Association of genetic variations in PTPN2 and CD122 with ocular Behcet's disease

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ABSTRACT

Background Protein tyrosine phosphatases (PTPs) play critical roles in human autoimmunity. Previous studies found that PTPN2 may be the key regulatory factor in the T-cell-mediated immune response. PTPN2 regulates the Janus kinase/signal transducers and activators of transcription pathway by inhibiting signalling via the interleukin (IL)-2 receptor (CD122). An association between genetic variations in PTPN2 and CD122 with ocular Behcet's disease (BD) has not yet been addressed and was therefore the purpose of this study.

Methods A two-stage case–control study was performed in 906 patients with ocular BD and 2178 healthy controls. Genotyping analysis of 11 single nucleotide polymorphisms was carried out. The expression of PTPN2 in peripheral blood mononuclear cells (PBMCs) was quantified by real-time PCR and cytokine production was measured by ELISA.

Results The frequency of the GG genotype of PTPN2-rs7234029 was significantly lower in patients with ocular BD ($p=1.94\times10^{-5}$, $p_c=8.34\times10^{-4}$, OR=0.466). Stratification according to gender showed that rs7234029 was significantly associated with BD in men. A stratified analysis according to the main clinical features showed that rs7234029 was significantly associated with genital ulcers, skin lesions and a positive pathergy test. No association could be detected between BD and CD122 gene polymorphisms. Functional studies showed that rs7234029 GG genotype carriers had a higher PNPT2 mRNA expression level than those which carrying the AA or AG genotype, and a decreased secretion of IL-17 and tumour necrosis factor-alpha was seen by PBMCs from GG carriers. No significant difference could be detected concerning IL-1β or IL-6 production by stimulated PBMCs between the different genotype groups.

Conclusions This study shows that a PTPN2-rs7234029 polymorphism is associated with ocular BD and is strongly influenced by gender. In addition, our results suggest that the genetic association with PTPN2 may involve the regulation of PTPN2 mRNA expression and cytokine secretion.

INTRODUCTION

Uveitis is a relatively common intraocular inflammatory eye disease which can lead to significant visual impairment. Behcet's disease (BD) is a common sight-threatening uveitis entity in China[1] and is currently considered to be a chronic, intractable autoinflammatory disorder. The main clinical symptoms of BD include a recurrent uveitis, recurrent oral ulceration, genital ulceration and skin lesions, whereby systemic vasculitis was recognised as the main pathological feature. However, musculoskeletal, neurological and gastrointestinal systems can also be affected. Cases of BD have been reported worldwide, but the prevalence of the disease is much higher in countries along the ancient 'Silk Road', from Mediterranean to the Far East, such as China, Japan and Turkey.[2]

The eye is the most commonly involved organ and ocular involvement belongs to the main disabling features of BD. The exact cause of BD remains unknown, but recent studies suggest that both genetic and environmental factors are involved. Two recent large genome-wide association studies (GWAS) reported an association of single nucleotide polymorphisms (SNPs) of interleukin *(IL)-10* and *IL-23R/IL-12RB2* genes with BD.[3 4] Other studies showed that a large number of other genetic factors were also involved in the susceptibility to BD, such as HLA-B51.[5] The study of the involvement of genetic factors not only enlarges our knowledge in the pathogenesis of BD, but also allows the development of novel therapeutic tools to prevent the sight-threatening consequences of this disease.

Recently attention has been drawn to the role of protein tyrosine phosphatases (PTPs) gene polymorphisms in the pathogenesis of several human autoimmune diseases, although little is known concerning their involvement with BD.[6] They are subdivided into receptor-type PTPs (CD45 and CD148) and non-receptor-type PTPs (PTPN22, SHP-1, PTPN2 and PTP-PEST).[7] PTPN22, located on chromosome 1p13, is an important negative regulator of T-lymphocyte function, and PTPN22 variants have been found to be correlated with various immune-related disorders.[8] In a previous study from our group, we were however unable to show an association between PTPN22 variants (rs2488457, rs1310182 and rs3789604) with ocular BD in Chinese Han.[9] PTPN2, which is located on chromosome 18p11, is a key regulatory factor in the T-cell-mediated immune response.[10] Its action is mediated via a negative regulation of the Janus kinase/signal transducers and activators of transcription (JAK/STAT) pathway following signalling via the IL-2 receptor (CD122).[10] The JAK1/STAT3 pathway is activated in BD, possibly through elevated expression of type 1 T helper (Th1)/type 17 T helper (Th17)-type cytokines such as IL-2.[11] Recently, the PTPN2 variant rs1893217 was found to be associated with the risk of BD development in a Han Chinese population.[12] In this latter study, only 28.5% of the patients had uveitis and we therefore decided to expand this study and to focus on a group of patients with BD with ocular involvement. In view of the known

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interaction between PTPN2 and CD122 signalling pathways, we also included an analysis of CD122 gene variants in our study. No association was found with CD122 but a polymorphism of PTPN2 rs7234029 was shown to affect predisposition to ocular BD.

MATERIALS AND METHODS

Study population

For this project, 906 patients with BD were recruited. Both the patients and healthy controls were recruited at the First Affiliated Hospital of Chongqing Medical University (Chongqing, China) from May 2008 to July 2015. All the enrolled patients with BD and healthy controls were Han Chinese. All the patients had uveitis, whereby most had panuveitis (95.25%), and only 43 patients (4.75%) had anterior uveitis. Patients with BD were strictly diagnosed according to the criteria of the International Study Group for Behcet's Disease.[13] The normal control group comprised 2178 unrelated normal Chinese Han volunteers with no history of any eye disorder or autoimmune disease, and originated from the same regions as the BD group. There was no significant difference in age distribution between the BD and control groups. All the investigated subjects provided their informed written consent to participate in this study before enrolment. The procedures of this study were conformed the ethical guidelines of the Declaration of Helsinki.

SNP selection

Based on the results of available literature reports,[12 14–20] we selected the SNPs which were earlier shown to be associated with a variety of autoimmune or autoinflammatory diseases. SNPs with a minor allele frequency of <5% in the Chinese Han population were excluded. Linkage disequilibrium was tested using Haploview software V.4.2. Finally, we selected five SNPs (rs2542151, rs1893217, rs657555, rs478582 and rs7234029) for PTPN2 and six SNPs for CD122 (rs743777, rs9622555, rs743776, rs228941, rs3218253 and rs2284033).

DNA extraction and genotyping

We extracted genomic DNA with the QIAamp DNA Blood Mini Kit (Qiagen) from peripheral blood samples of all the subjects. The SNP genotyping assay was performed by the Mass ARRAY System (Sequenom, San Diego, California, USA) in strict accordance with standard procedures.

Cell isolation and culture

PBMCs were obtained from venous blood samples of 40 healthy genotyped controls using Ficoll-Hypaque density-gradient centrifugation, seeded in 24-well plates (1×10^6 cells/well) and cultured in Roswell Park Memorial Institute (RPMI) 1640 medium including 100 U/mL penicillin, 10% FCS and 100 µg/mL streptomycin. PBMCs were treated with anti-CD3 antibody (5 µg/mL; eBioscience) and anti-CD28 antibody (1 µg/mL; eBioscience) (5:1) to simulate antigen presentation at 37°C for 72 hours.

Real-Time PCR

To examine the expression of the PTPN2 gene, we extracted total RNA from PBMCs using a commercial reagent (TRIzol; Life Technologies), and reverse transcription was performed using a commercial kit (ABI). Real-time quantitative PCR was carried out by a 7500 real-time instrument (ABI). The expression of PTPN2 was detected using the following primers (PTPN2, forward, 5′-CGGGAGTTCGAAGAGTTGGATA-3′; reverse, 5′-CGACTGTGATCATATGGGCTTA-3′) based on the SYBR-Green method. The primers of β-actin were as below: forward, 5′-GGATGCAGAAGGAGATCACTG-3′; reverse, 5′-CGATCCACACGGAGTACTT-3′. Relative expression levels of PTPN2 were quantified by the $2-\Delta\Delta Ct$ method.

Table 1 Clinical characteristics of patients with BD included in the current study

Extraocular findings	Total	%
Patients with BD	906	100
Mean age±SD	34.0±9.2	
Male	755	83.3
Female	151	16.7
Uveitis	906	100
Oral ulcer	906	100
Genital ulcer	511	56.4
Skin lesions	688	75.9
Hypopyon	223	24.6
Arthritis	154	17.0
Positive pathergy test	144	15.9
Controls	2178	
Mean age±SD	39.6±10.7	
Male	1222	56.1
Female	956	44.0

BD, Behcet's disease.

Measurement of cytokines by ELISA

Cytokine levels (tumour necrosis factor-alpha (TNF-α), IL-17, IL-1β and IL-6) in supernatants of PBMCs were quantified using Duoset ELISA development kits (R&D Systems).

Statistical methods

The Hardy-Weinberg equilibrium (HWE) of all tested SNP was determined by the χ^2 test. Genotype distributions and allele frequencies of candidate SNPs were evaluated using the χ^2 test with SPSS (V.17.0). The Bonferroni correction method was used for correcting the p values for multiple comparisons. The independent samples test or non-parametric Mann-Whitney U test was used to compare PTPN2 expression levels and cytokine levels (IL-1β, IL-6, IL-17 and TNF-α) among different genotype groups.

RESULTS

The clinical characteristics of the enrolled patients with BD and healthy controls are shown in table 1. All the patients with BD had uveitis and attended our uveitis clinic for their eye problems.

Genotype and allele frequencies of tested SNPs in the first stage study

In the first phase, 11 SNPs were genotyped in 375 patients with BD and 598 healthy controls. The AG genotype of rs7234029 was significantly associated with BD ($p_c=2.16\times10^{-2}$, OR=1.59) (table 2). The GG genotype of rs7234029 in BD patients showed a protective effect as compared with healthy controls ($p_c=4.43\times10^{-2}$, OR=0.42) (table 2). No significant association was observed in the other 10 SNPs between ocular BD and healthy controls.

Genotype and allele frequencies of SNPs in the replication phase and combined study

To validate the detected association of PTPN2 with BD that was observed in the first phase study, we recruited a separate set of 531 patients with BD and 1580 normal controls for a

Table 2 Genotype and allele frequencies of *PTPN2* and *CD122* genes in ocular BD

Gene	SNPs	Stage	Genotype allele	BD		Controls		P value	P_c value	OR	95% CI
				N	%	N	%				
PTPN2	rs7234029	First	A	522	0.696	827	0.691	0.833	NS	1.022	0.838 to 1.245
			AA	166	0.443	296	0.495	0.112	NS	0.81	0.625 to 1.050
			AG	190	0.507	235	0.393	5.02×10^{-4}	2.16×10^{-2}	1.586	1.222 to 2.059
			GG	19	0.051	67	0.112	1.03×10^{-3}	4.43×10^{-2}	0.423	0.250 to 0.716
		Replication	A	746	0.702	2266	0.717	0.361	NS	0.931	0.800 to 1.085
			AA	234	0.441	806	0.51	0.006	NS	0.757	0.621 to 0.922
			AG	278	0.524	654	0.414	1.08×10^{-5}	4.64×10^{-4}	1.556	1.277 to 1.896
			GG	19	0.036	120	0.076	1.24×10^{-3}	5.33×10^{-2}	0.451	0.275 to 0.740
		Combined	A	1268	0.7	3093	0.71	0.419	NS	0.952	0.844 to 1.073
			AA	400	0.442	1102	0.506	1.10×10^{-3}	4.73×10^{-2}	0.772	0.661 to 0.902
			AG	468	0.517	889	0.408	3.33×10^{-8}	1.43×10^{-6}	1.549	1.326 to 1.810
			GG	38	0.042	187	0.086	1.94×10^{-5}	8.34×10^{-4}	0.466	0.326 to 0.667
	rs1893217	First	C	129	0.172	188	0.163	0.626	NS	1.063	0.831 to 1.359
			CC	6	0.016	16	0.028	0.236	NS	0.568	0.220 to 1.465
			CT	117	0.312	156	0.271	0.175	NS	1.218	0.916 to 1.621
			TT	252	0.672	403	0.701	0.347	NS	0.874	0.661 to 1.157
	rs657555	First	C	230	0.307	327	0.283	0.264	NS	1.121	0.917 to 1.371
			CC	27	0.072	51	0.088	0.372	NS	0.802	0.493 to 1.303
			CT	176	0.469	225	0.389	0.014	NS	1.388	1.067 to 1.805
			TT	172	0.459	302	0.522	0.054	NS	0.774	0.597 to 1.005
	rs2542151	First	G	136	0.181	197	0.165	0.352	NS	1.121	0.881 to 1.426
			GG	12	0.032	20	0.034	0.898	NS	0.954	0.461 to 1.974
			GT	112	0.299	157	0.263	0.226	NS	1.193	0.896 to 1.589
			TT	251	0.669	420	0.704	0.262	NS	0.853	0.646 to 1.126
	rs478582	First	C	125	0.167	216	0.187	0.273	NS	0.873	0.685 to 1.113
			CC	12	0.032	19	0.033	0.947	NS	0.975	0.468 to 2.033
			CT	101	0.27	178	0.308	0.21	NS	0.831	0.623 to 1.110
			TT	261	0.698	381	0.659	0.213	NS	1.194	0.903 to 1.580
CD122	rs228941	First	C	252	0.342	414	0.353	0.628	NS	0.953	0.785 to 1.157
			CC	40	0.109	64	0.109	0.98	NS	0.995	0.655 to 1.512
			CG	172	0.467	286	0.488	0.534	NS	0.921	0.709 to 1.195
			GG	156	0.424	236	0.403	0.517	NS	1.091	0.838 to 1.422
	rs743777	First	A	659	0.879	1082	0.905	0.069	NS	0.763	0.570 to 1.022
			AA	285	0.76	488	0.816	0.035	NS	0.714	0.521 to 0.978
			AG	89	0.237	106	0.177	0.023	NS	1.444	1.052 to 1.984
			GG	1	0.003	4	0.007	0.362	NS	0.397	0.044 to 3.566
	rs3218253	First	C	686	0.92	1100	0.932	0.298	NS	0.832	0.587 to 1.178
			CC	313	0.839	510	0.864	0.279	NS	0.818	0.569 to 1.177
			CT	60	0.161	80	0.136	0.279	NS	0.818	0.569 to 1.177
	rs743776	First	C	103	0.138	118	0.099	0.007	NS	1.468	1.107 to 1.946
			CC	5	0.013	3	0.005	0.158	NS	2.702	0.642 to 11.373
			CT	93	0.25	112	0.187	0.02	NS	1.446	1.059 to 1.976
			TT	274	0.737	483	0.808	0.009	NS	0.666	0.489 to 0.905
	rs2284033	First	A	480	0.647	743	0.623	0.296	NS	1.107	0.915 to 1.340
			AA	159	0.429	239	0.401	0.397	NS	1.12	0.861 to 1.457
			AG	162	0.437	265	0.445	0.808	NS	0.968	0.746 to 1.257
			GG	50	0.135	92	0.154	0.403	NS	0.853	0.588 to 1.237
	rs9622555	First	G	654	0.872	1054	0.887	0.313	NS	0.866	0.655 to 1.146
			GG	282	0.752	464	0.781	0.294	NS	0.85	0.627 to 1.152
			GT	90	0.24	126	0.212	0.31	NS	1.173	0.862 to 1.596
			TT	3	0.008	4	0.007	0.821	NS	1.19	0.265 to 5.345

BD, Behcet's disease; NS, non-significant.

replication phase. In this replication study, we confined ourselves to the SNP rs7234029, which revealed a statistically significant result in the first phase. The AG genotype of rs7234029 again showed a significant susceptibility to ocular BD ($p=1.08\times10^{-5}$, $p_c=4.64\times10^{-4}$, OR=1.556, 95% CI 1.227 to 1.896), and the frequency of the GG genotype of rs7234029 in patients with BD was again significantly lower than that seen in the healthy controls ($p=1.24\times10^{-3}$, $p_c=5.33\times10^{-2}$, OR=0.451, 95% CI 0.275 to 0.740) (table 2). Combination of the data from the first and replication study also showed that rs7234029 was

Table 3 Genotype and allele frequencies of rs7234029 in patients and controls stratified by the main clinical features

Clinical features	Genotype allele	BD		Controls		P value	P_c value	OR	95% CI
		N	%	N	%				
Genital ulcer	A	722	0.706	3093	0.71	0.82	NS	0.983	0.846 to 1.141
	AA	230	0.45	1102	0.506	0.023	NS	0.799	0.659 to 0.970
	AG	262	0.513	889	0.408	1.72×10^{-5}	7.40×10^{-4}	1.526	1.257 to 1.851
	GG	19	0.037	187	0.086	1.97×10^{-4}	8.47×10^{-3}	0.411	0.254 to 0.666
Skin lesions	A	951	0.691	3093	0.71	0.179	NS	0.914	0.801 to 1.042
	AA	294	0.427	1102	0.506	3.21×10^{-4}	1.38×10^{-2}	0.729	0.613 to 0.866
	AG	363	0.528	889	0.408	3.66×10^{-8}	1.57×10^{-6}	1.619	1.363 to 1.924
	GG	31	0.045	187	0.086	4.33×10^{-4}	1.86×10^{-2}	0.502	0.340 to 0.742
Hypopyon	A	311	0.697	3093	0.71	0.573	NS	0.941	0.761 to 1.163
	AA	98	0.439	1102	0.506	0.059	NS	0.766	0.580 to 1.010
	AG	115	0.516	889	0.408	0.002	NS	1.544	1.171 to 2.035
	GG	10	0.045	187	0.086	0.034	NS	0.5	0.261 to 0.959
Arthritis	A	216	0.701	3093	0.71	0.744	NS	0.959	0.745 to 1.234
	AA	70	0.455	1102	0.506	0.217	NS	0.814	0.586 to 1.130
	AG	76	0.494	889	0.408	0.038	NS	1.413	1.018 to 1.960
	GG	8	0.052	187	0.086	0.142	NS	0.583	0.282 to 1.207
Positive pathergy test	A	203	0.705	3093	0.71	0.851	NS	0.975	0.751 to 1.267
	AA	61	0.424	1102	0.506	0.056	NS	0.718	0.510 to 1.009
	AG	81	0.562	889	0.408	2.76×10^{-4}	1.19×10^{-2}	1.864	1.327 to 2.620
	GG	2	0.014	187	0.086	0.002	NS	0.15	0.037 to 0.610

NS, non-significant.

significantly associated with BD (AG genotype, $p=3.33\times10^{-8}$, $p_c=1.43\times10^{-6}$, OR=1.549; AA genotype, $p=1.10\times10^{-3}$, $p_c=4.73\times10^{-2}$, OR=0.772; for GG genotype, $p=1.94\times10^{-5}$, $p_c=8.34\times10^{-4}$, OR=0.466).

Stratified analysis according to gender showed that rs7234029 was significantly associated with BD in men (AG genotype: $p=9.32\times10^{-6}$, $p_c=4.01\times10^{-4}$, OR=1.51; GG genotype: $p=1.66\times10^{-5}$, $p_c=7.14\times10^{-4}$, OR=0.408) but not in women (online supplementary table 1). Univariate and multivariate logistic regression analyses were also performed adjusting for age and gender. Logistic regression analysis showed that rs7234029 was associated with BD in a co-dominant model, dominant model, recessive model and overdominant model (online supplementary table 2). In the co-dominant model, compared with patients carrying the AA genotype, patients carrying the AG genotype have an increased risk (OR=1.38, $p=2.41\times10^{-4}$), while patients carrying the GG genotype have a decreased risk (OR=0.52, $p=8.88\times10^{-4}$). In the dominant model, patients carrying the AG or GG genotypes have an increased risk (OR=1.23, $p=1.58\times10^{-2}$) compared with those carrying the AA genotype. In the recessive model, patients carrying the GG genotype have a decreased risk (OR=0.44, $p=2.23\times10^{-5}$) compared with those carrying AA or AG genotypes. In the overdominant model, patients carrying the AG genotype have an increased risk (OR=1.49, $p=3.18\times10^{-6}$) compared with those carrying the AA or GG genotypes. We performed a stratified analysis to examine the association of rs7234029 with the main clinical features of BD. The result showed that rs7234029 was significantly associated in patients with BD with a positive pathergy test and with those having skin lesions and genital ulcers. No significant association was found for the other extraocular manifestations of BD with rs7234029 (table 3).

The influence of rs7234029 on the PTPN2 expression and cytokine secretion

Functional experiments were performed to determine whether PTPN2 mRNA expression was influenced by the different genotypes of rs7234029. Our results revealed that individuals with the AA and AG genotype had a significantly decreased PTPN2 mRNA expression when compared with GG individuals (p=0.014 and p=0.019) (figure 1). We further determined whether different genotypes of rs7234029 influenced PBMC cytokine secretion in healthy individuals. An elevated secretion

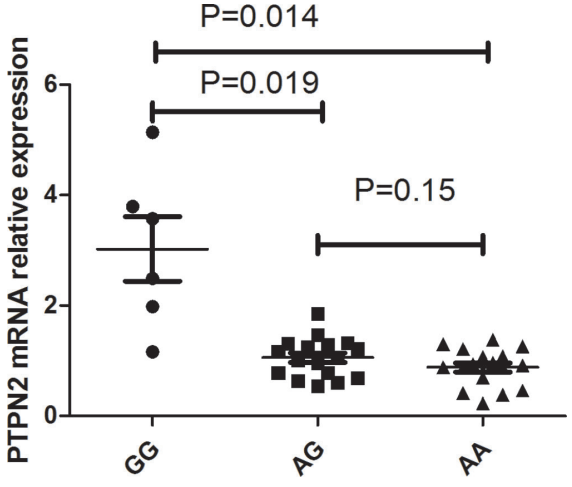

Figure 1 The influence of rs7234029 on the expression of PTPN2 mRNA. The expression of PTPN2 mRNA in PBMCs treated with anti-CD3/28 antibodies. PBMCs were obtained from healthy individuals with diverse genotypes of rs7234029 (GG=6, AG=17, AA=17). Data show the mean ±SD. PBMCs, peripheral blood mononuclear cells; PTP, protein tyrosine phosphatase.

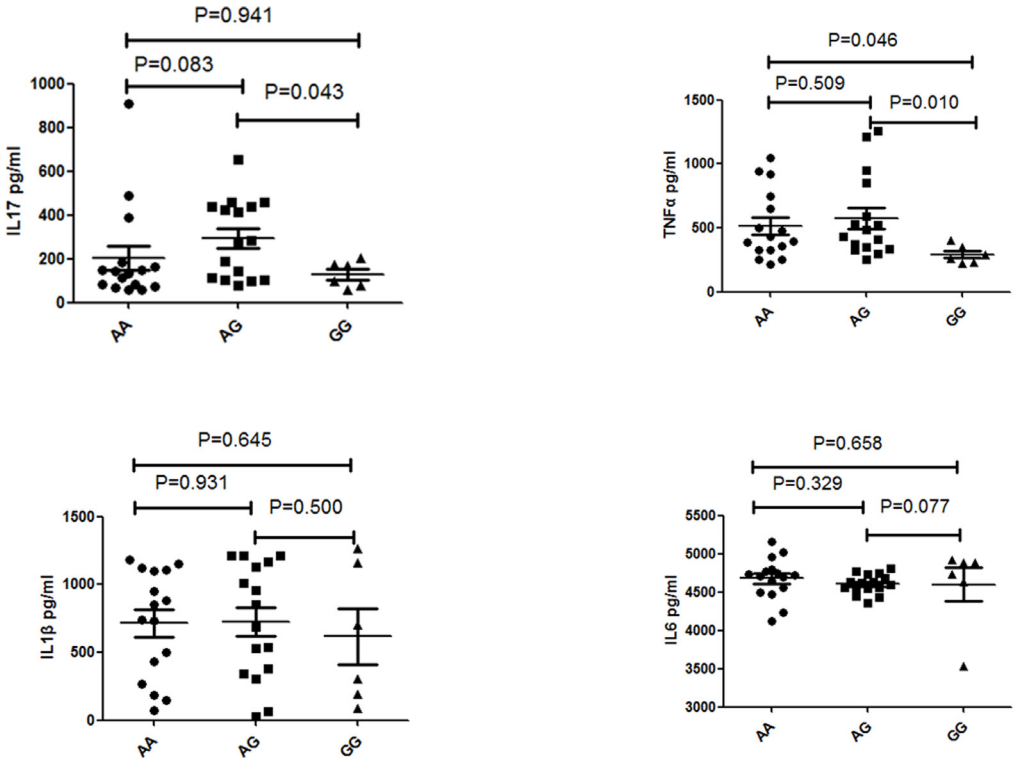

Figure 2 The influence of rs7234029 on the cytokine production. The production of IL-17, TNF-α, IL-1β, IL-6 in PBMCs treated with anti-CD3/28 antibodies from normal controls carrying different genotypes of rs7234029 (GG=6, AG=16, AA=16). IL, interleukin; PBMCs, peripheral blood mononuclear cell; TNF-α, tumour necrosis factor-alpha.

of IL-17 (p=0.018) and TNF-α (p=0.034) was detected in GG genotype as compared with AA genotype individuals. No significant difference could be detected concerning IL-1β or IL-6 production by stimulated PBMCs in different genotype individuals (figure 2).

DISCUSSION

We investigated the association of 11 SNPs of PTPN2 and CD122 with BD in a Chinese Han population. The results revealed that the AG genotype of PTPN2 rs7234029 conferred a disease risk for BD, whereas the GG genotype of this locus had a protective effect on BD. Functional studies showed GG genotype of rs7234029 carriers had a higher PNPT2 mRNA expression level than those carrying the AA or AG genotype. Additionally, the secretion of TNF-α by PBMCs was significantly lower in rs7234029 GG genotype cases as compared with the AA or AG genotype cases. Secretion of IL-17 by PBMCs was significantly lower in individuals with the GG genotype of rs7234029 than those with the AG genotype. The protective GG genotype is thus associated with an intrinsic lower capacity to respond with proinflammatory cytokines following an antigenic stimulus.

PTPN2 is a non-receptor tyrosine-protein phosphatase which is a member of the PTP family. It encodes the T-cell PTP (TC-PTP) that shows a ubiquitously high expression in haematopoietic cells. PTPN2 is involved in the IL-2 receptor-activated JAK-STAT signalling pathways as a negative regulator, whereby PTPN2 negatively regulates T-cell activation by inhibiting IL-2 receptor signalling.[21 22] It has been shown that PTPN2 plays a crucial role in the pathogenesis of chronic intestinal inflammation and dysfunction of PTPN2 was considered to play an important role in the development of autoimmunity.[23] This latter study showed that loss of PTPN2 causes increased levels of Th1 and Th17 cells but reduced levels of regulatory T cells (T_{regs}), and that the CD-associated PTPN2 variant led to increased Th1-associated and Th17-associated gene expression in intestinal samples from patients with inflammatory bowel disease (IBD).[23] Others have demonstrated that signalling of CD4 +T cells via the IL-2Rβ chain is affected by variants of PTPN2 rs1893217.[10]

GWAS studies have identified PTPN2 as a susceptibility gene for IBD. The Welcome Trust Case Control Consortium GWAS studies demonstrated an associations between Crohn's disease and variants in PTPN2 rs7234029 in a British population ($p=3.71\times10^{-7}$).[24] A Caucasian study also showed that PTPN2 SNP rs7234029 (G allele) was associated with CD ($p=1.30\times10^{-3}$, OR 1.35, 95% CI 1.13 to 1.62) and provided evidence showing that rs7234029 modulates the binding sites of transcription factors involved in inflammation.[16] In another study, rs7234029 was also observed to be associated with Crohn's disease.[25] Similar outcomes were recently found in European American individuals with juvenile idiopathic arthritis (JIA) (rs7234029, $p=7.19\times10^{-11}$, OR=1.59; rs1893217, $p=3.48\times10^{-8}$, OR=1.52; rs2542151, $p=3.05\times10^{-7}$, OR=1.45).[26] Our results confirm the previous study in which PTPN2 rs7234029 was shown to be associated with BD. We were not able to reproduce earlier findings from a Chinese group who found that PTPN2 SNP rs1893217 showed a weak association with the risk of BD in a Han Chinese population ($p_c=0.04$).[12] They also tested PTPN2 rs7234029 but did not find a significant association in their BD group. The discrepancy may be caused by the different clinical features of the patients included in the two studies. In their study,[12] the patients were all recruited at the Department of Rheumatology and Clinical Immunology and only 28.5% of patients exhibited ocular manifestations, whereas all the enrolled patients in our study had uveitis. There was no stratified analysis according to the main clinical features in the latter study, which means that it is not possible to determine the relationship between rs1893217

and ocular BD in that population. However, all the enrolled patients had uveitis in our study. Our results showed that a PTPN2-rs7234029 polymorphism is associated with ocular BD but is not associated with hypopyon. This might be due to the fact that only 223 patients (24.6%) had hypopyon in our study and that the sample size might have been too small to obtain statistical significance. Uveitis in patients with BD usually presents with a transient hypopyon in 25% of cases in our clinical practice. Further studies are needed to investigate the association between different uveitis disease manifestations and the tested gene polymorphisms.

In our study, we found that PTPN2 SNP rs7234029 was significantly associated with BD (AG genotype, $p=3.33\times10^{-8}$, $p_c=1.43\times10^{-6}$, OR=1.549; AA genotype, $p=1.10\times10^{-3}$, $p_c=4.73\times10^{-2}$, OR=0.772; for GG genotype, $p=1.94\times10^{-5}$, $p_c=8.34\times10^{-4}$, OR=0.466). Further analysis showed that rs7234029 was significantly associated with BD in men but not in women, although the trend was the same. Rs7234029 was significantly associated in patients with BD with genital ulcers, skin lesions and a positive pathergy test. This might seem discrepant since genital lesions for instance were observed more frequently in women than in men (64.2% vs 54.8%, p=0.033), whereas the frequency of skin lesions was similar between men and women (77.1% vs 70.2%, p>0.05) in our study. All the patients in our study had uveitis and men usually showed a more severe sight-threatening uveitis than women.[27] This combined with the fact that men were often referred to us preferentially might have resulted in a male patient bias in our study. The six SNPs of CD122 were not associated with BD in our study, although previous studies did show an association with autoimmune diseases. The disparity between the results may be due to the different genetic backgrounds for BD and the other autoimmune diseases.

PTPN2 variants have been demonstrated to be associated with an impairment of IL-2R signalling in CD4 +T cells,[10] and the rs7234029 variant may affect activation of proinflammatory transcription factors.[10] The JAK1/STAT3 signalling pathway is activated in BD, leading to an elevated expression of Th1/Th17-type cytokines.[11] Th1/Th17-type cytokines have been implicated in the pathogenesis of many autoimmune diseases. We performed functional assays to investigate whether SNP rs7234029 influenced gene expression and the results showed that the PTPN2 mRNA expression in rs7234029 AA and AG carriers was significantly decreased compared with those carrying the GG genotype. Further investigations found that a decreased secretion of IL-17 and TNF-α was seen by PBMCs from GG carriers. This suggests that more PTPN2 inhibits these cytokines. This is not completely supported by our data, since AG individuals produced significantly more IL-17 than GG individuals while AA individuals did not. We do not yet have an exact answer for this discrepancy. Phenotype–genotype interactions may be involved in the JAK1/STAT3 signalling pathway, and further research needs to be done to elucidate the exact mechanisms involved. Our results are in accordance with a recent study which showed that inflammation-induced interferon (IFN)-γ, TNF-α, IL-17 and IL-6 serum levels were elevated in mice harbouring PTPN2-deficient T cells, and with data showing that a loss-of-function PTPN2 variant led to increased serum levels of IFN-γ and IL-17 in patients with BD.[23]

Our study suffers from several limitations. First, our participants were all Chinese Han and the patients with ocular BD all came from a Department of Ophthalmology. Further studies should also enrol patients with BD without uveitis coming from other clinical departments and compare the PTPN2 disease association in patients with BD expressing various clinical features of the disease. Second, 83% of our patients with BD were men, while 56% of controls were men. Stratified analysis according to gender showed that rs7234029 was significantly associated with BD in men but not in women. This might be due to the fact that the sample size of our female patient group was too low. Our research was limited to uveitis in BD and should not be extrapolated to other uveitis entities. Further research is also needed to investigate the exact role of the IL-2 receptor-activated JAK-STAT signalling pathways in BD uveitis.

In conclusion, our results revealed that PTPN2 rs7234029 polymorphisms were associated with BD and were strongly influenced by gender. In addition, we provide evidence which suggests that the association with PTPN2 variants may be explained via a regulation of PTPN2 mRNA expression and cytokine secretion.

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Patient consent Obtained.

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