genetic studies, including GWAS, performed in patients with PBC have led to the identification of risk loci for this disease [9]. Data concerning genetic modifiers of PBC progression as well as variants that increase the risk of developing autoimmune conditions other than PBC are, in turn, scarce. Therefore in our study we aimed to find risk loci for PBC, but also to detect genetic polymorphisms that affect the progression of the disease and increase the odds for the coincidence of AITD with PBC. Indeed, detection of factors that are associated with a progressive PBC course might help to improve the fate of PBC patients and contribute to a better understanding of the PBC pathogenesis. Our candidate-gene study revealed a few new polymorphisms which are associated with the risk of developing PBC and with the disease progression.

We focused on two issues: genetic triggers of PBC and modifiers of the disease progression. Among tested variants, we found a significant association between the *PTPN22* polymorphism and PBC as well as PBC-AITD. Previous studies demonstrated the association of the rs2476601 polymorphism in the *PTPN22* gene, which encodes lymphoid-specific protein tyrosine phosphatase non-receptor type 22 (Lyp), with numerous autoimmune conditions, such as diabetes mellitus type 1 (DM1) [19], rheumatoid arthritis (RA) [20], systemic lupus erythematosus (SLE) [21], GD [22, 23] and myasthenia gravis [24]. The relationship between variants of *PTPN22* gene and autoimmunity in general is explicable by influence of Lyp on negative control of T-cell activation and T-cell development [25]. An early candidate gene study negated the association between *PTPN22* SNPs and predisposition to PBC [26], also in the GWAS studies no association between this variant and the PBC risk was reported [27-31]. The latest analysis from Japan however revealed that one of the *PTPN22* haplotypes might significantly modulate the risk of developing PBC [32]. Interestingly, our study showed significant association between *PTPN22* and concomitance of PBC and AITD. Previous studies connected the *PTPN22* polymorphism with an overlap between diabetes mellitus type 1 (DM1) and AITD [33, 34] as well

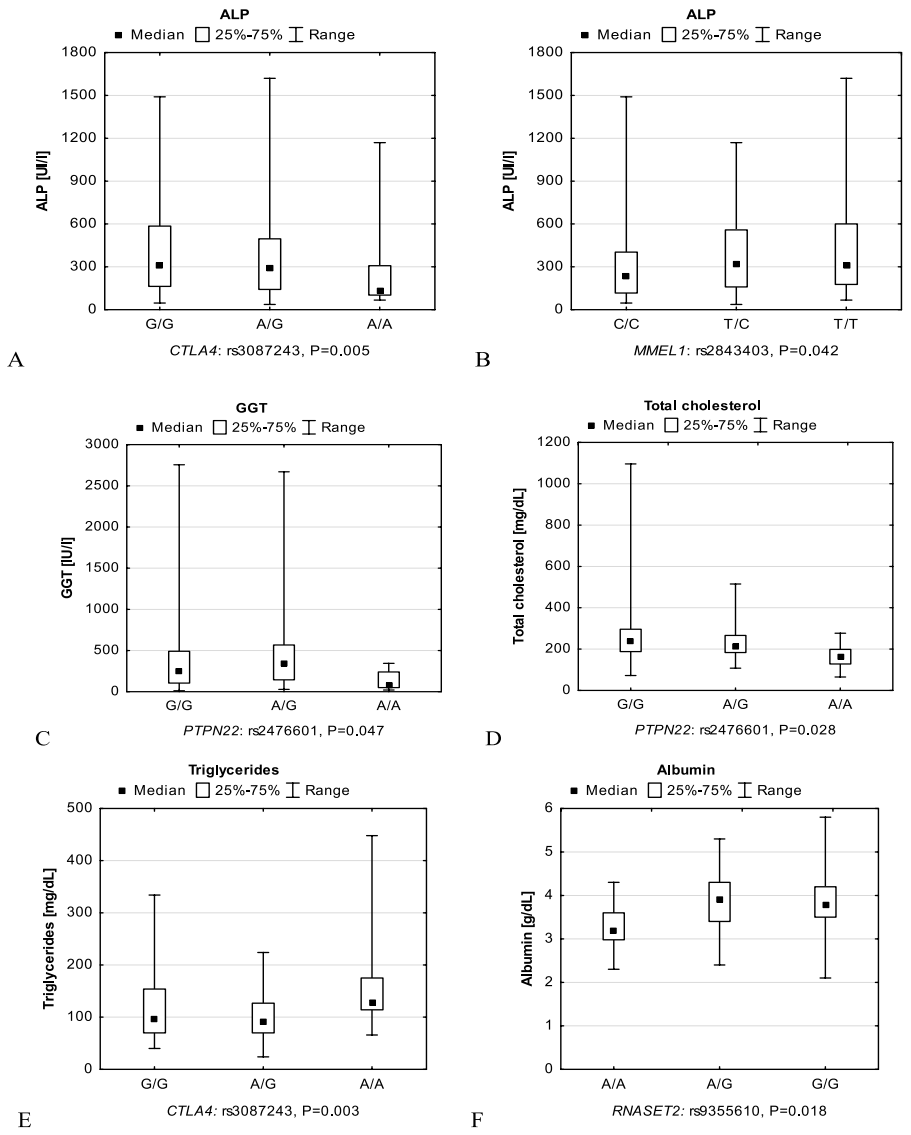

Fig. 1. Associations between the studied SNPs and biochemical parameters in PBC patients. Kruskal-Wallis ANOVA test was used to compare quantitative traits with non-normal distribution in PBC patients stratified by genotype for each SNP. Significant associations (all $P<0.05$) were observed between the CTLA4 (rs3087243) and MMEL1 (rs2843403) polymorphisms and alkaline phosphatase (ALP) levels (A, B), the PTPN22 (rs2476601) polymorphism and gamma-glutamyl transpeptidase (GGT) and cholesterol levels (C, D), the CTLA4 (rs3087243) polymorphism and serum triglycerides levels (E), and the RNASET2 (rs9355610) polymorphism and albumin concentrations (F), as presented below. In each plot, the black square within the box indicates the median value, boundaries of each box indicate the 25th-75th percentile range, and whiskers indicate the highest and lowest values in each subgroup of PBC patients.

as with a susceptibility to DM1, rheumatoid arthritis (RA), systemic lupus erythomatosus (SLE), and HT in members of the families with history of multiplex autoimmune diseases [35]. Therefore, the *PTPN22* gene polymorphism seems to play an important role in the shared susceptibility to autoimmune diseases.

Further analyses revealed that the rs41295061 polymorphism of the *IL2RA* gene, which encodes the alpha subunit of the interleukin 2 receptor, also known as CD25, is associated with increased risk of developing liver cirrhosis. The latest GWAS [36, 37] attributed the risk of hepatic autoimmunity to several new loci and among this group was the *IL2RA* gene. Of note, previously a homozygous mutation of *IL2RA* was described in a pediatric patient causing PBC-like syndrome with positive serological signs [38]. It has been proven that immunological pathways involving T-cell activation might play an important role in the pathogenesis of PBC [39, 40]. Interestingly, Hsu et al. [41] demonstrated that *IL2RA*-knockout mice spontaneously develop biliary inflammation. Hypothetically, the *IL2RA* gene variants associated with lower receptor affinity might also promote the progression of biliary inflammation and liver fibrosis in humans.

A further study [42] on the suggestive PBC risk variants from the first PBC GWAS [27] confirmed the *MMEL1* gene,

encoding membrane metalloendopeptidase like 1 protein, as PBC susceptibility locus. Previously reported associations of the *MMEL1* genetic variants with RA [43], celiac disease [44], multiple sclerosis [45], GD [46] and primary sclerosing cholangitis (PSC) [36] suggest that this locus modulates the susceptibility to develop autoimmune conditions. However, the link between the need of liver transplantation among carriers of this variant is a novel finding which requires confirmation in larger cohorts of patients with PBC. To our knowledge, there is no detailed description of *MMEL1* function that might explain the observed association. Given the involvement of the *MMEL1* in the pathogenesis of several autoimmune conditions, one could hypothesize that carriers of the *MMEL1* variant might have an increased tendency to autoimmune response leading to a rapid progression of the liver disease. Moreover, the analyzed *MMEL1* polymorphism is located in a region of strong linkage disequilibrium on chromosome 1, near the tumour necrosis factor receptor superfamily 14 (TNFRSF14) gene, involved in signal transduction pathways that activate inflammatory response [45]. We reckon that further investigations are needed to elucidate the role of the *MMEL1* polymorphism in the development of PBC and its overlap with other autoimmune conditions.

Of note, in our cohort of patients AITD per se did not seem to significantly influence the natural history of PBC. These results are in line with findings from other European groups, which demonstrated that presence of extrahepatic autoimmune conditions does not significantly affect the survival of patients with PBC [7, 8]. Furthermore, Muratori et al. [47] showed no differences between subgroups of PBC patients with and without concomitant autoimmune disorder in terms of age at diagnosis, serum level of antibodies and long-term complications of liver disease. On the other hand, Rigamonti et al. [48] reported a slower progression of liver disease in PBC patients with coexisting systemic sclerosis, whereas Wang et al. [49] observed certain differences in the laboratory features between Chinese PBC patients with and without overlapping connective tissue disease. These interesting results should be verified in future studies concerning presence of extrahepatic autoimmune conditions and progression of chronic cholestatic disorders.

There are several limitations of this study. First, whereas a number of studies suggest that the genetic background of many autoimmune conditions may be different in various populations, all PBC patients analyzed in the current study were Polish Caucasians: therefore, our results require confirmation in non-Caucasian populations and in cohorts from other European countries. Moreover, although the presence of TPO antibodies usually precedes the development of an overt thyroid disease, we do not have precise data on the thyroid status and thyroid hormones levels in the PBC patients included in our study. Finally, functional studies are necessary to elucidate the underlying mechanisms behind the reported associations

CONCLUSION

Our study suggests the presence of several polymorphisms that are associated with shared susceptibility to AITD and PBC. Some of them might contribute to the clinical course of PBC and determine the levels of biochemical parameters. These results underscore the existence of a shared genetic predisposition between AITD and PBC and might help to design new tailored strategies aiming at patients carrying genetic variants associated with progressive PBC. Further studies are warranted to investigate how shared genetic predisposition between cholestatic liver disease and extrahepatic autoimmune disorders might affect the progression of liver injury.

Conflicts of interest: No conflict to declare.

Authors' contribution: A.K. and M.A.-G. contributed equally to this work; A.K. and K.S. performed the genotyping procedures which were supervised by R.P.; M.A.-G., E.W., M.M. and P.M. recruited and characterized the patients; Z.S. and G.L.N. analyzed serum samples; T.B., P.M. and M.K. designed the study which was then supervised by M.K.; A.K., M.A.-G. and M.K. analyzed the data; A.K. and M.A.-G. wrote the first draft of the manuscript which was edited by M. K. All authors read and approved the final version of the manuscript.

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Supplementary Table A. A comparison of allele frequencies of the analyzed SNPs in the total group of PBC patients and control group.

Gene	SNP	Allele	PBC (N=230)		Controls (N=421)		OR (95% CI)	P-value
			n	%	n	%		
MMEL1	rs2843403	T	159	34.57	283	33.61	1.04	0.728
		C	301	65.43	559	66.39	(0.82-1.33)	
PTPN22	rs2476601	A	70	15.22	94	11.16	**1.43**	**0.035**
		G	390	84.78	748	88.84	**(1.02-1.99)**	
FCRL3	rs7528684	G	165	35.87	301	35.75	1.01	0.965
		A	295	64.13	541	64.25	(0.79-1.27)	
CTLA4	rs3087243	A	158	34.35	321	38.12	0.85	0.177
		G	302	65.65	521	61.88	(0.67-1.08)	
BACH2	rs10944479	A	103	22.39	178	21.14	1.08	0.600
		G	357	77.61	664	78.86	(0.82-1.42)	
RNASET2	rs9355610	A	135	29.35	289	34.32	0.79	0.067
		G	325	70.65	553	65.68	(0.62-1.02)	
TG	rs2076740	T	183	39.78	319	37.89	1.08	0.502
		C	277	60.22	523	62.11	(0.86-1.37)	
IL2RA	rs41295061	A	43	9.35	65	7.72	1.23	0.309
		C	417	90.65	777	92.28	(0.82-1.84)	
TSHR	rs12101255	T	125	27.17	243	28.86	0.92	0.518
		C	335	72.83	599	71.14	(0.71-1.19)	
CD40	rs1883832	T	128	27.83	222	26.37	1.08	0.570
		C	332	72.17	620	73.63	(0.83-1.39)	

CIŞ confidence interval; OR: odds ratio; PBC: primary biliary cholangitis; SNP:single nucleotide polymorphism; MMEL1: membrane metalloendopeptidase like 1; PTPN22: protein tyrosine phosphatase, non-receptor type 21; FCRL3: Fc receptor like 3; CTLA:, cytotoxic T-lymphocyte associated protein 4; BACH2: BTB domain and CNC homolog 2; RNASET2: ribonuclease T2; TG: thyroglobulin; IL2RA: interleukin 2 receptor subunit alpha; TSHR: thyroid stimulating hormone receptor; CD40: CD40 molecule. Bold entries indicate significant P-values (P<0.05).

Supplementary Table B. A comparison of allele frequencies of the analyzed SNPs in PBC patients with concomitant AITD and control group.

Gene	SNP	Allele	PBC-AITD (N=64)		Controls (N=421)		OR (95% CI)	P-value
			n	%	n	%		
MMEL1	rs2843403	T	41	32.03	283	33.61	0.93 (0.63-1.39)	0.724
		C	87	67.97	559	66.39		
PTPN22	rs2476601	A	23	17.97	94	11.16	**1.74 (1.06-2.87)**	**0.028**
		G	105	82.03	748	88.84		
FCRL3	rs7528684	G	46	35.94	301	35.75	1.01 (0.68-1.49)	0.967
		A	82	64.06	541	64.25		
CTLA4	rs3087243	A	38	29.69	321	38.12	0.69 (0.46-1.03)	0.066
		G	90	70.31	521	61.88		
BACH2	rs10944479	A	24	18.75	178	21.14	0.86 (0.54-1.38)	0.535
		G	104	81.25	664	78.86		
RNASET2	rs9355610	A	38	29.69	289	34.32	0.81 (0.54-1.21)	0.301
		G	90	70.31	553	65.68		
TG	rs2076740	T	44	34.38	319	37.89	0.86 (0.58-1.27)	0.444
		C	84	65.62	523	62.11		
IL2RA	rs41295061	A	11	8.59	65	7.72	1.12 (0.58-2.19)	0.732
		C	117	91.41	777	92.28		
TSHR	rs12101255	T	29	22.66	243	28.86	0.72 (0.47-1.12)	0.146
		C	99	77.34	599	71.14		
CD40	rs1883832	T	42	32.81	222	26.37	1.36 (0.91-2.03)	0.127
		C	86	67.19	620	73.63		

AITD: autoimmune thyroid disease; CI: confidence interval; OR: odds ratio; PBC: primary biliary cholangitis; SNP: single nucleotide polymorphism; MMEL1: membrane metalloendopeptidase like 1; PTPN22: protein tyrosine phosphatase, non-receptor type 22, FCRL3: Fc receptor like 3; CTLA4: cytotoxic T-lymphocyte associated protein 4; BACH2: BTB domain and CNC homolog 2; RNASET2: ribonuclease T2; TG: thyroglobulin; IL2RA: interleukin 2 receptor subunit alpha; TSHR: thyroid stimulating hormone receptor; CD40: CD40 molecule. Bold entries indicate significant P-values (P<0.05).

Supplementary Table C. A comparison of allele frequencies of the analyzed SNPs in PBC patients with liver cirrhosis (diagnosed either by liver biopsy or imaging screening) and control group.

Gene	SNP	Allele	PBC-cirrhosis (N=82)		Controls (N=421)		OR (95% CI)	P-value
			n	%	n	%		
MMEL1	rs2843403	T	60	36.59	283	33.61	1.14 (0.80-1.61)	0.462
		C	104	63.41	559	66.39		
PTPN22	rs2476601	A	25	15.24	94	11.16	1.43 (0.89-2.31)	0.139
		G	139	84.76	748	88.84		
FCRL3	rs7528684	G	55	33.54	301	35.75	0.91 (0.64-1.29)	0.588
		A	109	66.46	541	64.25		
CTLA4	rs3087243	A	55	33.54	321	38.12	0.82 (0.58-1.17)	0.267
		G	109	66.46	521	61.88		
BACH2	rs10944479	A	37	22.56	178	21.14	1.09 (0.73-1.62)	0.685
		G	127	77.44	664	78.86		
RNASET2	rs9355610	A	45	27.44	289	34.32	0.72 (0.50-1.05)	0.087
		G	119	72.56	553	65.68		
TG	rs2076740	T	63	38.41	319	37.89	1.02 (0.72-1.44)	0.898
		C	101	61.59	523	62.11		
IL2RA	rs41295061	A	21	12.80	65	7.72	**1.76 (1.04-2.96)**	**0.033**
		C	143	87.20	777	92.28		
TSHR	rs12101255	T	42	25.61	243	28.86	0.85 (0.58-1.24)	0.398
		C	122	74.39	599	71.14		
CD40	rs1883832	T	45	27.44	222	26.37	1.06 (0.73-1.54)	0.776
		C	119	72.56	620	73.63		

CI: confidence interval; OR: odds ratio; PBC: primary biliary cholangitis; SNP: single nucleotide polymorphism; MMEL1: membrane metalloendopeptidase like 1; PTPN22: protein tyrosine phosphatase, non-receptor type 22, FCRL3: Fc receptor like 3; CTLA4: cytotoxic T-lymphocyte associated protein 4; BACH2: BTB domain and CNC homolog 2; RNASET2: ribonuclease T2; TG: thyroglobulin; IL2RA: interleukin 2 receptor subunit alpha; TSHR: thyroid stimulating hormone receptor; CD40: CD40 molecule. Bold entries indicate significant P-values (P<0.05).

Supplementary Table D. A comparison of allele frequencies of the analyzed SNPs in PBC patients requiring liver transplantation and control group.

Gene	SNP	Allele	PBC-LTx (N=40)		Controls (N=421)		OR (95% CI)	P-value
			n	%	n	%		
MMEL1	rs2843403	T	37	46.25	283	33.61	**1.70 (1.07-2.70)**	**0.023**
		C	43	53.75	559	66.39		
PTPN22	rs2476601	A	12	15.00	94	11.16	1.40 (0.73-2.69)	0.304
		G	68	85.00	748	88.84		
FCRL3	rs7528684	G	26	32.50	301	35.75	0.87 (0.53-1.41)	0.562
		A	54	67.50	541	64.25		
CTLA4	rs3087243	A	26	32.50	321	38.12	0.78 (0.48-1.27)	0.321
		G	54	67.50	521	61.88		
BACH2	rs10944479	A	22	27.50	178	21.14	1.42 (0.84-2.38)	0.187
		G	58	72.50	664	78.86		
RNASET2	rs9355610	A	22	27.50	289	34.32	0.73 (0.44-1.21)	0.217
		G	58	72.50	553	65.68		
TG	rs2076740	T	37	46.25	319	37.89	1.41 (0.89-2.24)	0.142
		C	43	53.75	523	62.11		
IL2RA	rs41295061	A	4	5.00	65	7.72	0.63 (0.22-1.77)	0.377
		C	76	95.00	777	92.28		
TSHR	rs12101255	T	17	21.25	243	28.86	0.67 (0.38-1.16)	0.148
		C	63	78.75	599	71.14		
CD40	rs1883832	T	24	30.00	222	26.37	1.20 (0.72-1.98)	0.483
		C	56	70.00	620	73.63		

CI: confidence interval; LTx: liver transplantation; OR: odds ratio; PBC: primary billary cholangitis; SNP: single nucleotide polymorphism; MMEL1: membrane metalloendopeptidase like 1; PTPN22: protein tyrosine phosphatase, non-receptor type 22, FCRL3: Fc receptor like 3; CTLA4: cytotoxic T-lymphocyte associated protein 4; BACH2: BTB domain and CNC homolog 2; RNASET2: ribonuclease T2; TG: thyroglobulin; IL2RA: interleukin 2 receptor subunit alpha; TSHR: thyroid stimulating hormone receptor; CD40: CD40 molecule. Bold entries indicate significant P-values (P<0.05).

Supplementary Table E. A comparison of laboratory features between subgroups of PBC patients with different genotypes of the studied SNPs.

Gene	SNP	Geno.	AST IU/l		ALT IU/l		ALP IU/l		GGT IU/l		Bilirubin mg/dL		Albumin g/dL		INR		Total cholesterol mg/dL		Triglycerides mg/dL	
			N	median (range)	N	median (range)	N	median (range)	N	median (range)	N	median (range)	N	median (range)	N	median (range)	N	median (range)	N	median (range)
		TT	25	69 (16-188)	25	66 (16-202)	**25**	**309 (67-1620)**	25	368 (17-898)	25	1.5 (0.2-23.0)	22	3.9 (2.4-4.9)	25	1.0 (0.8-2.3)	19	210 (72-459)	15	115 (40-172)
MMEL1	rs2843403	TC	93	69 (13-493)	93	65 (10-605)	**92**	**321 (37-1170)**	91	239 (11-2670)	92	0.9 (0.2-32.8)	78	3.8 (2.1-5.0)	83	1.0 (0.6-2.3)	84	222 (128-582)	74	93 (48-448)
		CC	88	72 (16-1114)	88	71 (10-987)	**88**	**232 (47-1490)**	86	245 (12-2755)	81	1.1 (0.3-40.5)	76	3.8 (2.3-5.8)	81	1.1 (0.9-2.1)	77	240 (65-1096)	71	111 (24-334)
				P=0.979		P=0.620		**P=0.042**		P=0.339		P=0.567		P=0.206		P=0.234		P=0.872		P=0.094
		AA	6	59 (20-167)	6	56 (18-169)	6	207 (97-576)	**6**	**81 (20-344)**	6	9.4 (0.3-40.5)	6	3.6 (2.9-4.4)	6	1.2(1.0-1.8)	**6**	**166 (65-277)**	6	131 (63-260)
PTPN22	rs2476601	AG	49	63 (21-425)	49	67 (20-442)	49	314 (56-1170)	**48**	**349 (28-2670)**	48	1.0 (0.3-15.0)	44	3.9 (2.4-4.8)	46	1.0 (0.8-2.3)	**44**	**214 (108-515)**	41	99 (24-294)
		GG	151	72 (13-1114)	151	71 (10-987)	150	270 (37-1620)	**148**	**247 (11-2755)**	144	1.0 (0.2-30.0)	126	3.8 (2.1-5.8)	137	1.0 (0.6-2.3)	**130**	**240 (72-1096)**	113	96 (40-448)
				P=0.606		P=0.749		P=0.692		**P=0.047**		P=0.627		P=0.374		P=0.235		**P=0.028**		P=0.432
		GG	28	71 (13-1114)	29	71 (15-987)	28	325 (80-769)	27	241 (11-948)	29	0.9 (0.3-11.5)	25	3.8 (3.3-5.3)	25	1.0 (0.6-1.3)	26	226 (150-468)	23	92 (51-334)
FCRL3	rs7528684	GA	95	72 (16-931)	95	71 (10-605)	95	271 (47-1344)	94	254 (15-2755)	91	1.0 (0.2-32.8)	81	3.8 (2.1-5.8)	87	1.0 (0.7-2.3)	81	239 (124-871)	73	99 (24-448)
		AA	83	69 (16-467)	82	64 (12-539)	82	256 (37-1620)	81	262 (12-1700)	79	1.3 (0.3-40.5)	70	3.8 (2.4-4.9)	77	1.0 (0.8-2.3)	73	219 (65-1096)	64	97 (40-294)
				P=0.713		P=0.420		P=0.815		P=0.906		P=0.284		P=0.710		P=0.265		P=0.458		P=0.909
		AA	27	40 (16-1114)	27	59 (10-987)	**27**	**134 (67-1170)**	25	143 (22-2755)	25	0.8 (0.2-18.4)	23	3.8 (2.8-4.5)	26	1.0 (0.8-1.4)	23	237 (140-354)	**19**	**129 (66-448)**
CTLA4	rs3087243	AG	88	71 (13-493)	87	70 (12-605)	**87**	**293 (37-1620)**	86	259 (11-1932)	86	1.1 (0.2-40.5)	76	3.8 (2.4-5.8)	82	1.0 (0.8-2.3)	76	223 (65-1096)	**71**	**91 (24-224)**
		GG	91	77 (16-568)	92	71 (10-727)	**91**	**310 (47-1490)**	91	284 (15-2670)	87	1.0 (0.3-23.0)	77	3.9 (2.1-5.0)	81	1.0 (0.6-2.3)	81	226 (72-871)	**70**	**96 (40-334)**
				P=0.068		P=0.714		**P=0.005**		P=0.102		P=0.587		P=0.553		P=0.920		P=0.810		**P=0.003**
		AA	15	82 (21-262)	15	64 (20-331)	15	316 (94-584)	14	240 (33-810)	14	0.9 (0.3-18.0)	13	3.6 (2.8-4.2)	15	1.0 (0.9-1.3)	14	205 (130-369)	13	101 (51-260)
BACH2	rs10944479	AG	59	71 (16-931)	59	71 (10-727)	57	234 (47-935)	58	180 (18-1571)	55	1.1 (0.3-32.8)	47	3.7 (2.3-5.8)	53	1.0 (0.6-2.3)	49	219 (128-500)	41	114 (51-334)
		GG	132	71 (13-1114)	132	67 (12-987)	133	310 (37-1620)	130	272 (11-2755)	129	1.0 (0.2-40.5)	116	3.9 (2.1-5.0)	121	1.0 (0.8-2.3)	117	242 (65-1096)	106	92 (24-448)
				P=0.790		P=0.911		P=0.236		P=0.325		P=0.477		P=0.152		P=0.401		P=0.222		P=0.357
		AA	16	72 (16-262)	16	62 (10-306)	16	321 (74-900)	15	342 (29-782)	14	1.9 (0.4-30.0)	**11**	**3.2 (2.3-4.3)**	14	1.1 (0.9-2.1)	13	219 (72-500)	9	100 (63-186)
RNASET2	rs9355610	AG	87	63 (13-467)	88	64 (10-446)	87	250 (56-1221)	88	211 (11-2755)	84	0.8 (0.2-32.8)	**75**	**3.9 (2.4-5.3)**	82	1.0 (0.6-2.3)	76	240 (108-375)	71	101 (40-448)
		GG	103	80 (16-1114)	102	76 (12-987)	102	301 (37-1620)	99	279 (12-2670)	100	1.2 (0.3-40.5)	**90**	**3.8 (2.1-5.8)**	93	1.0 (0.8-1.9)	91	209 (65-1096)	80	93 (24-334)
				P=0.113		P=0.173		P=0.757		P=0.350		P=0.092		**P=0.018**		P=0.237		P=0.718		P=0.614
		TT	30	65 (20-266)	30	79 (10-361)	29	271 (80-1037)	29	279 (20-2755)	28	1.0 (0.3-40.5)	28	3.8 (2.8-5.0)	29	1.1 (0.8-1.8)	28	240 (65-459)	28	95 (48-175)
TG	rs2076740	TC	99	72 (16-1114)	100	69 (10-987)	99	310 (47-1490)	98	259 (12-2670)	96	1.0 (0.3-32.8)	83	3.8 (2.1-4.9)	87	1.0 (0.6-2.1)	88	242 (108-1096)	73	101 (24-448)
		CC	77	71 (13-568)	76	67 (15-727)	77	249 (37-1620)	75	201 (11-1524)	74	1.0 (0.2-23.0)	65	3.8 (2.4-5.8)	73	1.0 (0.8-2.3)	64	215 (72-371)	59	96 (51-334)
				P=0.949		P=0.912		P=0.337		P=0.358		P=0.947		P=0.904		P=0.399		P=0.241		P=0.483
		AA	1	112 (112-112)	1	43 (43-43)	1	1344 (1344-1344)	1	626 (626-626)	1	3.6 (3.6-3.6)	1	4.4 (4.4-4.4)	1	1.1 (1.1-1.1)	0	-	0	-
IL2RA	rs41295061	AC	38	61 (16-931)	38	60 (16-539)	38	242 (63-905)	38	176 (15-987)	36	1.0 (0.2-18.4)	30	3.7 (2.4-5.0)	37	1.1 (0.9-1.8)	34	209 (134-871)	32	98 (24-448)
		CC	167	71 (13-1114)	167	72 (10-987)	166	286 (37-1620)	163	265 (11-2755)	161	1.0 (0.2-40.5)	145	3.8 (2.1-5.8)	151	1.0 (0.6-2.3)	146	233 (65-1096)	128	96 (40-334)
				P=0.580		P=0.293		P=0.064		P=0.187		P=0.535		P=0.125		P=0.137		P=1.000		P=1.000
		TT	19	85 (20-217)	19	80 (10-349)	19	309 (103-1620)	19	347 (50-1524)	19	1.4 (0.3-22.0)	17	3.6 (2.3-4.3)	17	1.1 (0.9-1.8)	17	226 (171-329)	13	92 (51-222)
TSHR	rs12101255	TC	77	71 (16-1114)	78	66 (10-987)	77	279 (47-1344)	76	239 (17-1932)	75	0.9 (0.3-32.8)	68	4.0 (2.4-5.0)	71	1.0 (0.7-2.3)	69	235 (128-871)	65	96 (40-448)
		CC	110	70 (13-931)	109	65 (12-605)	109	271 (37-1170)	107	256 (11-2755)	104	1.1 (0.2-40.5)	91	3.8 (2.1-5.8)	101	1.0 (0.6-2.3)	94	226 (65-1096)	82	98 (24-320)
				P=0.615		P=0.711		P=0.600		P=0.589		P=0.381		P=.0173		P=0.265		P=0.903		P=0.888
		TT	12	71 (21-114)	12	48 (18-144)	12	271 (90-1620)	12	288 (18-1932)	11	0.6 (0.3-9.1)	11	3.9 (3.0-5.3)	11	1.0 (0.7-1.4)	7	254 (124-378)	6	83 (69-294)
CD40	rs1883832	TC	97	74 (13-931)	98	70 (10-605)	96	293 (63-1344)	97	256 (11-1571)	94	1.0 (0.2-40.5)	78	3.8 (2.1-5.0)	86	1.0 (0.8-2.3)	87	230 (65-500)	82	100 (40-448)
		CC	97	68 (16-1114)	96	71 (10-987)	97	269 (37-1490)	93	202 (12-2755)	93	1.0 (0.2-30.0)	87	3.8 (2.4-5.8)	92	1.0 (0.6-2.3)	86	226 (72-1096)	72	92 (24-334)
				P=0.838		P=0.403		P=0.766		P=0.499		P=0.226		P=0.798		P=0.542		P=0.554		P=0.727

PBC: primary billary cholangitis; SNP: single nucleotide polymorphism; Geno: genotype; MMEL1: membrane metalloendopeptidase like 1; PTPN22: protein tyrosine phosphatase, non-receptor type 22, FCRL3: Fc receptor like 3; CTLA4: cytotoxic T-lymphocyte associated protein 4; BACH2: BTB domain and CNC homolog 2; RNASET2: ribonuclease T2; TG: thyroglobulin; IL2RA: interleukin 2 receptor subunit alpha; TSHR: thyroid stimulating hormone receptor; CD40: CD40 molecule. Bold entries indicate significant P-values (P<0.05).

Supplementary Table F. A comparison of laboratory features between subgroups of PBC patients with and without concomitant AITD.

	AST IU/l		ALT IU/l		ALP IU/l		GGT IU/l		Bilirubin mg/dL		Albumin g/dL		INR		Total cholesterol mg/dL		Triglycerides mg/dL	
	N	**median (range)**	**N**	**median (range)**	**N**	**median (range)**	**N**	**median (range)**	**N**	**median (range)**	**N**	**median (range)**	**N**	**median (range)**	**N**	**median (range)**	**N**	**median (range)**
PBC-AITD	60	71 (16-467)	60	64 (10-442)	59	314 (47-1170)	58	277 (18-1700)	57	1.0 (0.2-14.8)	50	3.8 (2.4-4.9)	54	1.0 (0.8-2.3)	50	219 (128-1096)	44	90 (53-294)
PBC-nonAITD	126	71 (13-1114)	126	74 (12-987)	126	284 (57-1620)	124	264 (11-2755)	121	0.9 (0.3-32.8)	110	3.8 (2.3-5.8)	115	1.0 (0.6-1.8)	112	241 (108-582)	99	99 (24-448)
P-value		P=0.868		P=0.698		P=0.765		P=0.896		P=0.531		P=0.365		P=0.292		P=0.173		P=0.641

AITD: autoimmune thyroid disease; PBC: primary biliary cholangitis.