• Investigation •

A single nucleotide polymorphism in the *IL1RL1* gene is associated with Behcet's disease in a Chinese Han population

Xin-Shu Liu[1], Zi-Yan Wu[2], Si Chen[3], Chan Zhao[1], Fei Gao[1], Ming-Hang Pei[1], Shan-Shan Jia[1], Yong-Zhe Li[4], Pei-Zeng Yang[5], Mei-Fen Zhang[1]

[1]Department of Ophthalmology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100730, China
[2]Department of Rheumatology and Clinical Immunology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Key Laboratory of Rheumatology and Clinical Immunology, Ministry of Education, Beijing 100730, China
[3]Department of Clinical Laboratory, Beijing Anzhen Hospital, Capital Medical University, Beijing 100029, China
[4]Department of Clinical Laboratory, Peking Union Medical College Hospital, Peking Union Medical College and Chinese Academy of Medical Sciences, Beijing 100730, China
[5]The First Affiliated Hospital of Chongqing Medical University, Chongqing Key Laboratory of Ophthalmology and Chongqing Eye Institute, Chongqing 400016, China
Correspondence to: Mei-Fen Zhang. Department of Ophthalmology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100730, China. meifen_zhang@hotmail.com
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Abstract

● **AIM:** To explore the association of single nucleotide polymorphisms (SNPs) in the *IL33/IL1RL1* gene region with the susceptibility to Behcet's disease (BD) in a Chinese Han population.

● **METHODS:** A total of eight SNPs in the candidate gene region (rs11792633, rs7025417, rs10975519 and rs1048274 in *IL33*; rs2310220, rs12712142, rs13424006 and rs3821204 in *IL1RL1*) were genotyped in783 BD patients and 701 healthy controls by the Sequenom Mass Array iPLEX platform.

● **RESULTS:** A statistically significant association was observed between *IL1RL1* rs12712142 and BD patients. The frequency of *IL1RL1* rs12712142 variant allele A was significantly lower in BD patients than that in controls (OR=0.8, 95%CI: 0.69-0.94, *Pc*=0.039); the genotype distribution (*Pc*=0.043) and additive and dominant genetic model analyses (OR=0.8, 95%CI: 0.69-0.94, *Pc*=0.040 and OR=0.72, 95%CI: 0.58-0.88, *Pc*=0.011) also indicated a strong association between rs12712142 and BD patients.

● **CONCLUSION:** This is the first study to reveal the association between *IL1RL1* rs12712142 variant allele A and the decreased risk of BD in the Chinese Han population, indicating a protective role of *IL1RL1* in the pathogenesis of BD.

● **KEYWORDS:** Behcet's disease; single nucleotide polymorphism; Chinese Han population; IL33; *IL1RL1*

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INTRODUCTION

Behcet's disease (BD) is defined as a kind of chronic recurrent systemic vasculitis; its most common manifestations are aphthous ulceration, skin lesions, genital ulcers and ocular inflammation[1]. BD distributes worldwide and is especially prevalent in Mediterranean countries, the Middle East and Southeast Asia[2]. The etiology of BD remains poorly understood. Considerable evidence indicates that the immunopathogenesis of BD is critical for clarifying the initiation and progression of this disease. Cytokines involved in Th1 and Th17, such as IFN-γ, IL-12, and IL-17, were exhibited according to remissions and exacerbations of inflammation in BD patients[3]. Currently, it is supposed that the effect of environmental risk factors on genetically susceptible individuals may be a trigger of this pathological process. Polymorphisms in the *IL10*, *IL12*, *IL23* and *IL37* genes have been discovered to be associated with the disease[4-5].

IL-33 belongs to the IL1 cytokine family. After binding with ST2, the complex activates the downstream NF-κB and MAPK

pathways[6]. Recent studies indicate the degree of participation of the IL-33/ST2 axis in various diseases, especially in immune and inflammatory disorders, such as rheumatoid arthritis (RA)[7], giant cell arteritis (GCA)[8], Grave's disease (GD)[9], inflammatory bowel disease (IBD)[10] and systemic sclerosis (SSc)[11]. Correspondingly, genetic polymorphism studies have identified IL33 and/or IL1RL1 loci as susceptibility genes in RA[12], GCA[13], autoimmune thyroid diseases (AITD)[14] and IBD[15]. Several studies have displayed a significant change in IL-33 and/or ST2 levels in the peripheral circulation and/or at inflammatory sites[16], but few studies have been conducted to explore the genetic predisposition of IL33/IL1RL1 to BD.

In this study, we hypothesized that IL33/IL1RL1 gene polymorphisms may be associated with genetic susceptibility to BD in the Chinese Han population.

SUBJECTS AND METHODS

Ethical Approval This study was approved by the Institutional Review Board of the Peking Union Medical College Hospital and the First Affiliated Hospital of Chongqing Medical University and adhered to the tenets of the Declaration of Helsinki. All participants signed written informed consent forms.

Subjects A total of 783 BD patients and 701 ethnically matched healthy controls who visited Peking Union Medical College Hospital and the First Affiliated Hospital of Chongqing Medical University between October 2011 and October 2015, were consecutively recruited in this case-control study. All subjects were Han nationality Chinese and were not related to one another. Patients who fulfilled the criteria for the diagnosis of BD[17] were enrolled as cases, while those concomitant with other autoimmune or inflammatory diseases, such as systemic lupus erythematosus (SLE) and RA, were excluded. Healthy controls without any autoimmune or inflammatory disorders were included during their physical examination.

Selection of Single Nucleotide Polymorphisms Four single nucleotide polymorphisms (SNPs; rs11792633, rs7025417, rs10975519, and rs1048274) in the IL33 gene, which had previously shown associations with BD[18] or other autoimmune diseases[13,19], and four tag SNPs (rs2310220, rs12712142, rs13424006, and rs3821204) in the IL1RL1 gene were selected for subsequent analyses.

Tag SNPs in the IL1RL1 gene (chr2:102294394-102334929) were identified by Haploview 4.2 software from HapMap CHB data (HapMap Data Rel 27 PhaseII+III, Feb09), with a pairwise linkage disequilibrium (LD) of $r^2 \geq 0.8$ and minor allele frequency (MAF) values ≥0.1.

Genotyping Genomic DNA of the participants was extracted from EDTA peripheral venous blood by using a DNA isolation kit (Tiangen, Beijing, China) following the manufacturer's instructions. The SNPs were genotyped by the Sequenom MassARRAY system (San Diego, CA, USA) according to standard procedures. Specifically, first of all, designed the primers for multiplex polymerase chain reaction (PCR) and locus-specific single-base extension with the MassARRAY Assay Design 4.0 software; second, carried out the PCRs, the products were used for locus-specific single-base extension reactions; third, desalted and transferred the final products to a 384-element Spectro CHIP array for allele detection, which was performed by matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF MS). Finally, the resultant mass spectrometry data was analyzed by using MassARRAY Typer 4.0 software.

Statistical Analyses Statistical analyses were mainly accomplished by PLINKv1.07 software (http://pngu.mgh.harvard.edu/purcell/plink/)[20]. Hardy-Weinberg equilibrium (HWE) in the control population was assessed by the Chi-square (χ^2) test for each SNP. Any SNP with significant deviation from HWE was excluded from subsequent analyses. The basic analyses of allele frequencies and genotype distributions were performed by the χ^2 test. For additional genotype analyses under additive, dominant and recessive model, the Logistic regression test was used. Statistical power was calculated by a freely available power and sample size calculation program (PSv.3.1.2, http://biostat.mc.vanderbilt.edu/wiki/Main/PowerSampleSize)[21]. Haplotype analyses were performed by Haploviewv4.2 software (http://www.broadinstitute.org/haploview)[22]. *P* values less than 0.05 were considered statistically significant. *P* values for multiple comparisons were corrected by the Bonferroni method (*Pc*=P×n, n was the number of tested SNPs).

RESULTS

Clinical Features of the Participants The baseline demographic and clinical features of the participants were displayed in Table 1. A total of 783 BD patients (63.6% male) and 701 (55.5% male) ethnically matched healthy controls were recruited in the present study. The mean ages of the patients and controls were 35.8±11.2 and 38.4±10.2 years old, respectively.

Association Analyses of the Single Nucleotide Polymorphisms *IL1RL1* rs3821204 was excluded from further analyses because of deviation from HWE in the control group ($P<0.05$). The remaining seven SNPs were all in HWE. The average genotyping rate of the seven SNPs was over 98%. The sample size provided a statistical power of 80.1% (α=0.05) for detecting the association between rs12712142 and BD based on the odds ratio (OR) and MAF value of the present study.

The allele frequencies and genotype distributions of SNPs in the *IL33* and *IL1RL1* gene regions in BD patients and controls are presented in Table 2. The frequency of *IL1RL1* rs12712142

Table 1 Demographic and clinical data of BD patients and controls

Category	BD patients	Controls
Number	783	701
Male percentage	63.6%	55.5%
Averge age (y)	35.8±11.2	38.4±10.2
Clinical symptoms, *n* (%)		
Oral aphthous ulcer	773 (98.7)	-
Genital ulcer	585 (74.7)	-
Skin manifestation	464 (59.3)	-
Ocular manifestation	398 (50.8)	-

BD: Behcet's disease.

variant allele A was significantly lower in BD patients than that in controls (29.4% *vs* 34.1%, OR=0.8, 95%CI: 0.69-0.94, *Pc*=0.039; Table 2). The genotype distribution of rs12712142 was also significantly different between patients and controls (*Pc*=0.043; Table 2). Further Logistic regression analyses under additive, dominant and recessive model are displayed in Table 3, and rs12712142 was associated with BD based on the additive and dominant model (OR=0.8, 95%CI: 0.69-0.94, *Pc*=0.040 and OR=0.72, 95%CI: 0.58-0.88, *Pc*=0.011; Table 3). However, no significant difference was observed in allele frequencies or genotype distributions in the *IL33* SNPs (rs11792633, rs7025417, rs10975519 and rs1048274) and other *IL1RL1* SNPs (rs2310220 and rs13424006) between BD patients and controls (all *Pc*>0.05; Table 2), and further Logistic regression analyses based on additive, dominant and recessive model revealed no significant associations of the above SNPs with BD patients either (all *Pc*>0.05; Table 3).

Haplotype Analyses of the IL33 Single Nucleotide Polymorphisms The haplotype distributions of SNPs in *IL33* were analyzed by Haploview software. Pairwise LD was observed for rs11792633, rs10975519, and rs1048274 (r^2>0.8) in our data, which is shown in Figure 1. However, none of the distributions of the three haplotypes (TTA, CCG, TCG) formed by the above SNPs indicated any significant difference between BD patients and controls (all *Pc*>0.05; Table 4).

DISCUSSION

To the best of our knowledge, this is the first hospital-based case-control study conducted in China describing the relationship between *IL33/IL1RL1* gene polymorphisms and BD. In our study, the results demonstrated that the *IL1RL1* rs12712142 polymorphism was associated with BD in a Chinese Han population for the first time. In our cohort, the frequency of variant allele A of *IL1RL1* rs12712142 in BD patients was significantly lower than that in healthy controls. Accordingly, the basic genotype distribution and analyses under additive and dominant models also showed significant differences between BD patients and controls. This result suggested that variant allele A of *IL1RL1* rs12712142 seemed to be protective against BD.

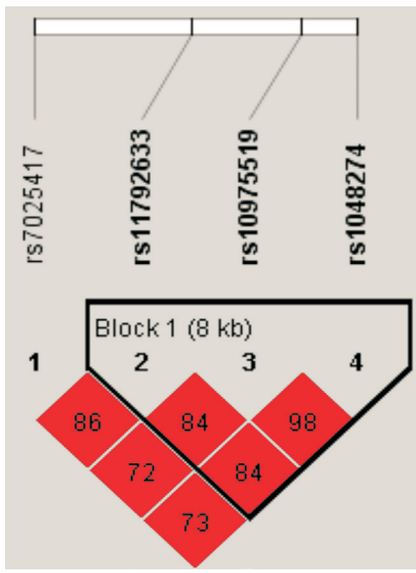

Figure 1 LD analyses of the SNPs in the *IL33* gene region The LD plots were generated by Haploview software v4.2 using our data. The numbers (divided by 100) in the small squares represent r^2 value and range from 0 to 1. The three SNPs (rs11792633, rs10975519, and rs1048274) in *IL33* reside in one LD block.

IL-33, encoded by the *IL33* gene, is expressed constitutively in endothelial and epithelial cells nucleus[23]. ST2, encoded by the *IL1RL1* gene, was identified as a receptor of IL-33[24]. ST2 has two isoforms: ST2 (a membrane-bound form) is expressed in immune cells such as dendritic cells, Th1 cells and Th2 cells, binds to IL-33 and then activates the NF-κB pathway, while sST2 (a soluble form) acts as a decoy receptor[24-25]. Emerging evidence suggests that the IL-33/ST2 pathway plays a vital role in autoimmune and inflammatory diseases. Increased levels of IL-33 were observed in the synovial fluid of RA patients[7]. Animal models showed that at the onset of collagen-induced arthritis, disease severity could be attenuated by administration of anti-ST2 antibody; at the meanwhile, joint destruction could be reduced and a marked decrease in IFN-γ production was observed[26]. Two of the previous studies showed elevated serum IL-33 and sST2 in BD patients compared to those in healthy controls; moreover, serum IL-33 levels were higher in active BD patients than those in inactive BD patients[27-28]. SNPs (rs1342326, rs7044343, and rs11792633) in the *IL33* gene region were also shown to be associated with BD[18,29].

In our cohort, the frequency of variant allele A of *IL1RL1* rs12712142 in BD patients was significantly lower than that in healthy controls. We assumed that the protective role of variant allele A of *IL1RL1* rs12712142 was mediated by decreasing level of ST2 and/or increasing level of sST2. A recent study supports our assumption that genetic polymorphisms may affect the expression of ST2. The study firstly showed a lower frequency of the *IL18R1* rs12987977 variant allele G in BD patients, then revealed a downregulation of *IL1RL1* in carriers

Table 2 Allele frequencies and genotype distributions of the *IL33* and *IL1RL1* gene markers in BD patients and controls

Gene	SNP	Group	Allele, *n* (%)		OR (95%CI)	*P*	*Pc*	Genotype, *n* (%)			χ^2	*P*	*Pc*
IL33			C	T				CC	CT	TT			
	rs11792633	Cases	692 (44.9)	850 (55.1)	1.09 (0.94-1.26)	0.249	1.000	141 (18.3)	410 (53.2)	220 (28.5)	2.03	0.363	1.000
		Controls	597 (42.8)	799 (57.2)				122 (17.5)	353 (50.6)	223 (31.9)			
			C	T				CC	CT	TT			
	rs7025417	Cases	641 (42.9)	855 (57.1)	1.09 (0.94-1.26)	0.283	1.000	133 (17.8)	375 (50.1)	240 (32.1)	1.26	0.532	1.000
		Controls	564 (40.9)	816 (59.1)				109 (15.8)	346 (50.1)	235 (34.1)			
			C	T				CC	CT	TT			
	rs10975519	Cases	737 (47.8)	805 (52.2)	1.07 (0.92-1.24)	0.368	1.000	159 (20.6)	419 (54.3)	193 (25.0)	1.77	0.412	1.000
		Controls	645 (46.1)	753 (53.9)				142 (20.3)	361 (51.6)	196 (28.0)			
			G	A				GG	GA	AA			
	rs1048274	Cases	734 (47.7)	804 (52.3)	1.06 (0.92-1.23)	0.434	1.000	159 (20.7)	416 (54.1)	194 (25.2)	1.41	0.494	1.000
		Controls	647 (46.3)	751 (53.7)				143 (20.5)	361 (51.6)	195 (27.9)			
IL1RL1			A	G				AA	AG	GG			
	rs2310220	Cases	677 (44.0)	861 (56.0)	0.90 (0.78-1.04)	0.155	1.000	152 (19.8)	373 (48.5)	244 (31.7)	1.99	0.369	1.000
		Controls	651 (46.6)	745 (53.4)				154 (22.1)	343 (49.1)	201 (28.8)			
			A	C				AA	AC	CC			
	rs12712142	Cases	451 (29.4)	1085 (70.6)	0.80 (0.69-0.94)	5.6×10^{-3}	0.039	73 (9.5)	305 (39.7)	390 (50.8)	10.16	6.2×10^{-3}	0.043
		Controls	477 (34.1)	921 (65.9)				75 (10.7)	327 (46.8)	297 (42.5)			
			C	T				CC	CT	TT			
	rs13424006	Cases	222 (14.5)	1314 (85.5)	1.22 (0.98-1.51)	0.068	0.476	11 (1.4)	200 (26.0)	557 (72.5)	3.49	0.169	1.000
		Controls	170 (12.2)	1228 (87.8)				7 (1.0)	156 (22.3)	536 (76.7)			

IL: Interleukin; BD: Behcet's disease; SNP: Single nucleotide polymorphism; OR: Odds ratio; CI: Confidence interval; *Pc*: *P* corrected by Bonferroni method; χ^2: Chi-square test.

Table 3 Analyses of the seven SNPs based on additive, dominant, and recessive genetic models

Gene	SNP	Additive model		Dominant model		Recessive model	
		OR (95%CI)	*Pc*	OR (95%CI)	*Pc*	OR (95%CI)	*Pc*
IL33	rs11792633	1.10 (0.94-1.27)	1.000	1.18 (0.94-1.47)	1.000	1.06 (0.81-1.38)	1.000
	rs7025417	1.09 (0.94-1.26)	1.000	1.09 (0.88-1.36)	1.000	1.15 (0.87-1.52)	1.000
	rs10975519	1.07 (0.92-1.25)	1.000	1.17 (0.93-1.47)	1.000	1.02 (0.79-1.31)	1.000
	rs1048274	1.06 (0.92-1.24)	1.000	1.15 (0.91-1.45)	1.000	1.01 (0.79-1.31)	1.000
IL1RL1	rs2310220	0.90 (0.78-1.04)	1.000	0.87 (0.70-1.09)	1.000	0.87 (0.68-1.12)	1.000
	rs12712142	0.80 (0.69-0.94)	0.040	0.72 (0.58-0.88)	0.011	0.87 (0.62-1.23)	1.000
	rs13424006	1.23 (0.99-1.54)	0.434	1.25 (0.98-1.58)	0.476	1.44 (0.55-3.73)	1.000

SNP: Single nucleotide polymorphism; OR: Odds ratio; CI: Confidence interval; *Pc*: *P* corrected by Bonferroni method.

Table 4 Haplotype analyses of *IL33* SNPs between BD patients and controls

Gene	Haplotype			Frequency (%)			χ^2	*Pc*
	rs11792633	rs10975519	rs1048274	Total	Cases	Controls		
IL33	T	T	A	52.3	51.5	53.2	0.81	1.000
	C	C	G	43.4	44.4	42.2	1.37	1.000
	T	C	G	3.6	3.3	3.9	0.64	1.000

SNP: Single nucleotide polymorphism; BD: Behcet's disease; χ^2: Chi-square test; *Pc*: *P* corrected by Bonferroni method.

of the protective homozygous rs12987977/GG genotype compared with the TT genotype in functional experiments[30]. Moreover, Ho *et al*[31] identified that genetic factors determined 45% sST2 production variation and that genetic variation in *IL1RL1* could lead to increased level of sST2. Higher decoy sST2 may be protective against BD, and increased level of serum sST2 in BD patients[28] may be the result of a compensatory protective reaction of the human body.

None of the SNPs in the *IL33* region showed a significant association with BD in our study at the allelic, genotypic or haplotypic levels. Although the variant allele T of rs11792633, which has been reported to be protective for BD in a Turkish population[18], was also lower in BD patients than in the controls from our data, the difference was not statistically significant. This inconsistency may be attributed to the genetic heterogeneity of different ethnic groups.

Despite the relatively large sample size in the current study, we only assessed four SNPs in *IL33* that have been previously reported to be associated with BD or other autoimmune diseases and four tag SNPs in *IL1RL1* that may not completely represent the whole genetic region. We did not examine the function of *IL1RL1* rs12712142 *in vivo* or *in vitro*, either. More genetic and mechanistic studies are warranted to determine the role of *IL33/IL1RL1* in BD pathogenesis.

In conclusion, this study clearly demonstrates the association of the *IL1RL1* polymorphism with BD in a Chinese Han population. The A variant in *IL1RL1* rs12712142 is correlated with a decreased risk of BD, which may suggest a protective role of the *IL1RL1* gene in the pathogenesis of BD.

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