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Interleukin-10 gene polymorphisms are associated with Behcet's disease but not with Vogt-Koyanagi-Harada syndrome in the Chinese Han population

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Purpose: This study aimed to investigate the association of interleukin (IL)-10 gene polymorphisms with Behcet's disease (BD) and Vogt–Koyanagi–Harada (VKH) syndrome in the Chinese Han population.
Methods: A two-stage association study was performed on 718 BD patients, 300 VKH patients, and 1,753 controls. Genotyping of the IL-10 gene was performed for six single nucleotide polymorphisms (SNPs), including rs1800871, rs1800872, rs1800896, rs3021094, rs3790622, and rs1554286 using PCR-restricted fragment length polymorphism or TaqMan SNP assays. Real-time PCR was performed to test the IL-10 mRNA expression of the associated polymorphisms.
Results: The first-stage result showed significantly increased frequencies of the rs1800871 T allele, rs1800872 A allele, and rs1554286 T allele in BD patients compared with controls ($P_{corrected}$ (P_{corr}) = 1.82×10^{-5}, OR = 1.837; P_{corr} = 6.1×10^{-5}, OR = 1.780; P_{corr} = 3.15×10^{-5}, OR = 1.794, respectively). There was no association of the tested six SNPs with VKH syndrome. A second-stage study was therefore performed in BD patients to validate the result of the first stage, showing a significantly increased frequency of the rs1800871 T allele (Second stage, P_{corr} = 5.59×10^{-5}, OR = 1.493; Combined data, P_{corr} = 3.65×10^{-11}, OR = 1.632). Compared to the controls, an increased frequency of the rs1800871 T allele was observed in BD patients with extraocular findings, including genital ulcers, skin lesions, and a positive pathergy test. No difference was found among the mRNA expressions of IL-10 in the peripheral blood mononuclear cells (PBMCs) of controls with different genotypes of rs1800871 after stimulation of lipopolysaccharide (LPS) or anti-CD3/CD28 antibodies.
Conclusions: The findings showed that IL-10 is a risk gene for BD but not for VKH syndrome.

Behcet's disease (BD) and Vogt–Koyanagi–Harada (VKH) syndrome have been proven as two of the most common and severe sight-threatening uveitis entities in China [1,2]. BD is a chronic, multisystem inflammatory disorder characterized by recurrent oral ulcerations, genital ulcerations, recurrent uveitis, and multiple skin lesions [3]. Similar to BD, VKH syndrome is a chronic, multisystem autoimmune disease characterized by bilateral granulomatous panuveitispoliosis, vitiligo, alopecia, and central nervous system and auditory signs [4]. So far, the prevalence of BD or VKH syndrome in China is unclear. Usually, the syndrome occurs more frequently in Turks, Chinese Han, and Japanese individuals, and it is uncommon in Caucasians. Turkey has the highest prevalence rate of BD (80–420 cases per 100,000 persons), whereas the disease is rare in Caucasian (0.27–7.5 cases per 100,000 persons) [5,6]. Although the pathogenesis of the two diseases remains unclear, it is currently thought a genetic predisposition coupled with a triggering event may play a pivotal role in its development [7,8]. Genetic predisposition is an important element, as evidenced by a strong association with the human leukocyte antigen (HLA) system [9-11]. More recently, studies have reported the association of many non-HLA genes with the two diseases, such as endoplasmic reticulum aminopeptidase 1 (ERAP1), killer cell lectin-like receptor subfamily C, member 4 (KLRC4), chemokine receptor 1 (CCR1), signal transducer and activator of transcription-4 (STAT4) [12-14], interleukin (IL)-2, IL-4, IL23R [15], transforming growth factor (TGF)-beta [16], tumor necrosis factor (TNF)-alpha [17], tumor necrosis factor alpha-induced pro3 (TNFAIP3) [18], and the small ubiquitin-like modifier 4 (SUMO4) [19] gene for BD, as well as IL23R-C1orf141 [20], ZNF365-ADO-EGR2 [20], CTLA-4, and the IL-17 gene for VKH syndrome [21-23]. As many of the proteins involved in the immune response appear to be polymorphic, it can be expected many more gene polymorphisms related to the immune system and its mediators will

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Table 1. Summary characteristic of Behcet's patients and normal control subjects in this study.

Analysis	Case			Control		
	Samples size	Mean age (S.D)	Male/Female	Samples size	Mean age (S.D)	Male/Female
StageI	300	33.52±8.73	257/43	350	36.57±10.89	217/133
Stage II	418	33.66±8.83	362/56	1403	40.44±11.14	789/614
Combined	718	33.60±8.78	619/99	1753	39.66±11.19	1006/747

SD, standard deviation

be identified in the predisposition of inflammatory diseases, such as BD and VKH syndrome.

IL-10, an anti-inflammatory cytokine, is produced by T lymphocytes (mainly Th2 subsets), B lymphocytes, NK cells, mast cells, eosinophils, dendritic cells, monocytes, and macrophages [24]. IL-10 was demonstrated to be an important cytokine-suppressing autoimmunity and inflammatory response [25-28]. IL-10 inhibits antigen-presenting cells by downregulating the cell surface expression of HLA molecules [29]. Moreover, it inhibits T cell function by suppressing the expressions of proinflammatory cytokines, such as TNFα, IL-1, IL-6, IL-8, and IL-12 [30,31]. In addition to these inhibitory actions, IL-10 inhibits naïve T cell differentiation into T helper 17 cells (Th17), as well as promotes the differentiation of CD4(+)CD25(-) T cells into regulatory T cells (Tregs) [32]. Gene polymorphisms of IL10 were associated with several immune-related disorders, including Crohn's disease (CD) [33], type 2 diabetes [34], systemic sclerosis [35], Sjogren's syndrome [36], and Graves' disease [37]. Several studies from Iran, Japan, and Turkey have also identified IL-10 as a risk gene for BD [38-40]. These studies have not yet been confirmed in China and we therefore performed a two-stage association study to examine whether IL-10 polymorphisms might also increase the susceptibility of BD and VKH syndrome in our population. We found the IL-10 gene was associated with BD in Chinese Han and in view of the independent confirmation by various independent groups, one may conclude IL-10 definitely plays a role in the pathogenesis of this disease. On the other hand, IL-10 does not seem to be a susceptibility gene for VKH syndrome.

METHODS

Subjects: The first-stage study cohort comprised 300 BD patients, 300 VKH syndrome patients, and 350 normal controls. As well, 418 BD patients and 1,403 normal controls were enrolled in the second stage (Table 1). All subjects were Chinese Han. The patients were continuously diagnosed at the Zhongshan Ophthalmic Center, Sun Yat-sen University, or the First Affiliated Hospital of Chongqing Medical University, China between April 2005 and November 2012. The diagnosis of BD was strictly based on the criteria of the International Study Group of BD [41]. The clinical characteristics of BD cases were assessed at the time of diagnosis and are summarized in Table 2. The normal controls consisted of spouses of the patients or accompanying people who had no genetic connection to the patients. This study was approved by the ethics committee of the First Affiliated Hospital of Chongqing Medical University, China. Every investigated subject provided informed consent before the collection of blood. The study was in agreement with the ethical principles described in the Declaration of Helsinki.

DNA extraction: Blood samples were collected in EDTA tubes and stored at −80 °C until use. Genomic DNA was extracted from the peripheral blood using the QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions.

SNP genotyping: The genotypes of rs1800871, rs1800872, rs1800896, rs3021094, rs3790622 were examined by

Table 2. The number and ratio of Behcet's patients with clinical features.

Clinical features	BD patients	
	Total (n=718)	Percentage (100)%
Female	99	13.8
Male	619	86.2
Age at onset (years [SD])	33.60 (8.78)	
Uveitis	718	100
Genital ulcers	413	57.5
Skin lesions	538	74.9
Hypopyon	188	26.2
Arthritis	99	15.2
Prick tests	164	22.8

SD, standard deviation

PCR-restricted fragment length polymorphism (PCR-RFLP). The target DNA sequence was amplified by the PCR method with proper primers for each single nucleotide polymorphism (SNP; Table 3). Each PCR was performed in a 7-µl reaction mixture containing 3.5 µl Premix Taq (Promega, Madison), 2 µl water, 0.5 pmol primers, and 0.2 µg genomic DNA for amplification of the DNA. The PCR conditions were as follows: initial denaturation at 95 °C for 5 min followed by 40 cycles of denaturation at 95 °C for 30 s, annealing at different temperatures (60 °C for rs1800871, 59 °C for rs1800872, 56 °C for rs1800896, 55 °C for rs3021094, and 60 °C for rs3790622) for 30 s, extension at 72 °C for 30 s, and a final extension at 72 °C for 5 min. PCR products were respectively digested with 2.5 U of EcoRV, Csp6I, BslI, BglI, and TasI (Fermentas, MBI) for 10–16 h. After digestion, PCR products were visualized on agarose gels at an appropriate concentration. The frequency of a given genotype was estimated by direct counting. In addition, rs1554286 was genotyped by the TaqMan genotyping assay (Applied Biosystems, Foster City, CA) on the 7500 real-time PCR system (Applied Biosystems), according to the supplier manual. Details of the TaqMan assay are listed in Table 3. A genotype analysis was performed using TaqMan® Genotyper Software. To confirm the accuracy of the two methods used, we randomly selected 20% of the samples to undergo direct sequencing to validate the results of genotyping by PCR-RFLP and TaqMan (Beijing Liuhe BGI Inc.).

Cell isolation and culture: The healthy study subjects used for the IL-10 gene expression assays were the partners of the patients or an accompanying person, totaling 18 individuals. Peripheral blood samples were obtained using vacuum tubes containing EDTA. Before the isolation of peripheral blood mononuclear cells (PBMCs), 1 ml of peripheral blood was stored in 1.5-ml EDTA tubes for genotyping. PBMCs were isolated from heparinized blood samples by Ficoll–Hypaque density-gradient centrifugation. Isolated PBMCs (2×10^6 cells per well) were seeded in 24-well plates and cultured in an RPMI medium 1640 supplemented with 10% fetal calf serum (FCS, Greiner, Wemmel, Belgium), 100 U/ml penicillin, and 100 µg/ml streptomycin. To detect IL-10 production, PBMCs were stimulated with a mix of the anti-CD3 antibody (5 µg/ml, eBioscience, San Diego, CA) and anti-CD28 antibody (1 µg/ml, eBioscience) for 72 h, or 100 ng/ml of lipopolysaccharide (LPS, 100 ng/ml; Sigma, MO) for 24 h.

DNA extraction, RNA extraction, and cDNA synthesis: DNA was extracted from whole blood using the QIAamp DNA Blood Mini Kit (Qiagen). Total RNA was extracted from PBMCs using TRIzol (Invitrogen, San Diego, CA), followed by reverse transcription using a transcriptase kit (Applied Biosystems, ABI).

Genotyping and RT-PCR: The genotype of rs1800871 was examined by PCR-RFLP, as mentioned above. Real-time quantitative PCR was performed using the Applied Biosystems 7500 System (Applied Biosystems) to quantify IL-10 mRNA transcripts. The relative expression level of IL-10 mRNA was normalized by the expression of β-actin mRNA in each sample. Real-time RT-PCR primers were as follows: for IL-10 transcripts, 5′AAGACCCAGACATCAAGGCG3′ (forward) and 5′AATCGATGACAGCGCCGTAG3′ (reverse),

Table 3. The details of primers of IL10 SNPs, restriction enzymes used for RFLP analysis and assay information from Applied Biosystems for TaqMan SNP assays analysis.

rs number	details		
rs1800871	Primers/Restriction enzyme	5′ TGTACCCTTGTACAGGTGATGTGA 3′	EcoRV
		5′ ACCCCGATTTCATTAGGATTCT 3′	
rs1800872	Primers/Restriction enzyme	5′ GAGAATCCTAATGAAATCGGG 3′	Csp6I
		5′ TTGCTAACTTAGGCAGTCACC 3′	
rs1800896	Primers/Restriction enzyme	5′ CAAGACAACACTACTAAGGCTCC 3′	BslI
		5′ CAGCACATAGAATGAAACCTTG 3′	
rs3021094	Primers/Restriction enzyme	5′CGGCCAGATTTTTTAAATAACTTGCCTCTG3′	BglI
		5′ TGAAATGCGGTCTTTTTGATGCCCT 3′	
rs3790622	Primers/Restriction enzyme	5′ TGCTTAGAGCGTTTCCAGACC 3′	TasI
		5′ TTTCTGCGGAGCTACATTCG 3′	
rs1554286	ABI assay ID	C___8828812_10	
	SNP type	Transition, Substitution, Intron, Intragenic	
	Assay Type	Validated	
	Chr site	Chr.1: 206,944,233	

and for β-actin, 5′GGATGCAGAAGGAGATCACTG3′ (forward) and 5′CGATCCACACGGAGTACTTG3′ (reverse). Relative expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method.

Statistical analysis: For each SNP, a deviation of genotype frequencies in controls from the Hardy–Weinberg equilibrium (HWE) was assessed using the chi-square test. No SNPs showed any significant deviation from the HWE (p>0.05). To ensure the accuracy of the genotyping of six SNPs in the investigated subjects, we compared the patterns of linkage disequilibrium (LD) of the six SNPs included in the study. Allele frequencies, genotype frequencies, HWE, and LD were calculated using the online platform SHEsis [42]. Allele and genotype frequencies were compared between patients and controls by the chi-square test. Risks were evaluated by odds ratios (ORs) with 95% confidence intervals (CIs). The expression of mRNA was analyzed by an independent samples *t* test. Data were analyzed using SPSS version 16.0 (Chicago, IL). P values were corrected ($P_{corrected}$(P_{corr})) for multiple comparisons with the Bonferroni correction by multiplying with the number of analyses performed, and P_{corr}<0.05 was considered statistically significant.

RESULTS

First-stage study for IL-10 in BD and VKH syndrome: Six SNPs, including rs1800871, rs1800872, rs1800896, rs3021094, rs3790622, and rs1554286, were successfully genotyped in 300 BD patients, 300 VKH syndrome patients, and 350 healthy controls. The distribution of genotype and allele frequencies of the six SNPs did not deviate from the HWE in the controls.

The frequency of the T allele of rs1800871 was obviously higher in BD patients than in the controls ($p = 1.01\times10^{-6}$, $P_{corr} = 1.82\times10^{-5}$, OR = 1.837, 95%CI = 1.437–2.347), whereas the CC genotype frequency was significantly lower in BD patients ($p = 4.6\times10^{-7}$, $P_{corr} = 8.28\times10^{-6}$, OR = 0.142, 95%CI = 0.060–0.338). Similar to rs1800871, the frequencies of the C allele of rs1800872 ($p = 3.39\times10^{-6}$, $P_{corr} = 6.1\times10^{-5}$, OR = 0.562, 95%CI = 0.440–0.717) and the C allele of rs1554286 ($p = 1.75\times10^{-6}$, $P_{corr} = 3.15\times10^{-5}$, OR = 0.558, 95%CI = 0.438–0.709) were lower in BD patients than in healthy controls. Alternatively, the frequencies of the AA genotype of rs1800872 ($p = 2.22\times10^{-5}$, $P_{corr} = 4.0\times10^{-4}$, OR = 1.962, 95%CI = 1.435–2.682) and the TT genotype of rs1554286 ($p = 1.51\times10^{-6}$, $P_{corr} = 2.72\times10^{-5}$, OR = 2.154, 95%CI = 1.572–2.950) were distinctly higher in BD patients. There was no statistically significant difference concerning the genotypes and alleles of the other three SNPs between patients with BD and the controls (Table 4). The result showed there were no differences in the allele and genotype frequencies of the six SNPs between VKH syndrome patients and controls (Table 5).

Second-stage and combined study for IL-10 in Behcet's disease: The LD analysis showed the patterns of the SNPs rs1800871, rs1800872, rs3021094, and rs1554286 were strongly linked (Figure 1). In addition, the p value of rs1800871 was the smallest among the aforementioned three positive SNPs in the first-stage study (Table 4). Therefore, we chose rs1800871 to conduct a replication study in an independent cohort including an additional set of 418 BD patients and 1,403 normal controls to confirm further the findings of the first-stage study.

The second-stage study showed the frequency of the T allele of rs1800871 was significantly higher in BD patients than in controls ($p = 3.73\times10^{-6}$, $P_{corr} = 5.59\times10^{-5}$, OR = 1.493, 95%CI = 1.259–1.770). Combining the data of the first-stage and second-stage studies showed that rs1800871 was convincingly associated with a susceptibility to BD ($p = 2.03\times10^{-12}$, $P_{corr} = 3.65\times10^{-11}$, OR = 1.632, 95%CI = 1.423–1.872; Table 4).

When the genotype and allele frequencies were analyzed according to the clinical features, the results showed significantly higher frequencies of the rs1800871 T allele in BD patients with extraocular findings, including genital ulcers, skin lesions, and a positive pathergy test, as compared to that observed in healthy controls. There was no difference between BD patients with arthritis and healthy controls (Table 6).

Haplotype analysis of IL-10 SNPs and Behcet's disease: An LD analysis was performed for six SNPs, including rs1800896, rs1800871, rs1800872, rs3790622, rs3021094, rs1554286, based on our data using Haploview software version 4.2. The results showed that haplotype GACCCA constructed by SNPs rs1554286, rs3021094, rs3790622, rs1800872, rs1800871, and rs1800896 was strongly associated with BD ($p = 1.76\times10^{-7}$; Table 7).

Meta-analysis of three IL-10 SNPs in the Chinese Han population: Recently, Wu et al. reported the association of IL-10 SNPs with BD [43]. In that study, the patients were recruited from the department of rheumatology, and only 28.5% patients had ocular manifestations. In our study, the BD patients were recruited from the department of ophthalmology and 100% of patients had uveitis. Wu et al.'s study included four candidate SNPs, namely rs1800871, rs1518111, rs3021094, and rs3790622. As rs1800871 was in a strong LD with rs1518111, the remaining three SNPs were included in our study. To understand better the association of IL-10 gene polymorphisms and BD in Chinese Han, a meta-analysis was conducted and the results showed rs1800871 and rs3790622

Table 4. Genotype and allele frequencies of IL10 polymorphisms in BD patients and controls.

SNPs	study	Allele	Case (frequency)	Control (frequency)	P value	Pc value	OR(95%CI)
rs1800871	StageI	C C	6(0.020)	44(0.126)	4.60×10^{-7}	8.28×10^{-6}	0.142(0.060–0.338)
		C T	126(0.420)	160(0.457)	0.342	NS	0.860(0.630–1.174)
		T T	168(0.560)	146(0.417)	2.80×10^{-4}	5.03×10^{-3}	1.778(1.302–2.429)
		C	138(0.230)	248(0.354)	1.01×10^{-6}	1.82×10^{-5}	0.544(0.426–0.696)
		T	462(0.770)	452(0.646)	1.01×10^{-6}	1.82×10^{-5}	1.837(1.437–2.347)
	StageII	C C	27(0.065)	166(0.118)	0.002	0.03	0.515(0.337–0.785)
		C T	175(0.419)	679(0.484)	0.019	NS	0.768(0.616–0.958)
		T T	216(0.517)	558(0.398)	1.55×10^{-5}	2.33×10^{-4}	1.619(1.300–2.017)
		C	229(0.274)	1011(0.360)	3.73×10^{-6}	5.59×10^{-5}	0.670(0.565–0.794)
		T	607(0.726)	1795(0.640)	3.73×10^{-6}	5.59×10^{-5}	1.493(1.259–1.770)
	Combined	C C	33(0.046)	210(0.120)	2.19×10^{-8}	3.95×10^{-7}	0.354(0.243–0.517)
		C T	301(0.419)	839(0.479)	0.007	NS	0.786(0.660–0.937)
		T T	384(0.535)	704(0.402)	1.39×10^{-9}	2.50×10^{-8}	1.713(1.438–2.041)
		C	367(0.256)	1259(0.359)	2.03×10^{-12}	3.65×10^{-11}	0.613(0.534–0.703)
		T	1069(0.744)	2247(0.641)	2.03×10^{-12}	3.65×10^{-11}	1.632(1.423–1.872)
rs1800872	StageI	A A	170(0.567)	140(0.400)	2.22×10^{-5}	4×10^{-4}	1.962(1.435–2.682)
		A C	120(0.400)	174(0.497)	0.013	NS	0.674(0.494–0.921)
		C C	10(0.033)	36(0.103)	0.001	0.018	0.301(0.147–0.617)
		A	460(0.767)	454(0.649)	3.39×10^{-6}	6.1×10^{-5}	1.780(1.394–2.274)
		C	140(0.233)	246(0.351)	3.39×10^{-6}	6.1×10^{-5}	0.562(0.440–0.717)
rs1800896	StageI	A A	271(0.903)	293(0.837)	0.013	NS	1.818(1.129–2.928)
		A G	29(0.097)	56(0.160)	0.017	NS	0.562(0.348–0.906)
		G G	0(0.000)	1(0.003)	0.354	NS	
		A	57(0.952)	642(0.917)	0.013	NS	1.779(1.123–2.817)
		G	29(0.048)	58(0.083)	0.013	NS	0.562(0.355–0.890)
rs3021094	StageI	A A	58(0.193)	100(0.286)	0.006	NS	0.599(0.414–0.866)
		A C	173(0.577)	199(0.569)	0.835	NS	1.034(0.757–1.412)
		C C	69(0.230)	51(0.146)	0.006	NS	1.751(1.173–2.614)
		A	289(0.482)	399(0.570)	0.001	0.018	0.701(0.563–0.873)
		C	311(0.518)	301(0.430)	0.001	0.018	1.426(1.146–1.776)

SNPs	study	Allele	Case (frequency)	Control (frequency)	P value	Pc value	OR(95%CI)
rs3790622	StageI	C C	260(0.867)	312(0.891	0.333	NS	0.792(0.493–1.271)
		C T	39(0.130)	38(0.109)	0.399	NS	1.227(0.762–1.975)
		T T	1(0.003)	0(0.000)	0.280	NS	
		C	559(0.932)	662(0.946)	0.291	NS	O.783(0.496–1.234)
		T	41(0.068)	38(0.054)	0.291	NS	1.278(0.810–2.015)
rs1554286	StageI	T T	168(0.560)	130(0.371)	1.51×10^{-6}	2.72×10^{-5}	2.154(1.572–2.950)
		C T	116(0.387)	181(0.517)	8.72×10^{-4}	0.015687	0.589(0.430–0.805)
		C C	16(0.053)	39(0.111)	0.080	NS	0.449(0.246–0.822)
		T	452(0.753)	441(0.630)	1.75×10^{-6}	3.15×10^{-5}	1.794(1.410–2.282)
		C	148(0.247)	259(0.370)	1.75×10^{-6}	3.15×10^{-5}	0.558(0.438–0.709)

OR, odds ratio; Pc, Bonferroni corrected p value; SNP, single-nucleotide polymorphisms; NS, not significant

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Table 5. The first-stage study Genotype and allele frequencies of IL10 polymorphisms in Han Chinese VKH syndrome patients and healthy controls.

SNPs	Allele	Case (frequency)	Control (frequency)	P value	Pc value	OR(95%CI)
rs1800871	C C	28(0.093)	44(0.126)	0.190	NS	0.716(0.434–1.182)
	C T	138(0.460)	160(0.457)	0.942	NS	1.012(0.742–1.378)
	T T	134(0.447)	146(0.417)	0.449	NS	1.128(0.826–1.540)
	C	194(0.323)	248(0.354)	0.240	NS	0.871(0.691–1.097)
	T	406(0.677)	452(0.646)	0.240	NS	1.148(0.912–1.446)
rs1800872	A A	120(0.400)	140(0.400)	1	NS	1.000(0.730–1.370)
	A C	160(0.533)	174(0.497)	0.357	NS	1.156(0.849–1.574)
	C C	20(0.067)	36(0.103)	0.101	NS	0.623(0.352–1.101)
	A	400(0.667)	454(0.649)	0.493	NS	1.084(0.861–1.364)
	C	200(0.333)	246(0.351)	0.493	NS	0.923(0.733–1.161)
rs1800896	A A	252(0.840)	293(0.837)	0.921	NS	1.021(0.672–1.553)
	A G	48(0.160)	56(0.160)	1	NS	1.000(0.657–1.523)
	G G	0(0.000)	1(0.003)	0.354	NS	
	A	552(0.920)	642(0.917)	0.851	NS	1.039(0.697–1.548)
	G	48(0.080)	58(0.083)	0.851	NS	0.963(0.646–1.434)
rs3021094	A A	76(0.253)	100(0.286)	0.354	NS	0.848(0.599–1.202)
	A C	170(0.567)	199(0.569)	0.961	NS	0.992(0.727–1.355)
	C C	54(0.180)	51(0.146)	0.236	NS	1.287(0.847–1.955)
	A	322(0.537)	399(0.570)	0.228	NS	0.874(0.702–1.088)
	C	278(0.463)	301(0.430)	0.228	NS	1.144(0.919–1.425)
rs3790622	C C	276(0.920)	312(0.891)	0.216	NS	1.401(0.819–2.394)
	C T	24(0.080)	38(0.109)	0.216	NS	0.714(0.418–1.220)
	T T				NS	
	C	576(0.960)	662(0.946)	0.228	NS	1.378(0.817–2.324)
	T	24(0.040)	38(0.054)	0.228	NS	0.726(0.0.430–1.225)
rs1554286	T T	124(0.413)	130(0.371)	0.275	NS	1.192(0.869–1.635)
	C T	150(0.500)	181(0.517)	0.663	NS	0.934(0.686–1.271)
	C C	26(0.087)	39(0.111)	0.294	NS	0.757(0.449–1.275)
	T	398(0.663)	441(0.630)	0.210	NS	1.157(0.921–1.454)
	C	202(0.337)	259(0.370)	0.210	NS	0.864(0.689–1.086)

OR, odds ratio; Pc, Bonferroni corrected p value; SNP, single-nucleotide polymorphisms; NS, not significant

were associated with BD, which is consistent with our study, whereas rs3021094 shows a discrepancy with our study (Figure 2). Further studies using larger samples are needed to address the inconsistent result.

Expression of IL-10 in the PBMCs of controls: The effect of the genotype on the IL-10 mRNA expression was tested in 18 normal genotyped subjects. We tested two individuals with rs1800871 CC, eight individuals with CT, and eight individuals with the TT genotype. After stimulation with LPS, no difference was found concerning the IL-10 mRNA expression by PBMCs in normal controls with different genotypes (Figure 3). Following anti-CD3 and anti-CD28 stimulation, a similar trend was evident (Figure 3).

DISCUSSION

This study aimed to investigate the association of six SNPs of IL-10 with two uveitis entities, BD and VKH, in the Chinese Han population. The results showed three IL-10 SNPs, including rs1800871, rs1800872, and rs1554286, were significantly associated with BD, but not with VKH syndrome. These three SNPs are in a strong LD with each other, and the IL-10 rs1800871 (−819 C/T) is considered a functional promoter gene. Our findings are in agreement with earlier studies demonstrating a genetic predisposition of certain IL-10 gene polymorphisms and BD in Iranian, Turkish, and Japanese cohorts [38-40]. In these latter studies, an association was found with the SNPs rs1554286, rs1518111, rs1800871, and rs1800872.

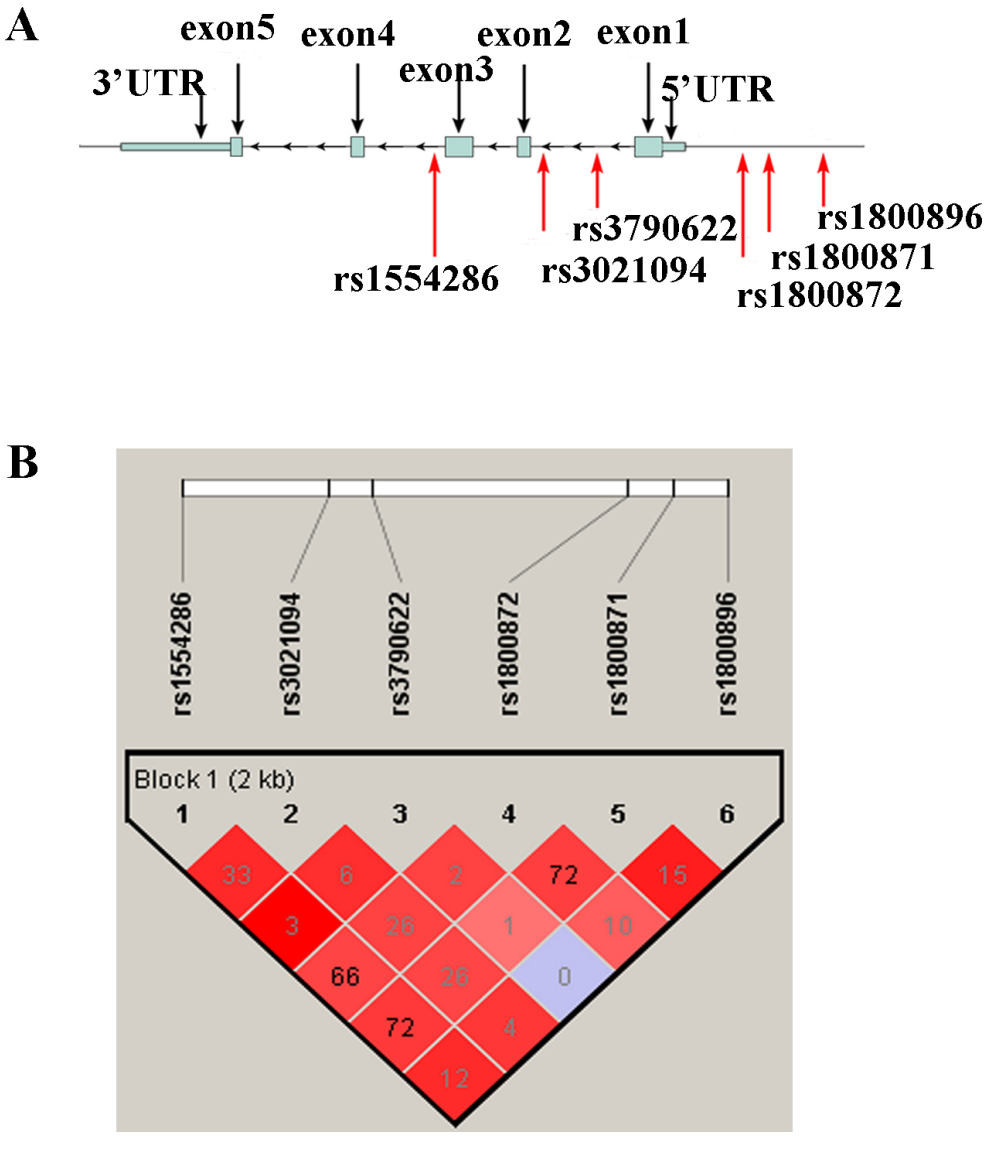


Figure 1. The relative positions of the investigated SNPs and the LD analysis of the examined SNPs of IL-10. **A**: The relative positions of the investigated SNPs of IL10 tested in this study (red arrows). **B**: An LD analysis was performed for SNPs rs1800896, rs1800871, rs1800872, rs3790622, rs3021094, and rs1554286 based on our data using Haploview software version 4.2. The number in the square indicates r^2 value.

Table 6. The combined study genotype and allele frequencies of rs1800871 in BD patients and controls according to the Clinical features.

SNPs	Allele	Case (frequency)	Control (frequency)	P value	Pc value	OR(95%CI)
Genital ulcers	C C	19(0.046)	210(0.120)	1.15×10^{-5}	1.38×10^{-4}	0.354(0.219–0.574)
	C T	17(0.431)	839(0.479)	0.081	NS	0.825(0.665–1.024)
	T T	21(0.523)	704(0.402)	7.11×10^{-6}	8.53×10^{-5}	1.634(1.317–2.026)
	C	21(0.262)	1259(0.359)	1.01×10^{-7}	1.21×10^{-6}	0.632(0.533–0.749)
	T	61(0.738)	2247(0.641)	1.01×10^{-7}	1.21×10^{-6}	1.582(1.335–1.875)
Skin lesions	C C	28(0.052)	210(0.120)	6.63×10^{-6}	7.96×10^{-5}	0.403(0.269–0.606)
	C T	21(0.405)	839(0.479)	0.003	0.036	0.742(0.610–0.903)
	T T	29(0.543)	704(0.402)	7.59×10^{-9}	9.11×10^{-8}	1.769(1.456–2.149)
	C	27(0.255)	1259(0.359)	2.13×10^{-10}	2.56×10^{-9}	0.610(0.523–0.711)
	T	80(0.745)	2247(0.641)	2.13×10^{-10}	2.56×10^{-9}	1.640(1.407–1.912)
Arthritis	C C	7(0.071)	210(0.120)	0.14	NS	0.559(0.256–1.222)
	C T	47(0.475)	839(0.479)	0.94	NS	0.985(0.656–1.477)
	T T	45(0.454)	704(0.402)	0.296	NS	1.242(0.827–1.865)
	C	61(0.308)	1259(0.359)	0.145	NS	0.795(0.583–1.083)
	T	137(0.692)	2247(0.641)	0.145	NS	1.258(0.923–1.715)
Positive pathergy test	C C	5(0.030)	210(0.120)	0.001	0.012	0.231(0.094–0.569)
	C T	71(0.433)	839(0.479)	0.263	NS	0.832(0.602–1.149)
	T T	88(0.537)	704(0.402)	0.001	0.012	1.725(1.251–2.379)
	C	81(0.247)	1259(0.359)	4.63×10^{-5}	5.56×10^{-4}	0.585(0.451–0.759)
	T	247(0.753)	2247(0.641)	4.63×10^{-5}	5.56×10^{-4}	1.709(1.317–2.216)

OR, odds ratio; Pc, Bonferroni corrected p value; SNP, single-nucleotide polymorphisms; NS, not significant

Further stratification of samples according to clinical features showed rs1800871 was significantly associated with extraocular findings in our BD patients, including genital ulcers, skin lesions, and a positive pathergy test. No significant association was observed in the group of patients manifesting arthritis. Earlier studies on the analysis of disease outcome showed an association between IL-10 rs1800896 AA homozygosity and poor outcomes in patients with intermediate uveitis [44]. We also included the SNP rs1800896 in our study but did not find a significant association with BD.

Animal models of experimental uveitis have shown IL-10 plays a protective role in the development of uveitis [45,46]. The role of IL-10 in BD is not yet exactly known, although lower levels of IL-10 were found in the aqueous humor of BD patients as compared to other uveitis entities, which indicated the BD intraocular microenvironment lacks immunosuppressive conditions [47]. As of yet, no data have been published concerning IL-10 plasma levels in BD patients.

The choice of IL-10 gene candidate SNPs was based on the associations reported for other autoimmune diseases [33-37]. A study on a Turkish cohort of BD patients identified an association of IL10 rs1518111 with a p value of 1.88×10^{-8} [40]. We did not use IL-10 rs1518111 in our study, but it is in a strong LD with rs1554286, which was one of the SNPs we used [38]. An association study on patients with white dot syndrome showed IL-10 rs2222202 was associated with multifocal choroiditis with panuveitis with a corrected p value of <0.012, but not in patients with punctate inner choroidopathy [48]. The SNP rs2222202 is linked with the SNP rs1800896 in Chinese Han (D' = 1.0, $r^2 = 0.33$), and we therefore chose to test the association of SNP rs1800896 with BD.

The three SNPs, including rs1800871, rs1800872, and rs1554286, showing an association in our study are in a strong LD with each other. In addition, the p value of rs1800871 was the smallest of the aforementioned three positive SNPs in the first-stage study. We therefore focused on rs1800871 in the second part of our study.

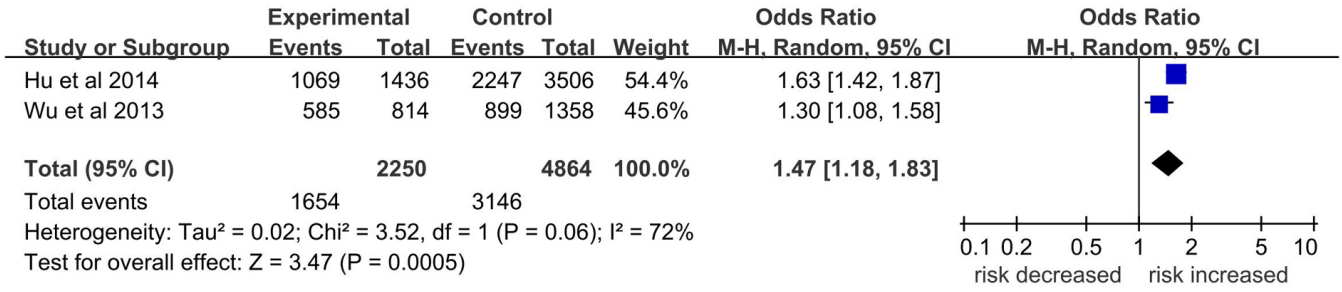
A recent study on IL-10 gene polymorphisms (including rs1800871) in uveitis patients from Austria did not identify a significant association in cases with HLA-B27-associated anterior uveitis or with intermediate uveitis [49]. The discrepancy with the association we found for this SNP in BD may be due to disease heterogeneity among different uveitis entities. The SNP rs1800871 is located in the 5′UTR region of IL-10, implying a functional role that regulates its gene expression. An expression analysis did not, however, reveal a significant association between genotype and the IL-10 mRNA expression. We did not include patients in the expression analysis due to confounding factors, such as disease activity and variable immunosuppressive treatment regimens. Our results agree with previous reports that also did not observe significant differences in the levels of IL-10 mRNA among healthy controls with different genotypes of rs1800871 [36]. Nonetheless, they did observe an association between IL-10 genotypes and plasma IL-10 levels in patients with Sjogren's syndrome. A study on patients with H pylori-associated gastritis showed individuals carrying the rs1800871 C allele had higher mucosal IL-10 mRNA expression levels [50]. As mentioned above, such studies have not yet been performed in patients with ocular BD and should be addressed in the future. Furthermore, studies should not only be focused on the mRNA expression of IL-10 but they should also deal with functional immunosuppressive properties of the various IL-10 protein variants.

Unexpectedly, the other three SNPs were not found to be associated with BD, and none of the tested SNPs were found to be associated with VKH syndrome. The number of samples in our study seems to be sufficient to ensure the accuracy of our observations. The absence of an IL-10 gene association was also observed in patients with metabolic syndrome in Taiwan [43], as well as in non-Hodgkin's lymphoma patients

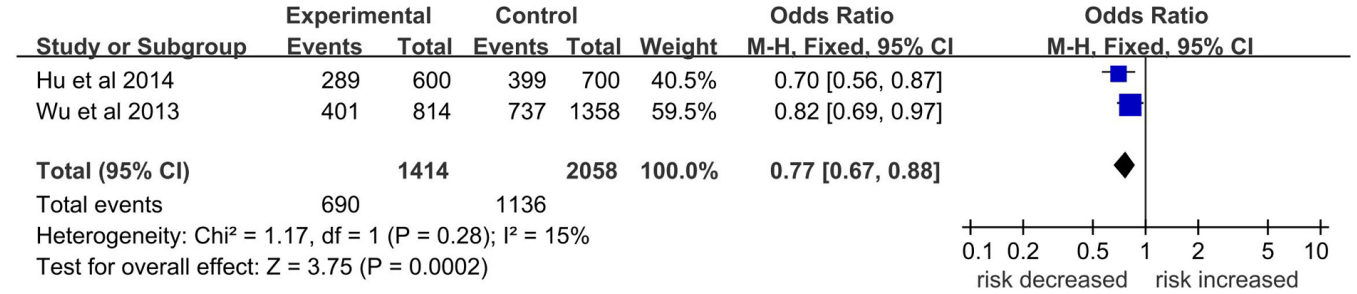
Table 7. Haplotype results for IL10 SNPs including rs1800896, rs1800871, rs1800872, rs3790622, rs3021094, rs1554286.

Block	Case, Control (%)	Chi Square	P Value
ACCATA	0.397, 0.337	5.191	0.023
AACATA	0.228, 0.205	0.999	0.318
GACCCA	0.138, 0.253	27.279	**1.76×10^{-7}**
GACCCG	0.035, 0.070	7.83	0.0051
ACTATA	0.056, 0.051	0.175	0.675
GACATA	0.034, 0.025	1.164	0.281
ACCCTA	0.033, 0.011	8.347	0.0039
ACCACA	0.013, 0.010	0.348	0.555

A



B



C

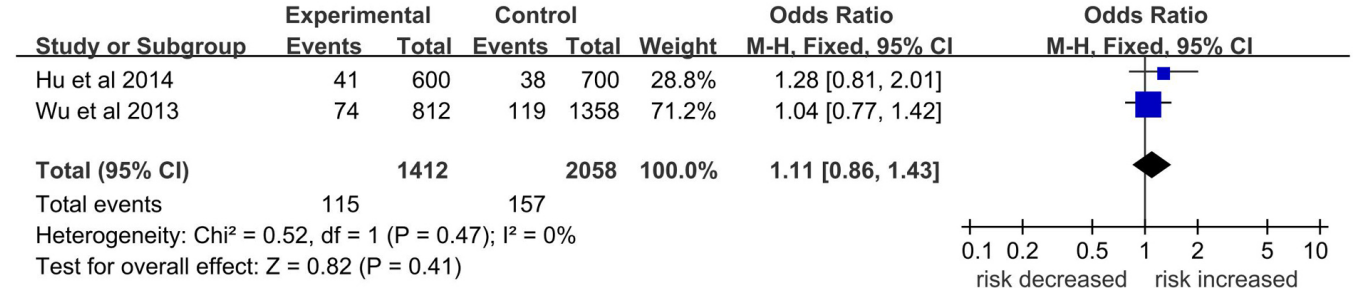


Figure 2. Forest plots illustrate the association between IL-10 gene polymorphisms and BD in Chinese Han. For each study, the odds ratio (OR) and 95% confidence interval (CI) values are indicted. The size of each box is proportional to the weight of each study. The squares and horizontal lines correspond to the OR and 95% CI, and the diamond represents the summary OR and 95% CI. A: The rs1800871 T allele versus C allele. **B**: The rs3021094 A allele versus C allele. **C**: The rs3790622 T allele versus C allele.

in the German population [44]. These results indicate there is a significant difference in gene susceptibility among different diseases. The inconsistent result between IL-10 SNPs and susceptibility to VKH syndrome and BD may be partly explained by the different etiology and manifestations of these two uveitis entities. VKH syndrome is mediated by an autoimmune response against melanocytes (autoimmune disease), whereas BD is considered mediated by an aberrant response against invading viruses or microbes (autoinflammatory disease) [51,52]. BD is a multisystem inflammatory disorder characterized by recurrent oral ulcerations, genital ulcerations, recurrent uveitis, and multiple skin lesions. VKH syndrome is a multisystem autoimmune disease characterized by bilateral granulomatous panuveitis, poliosis, vitiligo, alopecia, and central nervous system and auditory signs. To avoid confounding factors, including a selection of patients

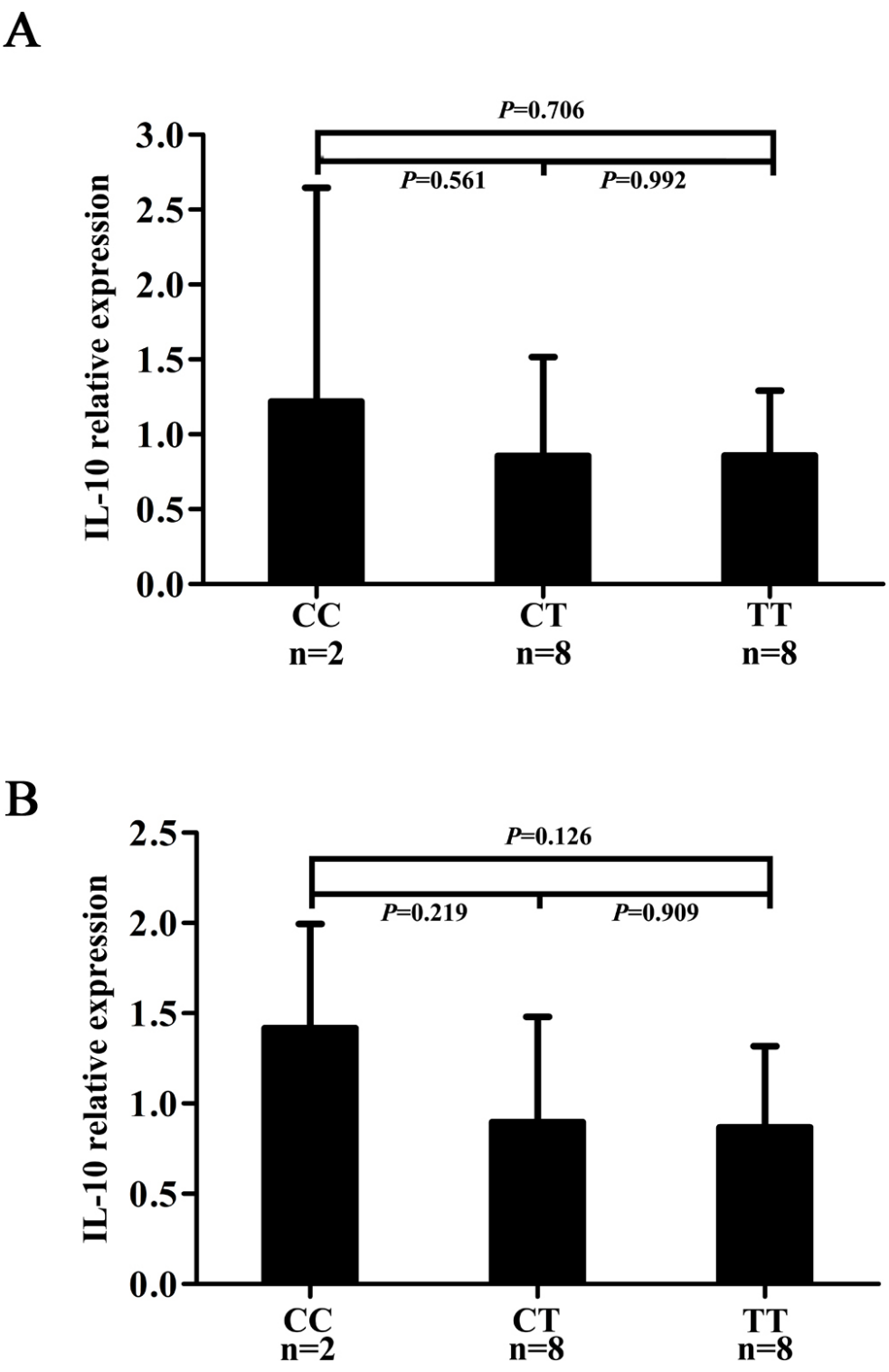


Figure 3. The expression of different genotypes of rs1800871/IL-10 at mRNA level in a stimulated condition by LPS or anti-CD3/CD28 antibodies. **A**: The relative expression of IL-10 mRNA in LPS-stimulated PBMCs obtained from healthy controls with the known IL-10 rs1800871 genotype. **B**: The relative expression of IL-10 mRNA by anti-CD3/CD28 antibody-stimulated PBMCs obtained from healthy controls with the known IL-10 rs1800871 genotype. The y-axis represents the IL-10 mRNA relative expression level through the real-time PCR of each genotype. Data are shown as mean ± SD. PBMCs included lymphocytes, such as T, B, and NK cells (90–95%), monocytes (3%), and Dendritic cells.

and healthy controls, as well as ethnic factors, in the results of gene susceptibility to disease in a case-control study, we made several efforts to ensure the accuracy of the results. We paid particular attention to the selection of patients with a definite diagnosis and control individuals to ensure they were from the same ethnic background. To ensure a balance in genotype and allele distributions among the tested patients and the controls, the HWE was examined. Direct sequencing was performed in 20% of the total samples, and the results were in complete accordance with the previous genotyping outcomes.

Taken together, our study confirms the role of IL-10 polymorphisms in BD and suggests a lack of endogenous control of inflammation may play an important role in the pathogenesis of this disease.

Conclusion: In conclusion, our study demonstrated rs1800871, rs1800872, and rs1554286 of IL-10 were possibly associated with BD. However, it is unclear whether or how these SNPs play a role in BD. To clarify this issue, further studies should be conducted. Our study found none of the tested six SNPs was associated with VKH syndrome. Considering only six SNPs of the IL-10 gene were tested in this study, it is unclear whether other SNPs of IL-10 are associated with VKH syndrome. Further studies are needed to address this issue.

ACKNOWLEDGMENTS

JH, SH and PY conceived of the study and participated in its design and coordination. XZ performed the genotyping, expression studies and the statistical analysis and drafted the manuscript. JF, YZ, YL, and LB provided technical support, participated in the samples collection and the interpreted the data. JH, SH, AK, and PY critically revised the manuscript. All authors read and approved the final manuscript. This work was supported by Natural Science Foundation Major International (Regional) Joint Research Project (81320108009), Key Project of Natural Science Foundation (81130019), National Natural Science Foundation Project (31370893, 81270990),National Basic Research Program of China (973 Program; 2011CB510200), Research Fund for the Doctoral Program of Higher Education of China (20115503110002), Clinic Key Project of Ministry of Health, Basic Research program of Chongqing (cstc2013jcyjC10001), Chongqing Key Laboratory of Ophthalmology (CSTC, 2008CA5003), Key Project of Health Bureau of Chongqing (2012–1-003) and Fund for PAR-EU Scholars Program.

REFERENCES

1. Yang P, Fang W, Meng Q, Ren Y, Xing L, Kijlstra A. Clinical features of chinese patients with Behcet's disease. Ophthalmology 2008; 115:312-8. [PMID: 17692378].
2. Yang P, Zhang Z, Zhou H, Li B, Huang X, Gao Y, Zhu L, Ren Y, Klooster J, Kijlstra A. Clinical patterns and characteristics of uveitis in a tertiary center for uveitis in China. Curr Eye Res 2005; 30:943-8. [PMID: 16282128].
3. Sakane T, Takeno M, Suzuki N, Inaba G. Behcet's disease. N Engl J Med 1999; 341:1284-91. [PMID: 10528040].
4. Yang P, Ren Y, Li B, Fang W, Meng Q, Kijlstra A. Clinical characteristics of Vogt-Koyanagi-Harada syndrome in Chinese patients. Ophthalmology 2007; 114:606-14. [PMID: 17123618].
5. Azizlerli G, Kose AA, Sarica R, Gul A, Tutkun IT, Kulac M, Tunc R, Urgancioglu M, Disci R. Prevalence of Behcet's disease in Istanbul, Turkey. Int J Dermatol 2003; 42:803-6. [PMID: 14521694].
6. Khairallah M, Accorinti M, Muccioli C, Kahloun R, Kempen JH. Epidemiology of Behcet disease. Ocul Immunol Inflamm 2012; 20:324-35. [PMID: 23030353].
7. Pineton de Chambrun M, Wechsler B, Geri G, Cacoub P, Saadoun D. New insights into the pathogenesis of Behcet's disease. Autoimmun Rev 2012; 11:687-98. [PMID: 22197900].
8. Greco A, Fusconi M, Gallo A, Turchetta R, Marinelli C, Macri GF, De Virgilio A, de Vincentiis M. Vogt-Koyanagi-Harada syndrome. Autoimmun Rev 2013; 12:1033-8. [PMID: 23567866].
9. Ohno S, Ohguchi M, Hirose S, Matsuda H, Wakisaka A, Aizawa M. Close association of HLA-Bw51 with Behcet's disease. Arch Ophthalmol 1982; 100:1455-8. [PMID: 6956266].
10. Meguro A, Inoko H, Ota M, Katsuyama Y, Oka A, Okada E, Yamakawa R, Yuasa T, Fujioka T, Ohno S, Bahram S, Mizuki N. Genetics of Behcet disease inside and outside the MHC. Ann Rheum Dis 2010; 69:747-54. [PMID: 19684014].
11. Islam SM, Numaga J, Fujino Y, Hirata R, Matsuki K, Maeda H, Masuda K. HLA class II genes in Vogt-Koyanagi-Harada disease. Invest Ophthalmol Vis Sci 1994; 35:3890-6. [PMID: 7928186].
12. Hou S, Xiao X, Li F, Jiang Z, Kijlstra A, Yang P. Two-stage association study in Chinese Han identifies two independent associations in CCR1/CCR3 locus as candidate for Behcet's disease susceptibility. Hum Genet 2012; 131:1841-50. [PMID: 22829007].
13. Kirino Y, Bertsias G, Ishigatsubo Y, Mizuki N, Tugal-Tutkun I, Seyahi E, Ozyazgan Y, Sacli FS, Erer B, Inoko H, Emrence Z, Cakar A, Abaci N, Ustek D, Satorius C, Ueda A, Takeno M, Kim Y, Wood GM, Ombrello MJ, Meguro A, Gul A, Remmers EF, Kastner DL. Genome-wide association analysis identifies new susceptibility loci for Behcet's disease and epistasis between HLA-B*51 and ERAP1. Nat Genet 2013; 45:202-7. [PMID: 23291587].

14. Hou S, Yang Z, Du L, Jiang Z, Shu Q, Chen Y, Li F, Zhou Q, Ohno S, Chen R, Kijlstra A, Rosenbaum JT, Yang P. Identification of a susceptibility locus in STAT4 for Behcet's disease in Han Chinese in a genome-wide association study. Arthritis Rheum 2012; 64:4104-13. [PMID: 23001997].

15. Jiang Z, Yang P, Hou S, Du L, Xie L, Zhou H, Kijlstra A. IL-23R gene confers susceptibility to Behcet's disease in a Chinese Han population. Ann Rheum Dis 2010; 69:1325-8. [PMID: 20375120].

16. Shahram F, Nikoopour E, Rezaei N, Saeedfar K, Ziaei N, Davatchi F, Amirzargar A. Association of interleukin-2, interleukin-4 and transforming growth factor-beta gene polymorphisms with Behcet's disease. Clin Exp Rheumatol 2011; 29:Suppl 67S28-31. [PMID: 21640045].

17. Bonyadi M, Jahanafrooz Z, Esmaeili M, Kolahi S, Khabazi A, Ebrahimi AA, Hajialilo M, Dastgiri S. TNF-alpha gene polymorphisms in Iranian Azeri Turkish patients with Behcet's Disease. Rheumatol Int 2009; 30:285-9. [PMID: 19774383].

18. Wang J, Yang Y, Guo S, Chen Y, Yang C, Ji H, Song X, Zhang F, Jiang Z, Ma Y, Li Y, Du A, Jin L, Reveille JD, Zou H, Zhou X. Association between copy number variations of HLA-DQA1 and ankylosing spondylitis in the Chinese Han population. Genes Immun 2013; [PMID: 24048351].

19. Hou S, Yang P, Du L, Zhou H, Lin X, Liu X, Kijlstra A. SUMO4 gene polymorphisms in Chinese Han patients with Behcet's disease. Clin Immunol 2008; 129:170-5. [PMID: 18657476].

20. Hou S, Du L, Lei B, Pang CP, Zhang M, Zhuang W, Zhang M, Huang L, Gong B, Wang M, Zhang Q, Hu K, Zhou Q, Qi J, Wang C, Tian Y, Ye Z, Liang L, Yu H, Li H, Zhou Y, Cao Q, Liu Y, Bai L, Liao D, Kijlstra A, Xu J, Yang Z, Yang P. Genome-wide association analysis of Vogt-Koyanagi-Harada syndrome identifies two new susceptibility loci at 1p31.2 and 10q21.3. Nat Genet 2014; 46:1007-11. [PMID: 25108386].

21. Hu K, Yang P, Jiang Z, Hou S, Du L, Li F. STAT4 polymorphism in a Chinese Han population with Vogt-Koyanagi-Harada syndrome and Behcet's disease. Hum Immunol 2010; 71:723-6. [PMID: 20438790].

22. Du L, Yang P, Hou S, Lin X, Zhou H, Huang X, Wang L, Kijlstra A. Association of the CTLA-4 gene with Vogt-Koyanagi-Harada syndrome. Clin Immunol 2008; 127:43-8. [PMID: 18282809].

23. Shu Q, Yang P, Hou S, Li F, Chen Y, Du L, Jiang Z. Interleukin-17 gene polymorphism is associated with Vogt-Koyanagi-Harada syndrome but not with Behcet's disease in a Chinese Han population. Hum Immunol 2010; 71:988-91. [PMID: 20620187].

24. Iyer SS, Cheng G. Role of interleukin 10 transcriptional regulation in inflammation and autoimmune disease. Crit Rev Immunol 2012; 32:23-63. [PMID: 22428854].

25. Rennick DM, Fort MM, Davidson NJ. Studies with IL-10−/− mice: an overview. J Leukoc Biol 1997; 61:389-96. [PMID: 9103224].

26. Spencer SD, Di Marco F, Hooley J, Pitts-Meek S, Bauer M, Ryan AM, Sordat B, Gibbs VC, Aguet M. The orphan receptor CRF2–4 is an essential subunit of the interleukin 10 receptor. J Exp Med 1998; 187:571-8. [PMID: 9463407].

27. Barbara G, Xing Z, Hogaboam CM, Gauldie J, Collins SM. Interleukin 10 gene transfer prevents experimental colitis in rats. Gut 2000; 46:344-9. [PMID: 10673295].

28. van Deventer SJ, Elson CO, Fedorak RN. Multiple doses of intravenous interleukin 10 in steroid-refractory Crohn's disease. Crohn's Disease Study Group. Gastroenterology 1997; 113:383-9. [PMID: 9247454].

29. Ding L, Linsley PS, Huang LY, Germain RN, Shevach EM. IL-10 inhibits macrophage costimulatory activity by selectively inhibiting the up-regulation of B7 expression. J Immunol 1993; 151:1224-34. [PMID: 7687627].

30. de Waal Malefyt R, Haanen J, Spits H, Roncarolo MG, te Velde A, Figdor C, Johnson K, Kastelein R, Yssel H, de Vries JE. Interleukin 10 (IL-10) and viral IL-10 strongly reduce antigen-specific human T cell proliferation by diminishing the antigen-presenting capacity of monocytes via downregulation of class II major histocompatibility complex expression. J Exp Med 1991; 174:915-24. [PMID: 1655948].

31. Fiorentino DF, Bond MW, Mosmann TR. Two types of mouse T helper cell. IV. Th2 clones secrete a factor that inhibits cytokine production by Th1 clones. J Exp Med 1989; 170:2081-95. [PMID: 2531194].

32. Flores-Borja F, Bosma A, Ng D, Reddy V, Ehrenstein MR, Isenberg DA, Mauri C. CD19+CD24hiCD38hi B cells maintain regulatory T cells while limiting TH1 and TH17 differentiation. Sci Transl Med 2013; 5: 73ra23-[PMID: 23427243].

33. Amre DK, Mack DR, Morgan K, Israel D, Lambrette P, Costea I, Krupoves A, Fegury H, Dong J, Grimard G, Deslandres C, Levy E, Seidman EG. Interleukin 10 (IL-10) gene variants and susceptibility for paediatric onset Crohn's disease. Aliment Pharmacol Ther 2009; 29:1025-31. [PMID: 19210299].

34. Forte GI, Pilato G, Vaccarino L, Sanacore M, Candore G, Romano GC, Testa R, Franceschi C, Capri M, Marra M, Bonfigli AR, Caruso C, Scola L, Lio D. Risk profiles in type 2 diabetes (metabolic syndrome): integration of IL-10 polymorphisms and laboratory parameters to identify vascular damages related complications. Curr Pharm Des 2010; 16:898-903. [PMID: 20388104].

35. Peng WJ, Wang BX, Pan HF, Tao JH, Zhang JQ, He Q, Xiao CC, Wang J. Association of the interleukin-10 1082G/A, 819C/T and 3575T/A gene polymorphisms with systemic sclerosis: a meta-analysis. Mol Biol Rep 2012; 39:6851-5. [PMID: 22307790].

36. Hulkkonen J, Pertovaara M, Antonen J, Lahdenpohja N, Pasternack A, Hurme M. Genetic association between interleukin-10 promoter region polymorphisms and primary Sjogren's syndrome. Arthritis Rheum 2001; 44:176-9. [PMID: 11212157].

37. Liu N, Lu H, Tao F, Guo T, Liu C, Cui B, Ning G. An association of interleukin-10 gene polymorphisms with Graves' disease in two Chinese populations. Endocrine 2011; 40:90-4. [PMID: 21424183].

38. Xavier JM, Shahram F, Davatchi F, Rosa A, Crespo J, Abdollahi BS, Nadji A, Jesus G, Barcelos F, Patto JV, Shafiee NM, Ghaderibarim F, Oliveira SA. Association study of IL10 and IL23R–IL12RB2 in Iranian patients with Behcet's disease. Arthritis Rheum 2012; 64:2761-72. [PMID: 22378604].

39. Mizuki N, Meguro A, Ota M, Ohno S, Shiota T, Kawagoe T, Ito N, Kera J, Okada E, Yatsu K, Song YW, Lee EB, Kitaichi N, Namba K, Horie Y, Takeno M, Sugita S, Mochizuki M, Bahram S, Ishigatsubo Y, Inoko H. Genome-wide association studies identify IL23R–IL12RB2 and IL10 as Behcet's disease susceptibility loci. Nat Genet 2010; 42:703-6. [PMID: 20622879].

40. Remmers EF, Cosan F, Kirino Y, Ombrello MJ, Abaci N, Satorius C, Le JM, Yang B, Korman BD, Cakiris A, Aglar O, Emrence Z, Azakli H, Ustek D, Tugal-Tutkun I, Akman-Demir G, Chen W, Amos CI, Dizon MB, Kose AA, Azizlerli G, Erer B, Brand OJ, Kaklamani VG, Kaklamanis P, Ben-Chetrit E, Stanford M, Fortune F, Ghabra M, Ollier WE, Cho YH, Bang D, O'Shea J, Wallace GR, Gadina M, Kastner DL, Gul A. Genome-wide association study identifies variants in the MHC class I, IL10, and IL23R–IL12RB2 regions associated with Behcet's disease. Nat Genet 2010; 42:698-702. [PMID: 20622878].

41. Criteria for diagnosis of Behcet's disease. International Study Group for Behcet's Disease. Lancet 1990; 335:1078-80. [PMID: 1970380].

42. Shi YY, He L. SHEsis, a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci. Cell Res 2005; 15:97-8. [PMID: 15740637].

43. Wu Z, Zheng W, Xu J, Sun F, Chen H, Li P, Chen S, Shen M, Zhang W, You X, Wu Q, Zhang F, Li Y. IL10 polymorphisms associated with Behcet's disease in Chinese Han. Hum Immunol 2014; 75:271-6. [PMID: 24269690].

44. Stanford MR, Vaughan RW, Kondeatis E, Chen Y, Edelsten CE, Graham EM, Wallace GR. Are cytokine gene polymorphisms associated with outcome in patients with idiopathic intermediate uveitis in the United Kingdom? Br J Ophthalmol 2005; 89:1013-6. [PMID: 16024856].

45. Rizzo LV, Xu H, Chan CC, Wiggert B, Caspi RR. IL-10 has a protective role in experimental autoimmune uveoretinitis. Int Immunol 1998; 10:807-14. [PMID: 9678762].

46. Fang IM, Lin CP, Yang CH, Chiang BL, Yang CM, Chau LY, Chen MS. Inhibition of experimental autoimmune anterior uveitis by adenovirus-mediated transfer of the interleukin-10 gene. J Ocul Pharmacol Ther 2005; 21:420-8. [PMID: 16386083].

47. El-Asrar AM, Struyf S, Kangave D, Al-Obeidan SS, Opdenakker G, Geboes K, Van Damme J. Cytokine profiles in aqueous humor of patients with different clinical entities of endogenous uveitis. Clin Immunol 2011; 139:177-84. [PMID: 21334264].

48. Atan D, Fraser-Bell S, Plskova J, Kuffova L, Hogan A, Tufail A, Kilmartin DJ, Forrester JV, Bidwell J, Dick AD, Churchill AJ. Cytokine polymorphism in noninfectious uveitis. Invest Ophthalmol Vis Sci 2010; 51:4133-42. [PMID: 20335604].

49. Lindner E, Steinwender G, Plainer S, Poeschl EM, Weger M, Ardjomand N, Renner W, El-Shabrawi Y. Role of IL-10 gene polymorphisms in intermediate and HLA-B27-associated uveitis. Acta Ophthalmol (Copenh) 2013; 91:e415-7. [PMID: 23506437].

50. Rad R, Dossumbekova A, Neu B, Lang R, Bauer S, Saur D, Gerhard M, Prinz C. Cytokine gene polymorphisms influence mucosal cytokine expression, gastric inflammation, and host specific colonisation during Helicobacter pylori infection. Gut 2004; 53:1082-9. [PMID: 15247172].

51. Gul A. Behcet's disease as an autoinflammatory disorder. Curr Drug Targets Inflamm Allergy 2005; 4:81-3. [PMID: 15720240].

52. McGonagle D, McDermott MF. A proposed classification of the immunological diseases. PLoS Med 2006; 3:e297- [PMID: 16942393].

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