Association of *ERAP1* Gene Polymorphisms With Behçet's Disease in Han Chinese

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Purpose. Behçet's disease (BD) is a common uveitis entity in China. The endoplasmic reticulum aminopeptidase 1 (ERAP1), has a significant influence on the stability and immunological properties of MHC-I loaded peptides. In the present study, we investigated the association of *ERAP1* gene polymorphisms with BD in a Chinese Han population.

Methods. A two-stage case-control study was carried out in 930 BD patients and 1704 healthy controls. Seven single nucleotide polymorphisms (SNPs) of the ERAP1 gene were determined using a PCR restriction fragment length polymorphism (PCR-RFLP) assay and one SNP was genotyped by TaqMan SNP genotyping assay. Furthermore, *ERAP1* expression in peripheral blood mononuclear cells (PBMCs) was examined in genotyped individuals by real-time PCR.

Results. The result demonstrated that the frequencies of the A allele of rs1065407 and C allele of rs10050860 were significantly decreased in BD patients ($Pc = 8.5 \times 10^{-8}$, OR = 0.51; $Pc = 1.1 \times 10^{-5}$, OR = 0.54, respectively). No significant association was observed for the other six SNPs. ERAP1 expression in AA carriers of rs1065407 and CC carriers of rs10050860 was higher than that observed in AC/CC carriers ($P = 0.022$) or CT/TT carriers ($P = 0.018$) by LPS-stimulated PBMCs, respectively. In addition, the expression of ERAP1 in active BD patients not receiving immunosuppression was significantly lower than that in healthy controls ($P = 3.8 \times 10^{-4}$).

Conclusions. Our study showed that rs1065407 and rs10050860 of the *ERAP1* gene may contribute to the genetic susceptibility of BD by modulating the expression of *ERAP1*.

Keywords: Behçet's disease, *ERAP1*, single nucleotide polymorphism (SNP)

Behçet's disease (BD) is one of the multisystem autoimmune or autoinflammatory diseases of complex pathogenesis characterized by recurrent ulcerations of the oral cavity and genitalia, representative skin lesions, and severe ocular inflammation. It can also affect digestive, cardiovascular, and nervous systems.[1,2] The pathogenic pathways of BD are largely unknown. Besides geographic tendency, numerous studies have indicated that the occurrence of BD is associated with genetic, immune, and environmental factors. Among all genetic factors, HLA-B51 has been proven as the strongest risk gene for BD, which was confirmed in various populations.[3] However, other factors besides *HLA-B51* are also involved in the inheritance of BD.[4–6]

Endoplasmic reticulum amino peptidase1 (*ERAP1*), which is located at 5q15, has two major functions. First, it degrades intracellular precursors to small peptides that can subsequently attach to major histocompatibility complex (MHC) class I molecules forming the MHC-I-antigenic peptide complex.[7–10] Second, ERAP1 is involved in cleaving cytokine receptors on the surface of cells, such as TNF receptor (TNFR1), IL-1 receptor-2 (IL-1R2), and IL-6 receptorα (IL-6Rα).[11–13] It may be involved in pathogenesis of BD either through its function to trim peptides before loading into HLA class I molecules, such as HLA-B51, or through its proposed role in shedding proinflammatory cytokine receptors from the cell wall. Recently, various groups have shown that polymorphisms of the *ERAP1* gene are associated with autoimmune disease susceptibility in various populations.[14–29] For instance, genome-wide association studies (GWAS) have identified *ERAP1* as a susceptibility gene for psoriasis in Chinese individuals[14,15] and ankylosing spondylitis (AS) in Europeans.[27,28] In addition, genetic surveys for multiple sclerosis from Italy[16]; Kawasaki disease in China[29]; and AS in China,[17–20] Spain,[21] Turkey,[22] Iran,[23] Portugal,[24] Korea,[25] and Europe,[26] have shown an association with ERAP1 polymorphisms. Moreover, a GWAS in a Turkish population found that two single nucleotide polymorphisms (SNPs) (rs10050860 and rs17482078) in *ERAP1*, which are in strong linkage disequilibrium (LD), were both associated with risk for BD with uveitis.[30] The association of genetic variants of *ERAP1* with the susceptibility of BD in a Chinese Han population has, however, not yet been reported and was therefore the subject of the study presented here.

Materials and Methods

Subjects

For the case-control study, a total of 930 (first stage: 382, second stage: 548) unrelated Chinese Han patients with BD and 1704 (first stage: 570, second stage: 1134) Chinese Han healthy

Table 1. Primers and Restriction Enzymes Used for RFLP Analysis

Gene	SNP	Primers	Tm, °C	Enzyme
ERAP1	rs27044	5′-ACACAGGCGAGGAGTAGTAGT-3′ 5′-TGTTTCCCTGTACAACGCCC-3′	60	HinfI
ERAP1	rs149481	5′-CACTGGATCTTGCGCACTTC-3′ 5′-GAGGGATTTGGAATTTACTGCAAGAAGAAT-3′	60	HinfI
ERAP1	rs1065407	5′-CCGTTGTGTCCAACAGACAA-3′ 5′-TGGTAGCGATAGCCCATTCA-3′	58	MvaI
ERAP1	rs10050860	5′-TCCTACACATTTTCACATTCCTCCT-3′ 5′-GAGAGCTTGGCTGTAGCAGT-3′	62	Tth111I
ERAP1	rs27038	5′-CAGATAGGCTGGCTTTGCCCCA-3′ 5′-GAACTGGCTGTCTGGAATCTATGCT-3′	62	MvaI
ERAP1	rs27980	5′-ATGCCATCACTTAACTATAACTGT-3′ 5′-AAACAAACCGTGGAACTCTA-3	54	HincII
ERAP1	rs13167972	5′-TGAAAAATCATTATTTTATGCTTTTGTGTAAC-3′ 5′-ACCCTGTAAAGCCGTTTTAGAAT-3′	56	TaiI

controls were recruited from the Zhongshan Ophthalmic Center of Sun Yat-sen University (Guangzhou, China) and the department of ophthalmology in the First Affiliated Hospital of Chongqing Medical University (Chongqing, PR China) from April 2007 to November 2014. All patients were diagnosed according to the international criteria for diagnosis of BD.[31] The individuals studied in the first stage and second stage were randomly selected. This study was approved by the Ethical Committee of Chongqing Medical University and written informed consent was obtained from all subjects.

Genotyping

Genomic DNA was isolated from peripheral blood cells of patients and healthy controls using the QIAamp DNA Blood Mini Kit (250) (QIAGEN, Valencia, CA, USA). The choice of SNPs of the *ERAP1* gene was based on previously reported associations with susceptibility to other autoimmune diseases[14–29] and from the GWAS report for BD in Turkish patients.[30] Linkage equilibrium (LD) and minor allele frequency (MAF) were analyzed by Haploview software 4.2 (Broad Institute, Cambridge, MA, USA). We eliminated one SNP (rs28366066) that was not polymorphic in the Chinese population and finally selected eight SNPs (the MAF at each locus was required to be >0.05 in Han Chinese in Beijing, with an r^2-value of LD <0.8 between adjacent markers). Seven SNPs (rs27038, rs1065407, rs149481, rs10050860, rs27044, rs27980, and rs13167972) were genotyped using the PCR restriction fragment length polymorphism assay (PCR-RFLP) (specific restriction enzymes for each SNP are shown in Table 1). The rs7711564 polymorphism was genotyped by TaqMan SNP Genotyping Assay (TagMan assay ID: C_3056830_20). Moreover, the assay validity of genotyping was checked using direct sequencing in 3% of the samples (Sangon Biotech, ShangHai, China). The genotyping success rate for all the SNPs tested in our study ranged from 94.9% to 100%.

Cell Isolation and Culture

Ficoll-Hypaque density-gradient centrifugation was used to obtain peripheral blood mononuclear cells (PBMCs) from venous blood samples. The PBMCs were counted by microscope and then seeded into 24-well culture plates (1×10^6 cells per well). The culture medium consisted of RPMI medium 1640, 10% fetal calf serum (Greiner, Wemmel, Belgium), 100 U/mL penicillin, and 100 μg/mL streptomycin. To investigate the effect of gene polymorphisms on the expression of *ERAP1*, PBMCs were cultured with 100 ng/mL lipopolysaccharide to simulate an inflammatory signal (LPS; Sigma-Aldrich Corp., St. Louis, MO, USA) and harvested at 24 hours.[32]

Real-Time PCR

Total RNA was extracted from PBMCs using TRIzol Reagent (Invitrogen, San Diego, CA, USA). The purified RNA was reverse transcribed to single-stranded cDNA using the reverse transcription reagent kit (Takara Biotechnology Co., Ltd., Ostu, Japan) and then the expression of *ERAP1* was measured by 7500 real-time instrument (ABI, Foster City, CA, USA). The following sequences of the sense and antisense primers of *ERAP1* were used: forward 5′-ACAGATGGTGTAAAAGG GATGG-3′ and reverse 5′-GCAGTGTCCAAGTGTTCATCAT-3′. For the internal reference gene, *β-actin* was chosen and its expression was detected by the following primers: forward 5′-GGATGCAGAAGGAGATCACTG-3′ and reverse 5′-CGATCCA CACGGAGTACTT-3′. Relative expression levels of *ERAP1* were calculated using the $2^{-\triangle\triangle Ct}$ method.

Statistical Analysis

Genotype frequencies were estimated by direct counting. Allele and genotype frequencies in both cases and controls were tested for Hardy-Weinberg equilibrium (HWE) by the SHEsis method (available in the public domain at http://analysis.bio-x.cn/myAnalysis.php). The odds ratio (OR) and 95% confidence intervals (95% CIs) were calculated by SPSS 19.0 software (IBM SPSS Statistics, IBM Corporation, Chicago, IL, USA). The *P* values were corrected (*Pc*) for multiple comparisons with the Bonferroni correction method by multiplying with the number of analyses performed. The number of independent comparisons is 24. A *Pc* value less than 0.05 was considered statistically significant. The nonparametric Mann-Whitney test or *t*-test was used to compare *ERAP1* expression among the genotype groups. Two-tailed *P* values less than 0.05 were considered to be statistically significant. Data were shown as mean ± SD.

Results

Allele and Genotype Frequencies of Tested SNPs in Patients and Controls

Characteristics of the BD patients are shown in Table 2. Overall, genotype and distribution of allele frequencies in patients and controls for the two SNPs in *ERAP1* are displayed in Table 3. The other six SNPs are shown as Supplementary

Table 2. Clinical Characteristics, Sex, and Age of BD Patients With Uveitis

Phenotype	Total	%
Patients with BD	930	
Mean age ± SD	34.1 ± 9.2	
Male	781	84.0
Female	149	16.0
Uveitis	930	100.0
Oral ulcer	892	95.9
Genital ulcer	528	56.8
Skin lesion	698	75.1
Arthritis	163	17.5
Controls	1704	
Mean age ± SD	39.2 ± 10.7	
Male	955	56.0
Female	749	44.0

Material. The genotype frequencies of all SNPs did not deviate from HWE equilibrium (both Fisher's P and Pearson's $P > 0.05$). The results of direct sequencing were consistent with the results of the PCR-RFLP method.

Our results showed that there was a significant difference between patients with BD and healthy controls concerning the frequencies of both rs1065407 and rs10050860. As shown in Table 3, in the first-stage study, the frequency of the A allele and AA genotype of rs1065407 in BD patients was significantly lower than in healthy controls ($Pc = 0.011$, OR = 0.53; $Pc = 0.023$, OR = 0.53, respectively). In the second-stage study, we tested a different set of BD patients and controls and restricted the study to the two SNPs that showed a significant association in the first stage study. A similar result as found in the first stage was observed in the second-stage study ($Pc = 1.150 \times 10^{-4}$, OR = 0.51; $Pc = 1.315 \times 10^{-4}$, OR = 0.49, respectively). Stronger significance became apparent after combining the data from the two stages ($Pc = 9.677 \times 10^{-8}$, OR = 0.51; $Pc = 2.714 \times 10^{-7}$, OR = 0.50, respectively). The frequency of the rs1065407 AC genotype was significantly increased in BD patients compared with healthy controls only in the second stage ($Pc = 2.981 \times 10^{-4}$, OR = 1.99). The same increase in the AC genotype frequency was observed in the combination of the two stages ($Pc = 2.429 \times 10^{-6}$, OR = 1.92). In addition, decreased frequencies of the rs10050860 C allele and CC genotype were observed in BD patients as compared with normal controls in the first stage ($Pc = 0.012$, OR = 0.51; $Pc = 0.018$, OR = 0.50 respectively). The result was confirmed both in the second stage ($Pc = 8.724 \times 10^{-3}$, OR = 0.57; $Pc = 0.016$, OR = 0.57, respectively) and after combining the data ($Pc = 1.059 \times 10^{-5}$, OR = 0.54; $Pc = 3.086 \times 10^{-5}$, OR = 0.54, respectively). The frequency of the rs10050860 CT genotype was significantly higher in BD patients when compared with healthy controls in the first stage ($Pc = 0.043$, OR = 1.91), second stage ($Pc = 0.040$, OR = 1.69), and the combination ($Pc = 1.823 \times 10^{-4}$, OR = 1.78). The genotype and allele frequencies for the other six SNPs tested showed no significant difference between patients and controls in the first stage. Taking into account the finance saving, we did not perform a second-stage study on these SNPs.

A stratified analysis of the main associations by sex was also performed in this study. The result showed that the genotype and allele frequencies for rs1065407 showed significant differences between patients and controls in both male (A allele: $P = 7.644 \times 10^{-6}$, OR = 0.54; AA genotype: $P = 3.484 \times 10^{-5}$, OR = 0.55) and female patients (A allele: $P = 1.616 \times 10^{-4}$, OR = 0.43; AA genotype: $P = 8.590 \times 10^{-5}$, OR = 0.39) (Table 4). However, the genotype and allele frequencies for rs10050860 showed more significant differences in male (C allele: $P = 1.012 \times 10^{-5}$, OR = 0.52; CC genotype: $P = 7.617 \times 10^{-5}$, OR = 0.52) compared with that in female patients (C allele: $P = 0.030$, OR = 0.58; CC genotype $P = 0.040$, OR = 0.58) (Table 4).

Table 3. Association of Two SNPs of *ERAP1* With BD

SNP	Stage	Allele/Genotype	BD	Controls	*P* Value	*Pc* Value	OR (95% CI)
rs1065407	First	A	692	1080	4.592×10^{-4}	0.011	0.53 (0.37–0.76)
		AA	315	512	9.755×10^{-4}	0.023	0.53 (0.37–0.78)
		AC	62	56	0.003	NS	1.78 (1.21–2.62)
		CC	5	2	0.090	NS	3.77 (0.73–19.51)
	Second	A	1005	2168	4.791×10^{-6}	1.150×10^{-4}	0.51 (0.38–0.68)
		AA	460	1036	5.480×10^{-6}	1.315×10^{-4}	0.49 (0.36–0.67)
		AC	85	96	1.242×10^{-5}	2.981×10^{-4}	1.99 (1.45–2.71)
		CC	3	2	0.190	NS	3.12 (0.52–18.70)
	Combined	A	1697	3248	4.032×10^{-9}	9.677×10^{-8}	0.51 (0.41–0.64)
		AA	775	1548	1.131×10^{-8}	2.714×10^{-7}	0.50 (0.40–0.64)
		AC	147	152	1.012×10^{-7}	2.429×10^{-6}	1.92 (1.50–2.44)
		CC	8	4	0.023	NS	3.69 (1.11–12.28)
rs10050860	First	C	702	1091	4.937×10^{-4}	0.012	0.51 (0.35–0.75)
		CC	323	522	7.705×10^{-4}	0.018	0.50 (0.34–0.76)
		CT	56	47	1.791×10^{-3}	0.043	1.91 (1.27–2.89)
		TT	3	1	0.154	NS	4.50 (0.47–43.46)
	Second	C	1019	2174	3.365×10^{-4}	8.724×10^{-3}	0.57 (0.42–0.78)
		CC	474	1041	6.538×10^{-4}	0.016	0.57 (0.41–0.79)
		CT	71	92	1.650×10^{-3}	0.040	1.69 (1.21–2.34)
		TT	3	1	0.070	NS	6.24 (0.65–60.10)
	Combined	C	1721	3265	4.411×10^{-7}	1.059×10^{-5}	0.54 (0.43–0.69)
		CC	797	1563	1.286×10^{-6}	3.086×10^{-5}	0.54 (0.42–0.70)
		CT	127	139	7.599×10^{-6}	1.823×10^{-4}	1.78 (1.38–2.30)
		TT	6	2	0.019	NS	5.53 (1.11–27.43)

Table 4. The Distribution of Two SNPs of *ERAP1* in Patients With BD and Healthy Controls by Sex Basis

		Male				Female			
SNP	Allele/ Genotype	BD, $n = 781$	Controls, $n = 955$	*P* Value	OR (95% CI)	BD, $n = 149$	Controls, $n = 749$	*P* Value	OR (95% CI)
rs1065407	A	1429	1819	7.644×10^{-6}	0.54 (0.41–0.71)	268	1429	1.616×10^{-4}	0.43 (0.28–0.68)
	AA	655	864	3.484×10^{-5}	0.55 (0.41–0.73)	120	684	8.590×10^{-5}	0.39 (0.24–0.64)
	AC	119	91	2.853×10^{-4}	1.71 (1.28–2.28)	28	61	7.113×10^{-5}	2.61 (1.60–4.25)
	CC	7	0	—	—	1	4	NS	1.26 (0.14–11.33)
rs10050860	C	1445	1833	1.012×10^{-5}	0.52 (0.39–0.70)	276	1432	0.030	0.58 (0.35–0.95)
	CC	669	879	2.070×10^{-5}	0.52 (0.38–0.70)	128	684	0.040	0.58 (0.34–0.98)
	CT	107	75	7.599×10^{-6}	1.86 (1.36–2.54)	20	64	NS	1.66 (0.97–2.84)
	TT	5	1	NS	6.15 (0.72–52.72)	1	1	NS	5.05 (0.31–81.26)

The Expression of *ERAP1*

As shown above, rs1065407 and rs10050860 of *ERAP1* were shown to be associated with BD. We further evaluated the expression of *ERAP1* in PBMCs derived from 16 healthy individuals with a known SNP rs1065407 genotype and 16 healthy individuals with a known SNP rs10050860 genotype. The expression of *ERAP1* showed no significant differences between AA carriers and AC/CC carriers of rs1065407 ($P = 0.963$) and CC carriers and CT/TT carriers of rs10050860 ($P = 0.674$) in nonstimulated PBMCs (Fig. 1). However, after stimulation by LPS, the *ERAP1* expression in AA carriers was higher than that in AC or CC carriers ($P = 0.022$) for rs1065407, and expression in CC carriers was higher than CT or TT carriers ($P = 0.018$) for rs10050860 (Fig. 1). We also observed that *ERAP1* mRNA levels of AA individuals and AC or CC individuals of rs1065407 were significantly increased following stimulation with LPS ($P = 2.059 \times 10^{-5}$; $P = 0.006$, respectively) when compared with nonstimulated PBMCs (Fig. 1). Similarly, *ERAP1* expression of CC individuals and CT or TT individuals of rs10050860 was significantly higher when PBMCs had been stimulated with LPS as compared to nonstimulated PBMCs of CC and CT or TT carriers ($P = 8.118 \times 10^{-5}$; $P = 7.932 \times 10^{-5}$, respectively) (Fig. 1).

We also measured the expression of *ERAP1* in PBMCs obtained from active BD patients before they received immunosuppressive treatment ($n = 9$), and found that the mean expression of ERAP1 in active BD patients was significantly lower than that observed in healthy controls ($P = 3.762 \times 10^{-4}$) (Fig. 2).

Discussion

In this study, we show an association of *ERAP1* polymorphisms with BD in a Chinese Han population. Individuals carrying the AA genotype of rs1065407 and CC genotype of rs10050860 had a lower risk of developing BD. Functional assays showed that carriers of the protective variant had a higher *ERAP1* expression.

Behçet's disease is a systemic, immune-mediated vasculitis of small and large vessels affecting veins and arteries. Behçet's disease occurs worldwide, but the strongest incidence is found among people from the Middle and Far East, including Turkey, Iran, Japan, and China, countries also showing the highest frequency of *HLA-B*51*.[3] Behçet's disease is one of the most commonly seen uveitis entities (16.5%) in China, affecting young people and often leading to visual impairment despite aggressive immunosuppressive treatment.[33] Studies that may unravel the pathogenic pathways leading to this disease are therefore of utmost importance because they may offer new tools to prevent the blinding complications of this disease.

Recent GWAS and case-control studies have revealed that naturally occurring *ERAP1* polymorphisms are linked with

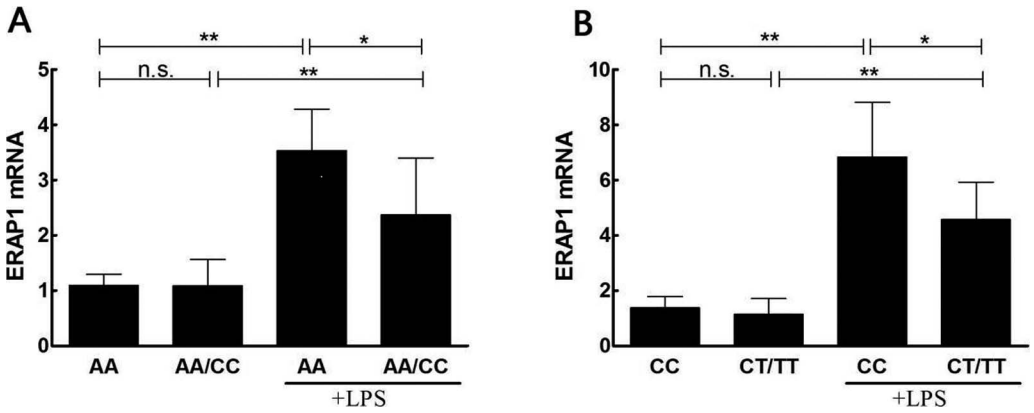

Figure 1. Effect of genotype on *ERAP1* mRNA expression of rs1065407 (**A**) and rs10050860 (**B**) by PBMCs. Peripheral blood mononuclear cells stimulated by LPS show an increased *ERAP1* expression compared with nonstimulated PBMCs. Endoplasmic reticulum aminopeptidase 1 mRNA levels in AA individuals of SNP rs1065407 (**A**) and CC individuals of SNP rs10050860 (**B**) were significantly higher than AC/CC individuals and CT/TT individuals, respectively. Peripheral blood mononuclear cells were derived from healthy individuals ($n = 8$ per group). Data are shown as mean ± SD. $^{**}P < 0.01$; $^{*}P < 0.05$.

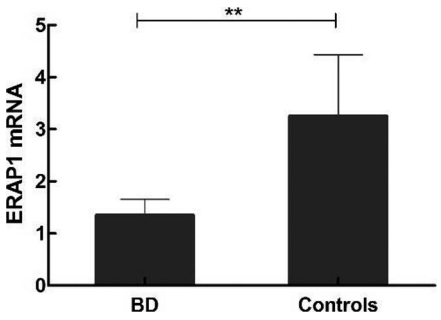

FIGURE 2. Endoplasmic reticulum aminopeptidase 1 expression in PBMCs derived from untreated patients with active BD ($n = 9$) was significantly lower than in healthy individuals ($n = 16$). Data are shown as mean ± SD. $^{**}P < 0.01$.

several MHC class I–associated diseases, including AS,[34,35] psoriasis,[15] and BD.[30,36] We undertook the study presented here in view of the fact that an association of *ERAP1* with BD has not yet been reported for patients from China. Our results confirm the *ERAP1* rs10050860 association with BD reported in BD patients from Turkey[30] and Spain.[36] Besides, the stratified analysis of *ERAP1* rs10050860 association with BD by sex showed that more significant differences were found in the male population than in the female population, which suggests that *ERAP1* rs10050860 may have a sex-skewed impact on BD in males.

European studies in AS have shown that the A allele of rs1065407 and C allele of rs10050860 were reported as risk factors.[21,26] The difference in risk allele of *ERAP1* gene polymorphisms with AS as compared with BD is remarkable, but may be due to differences in the pathogenesis of these two disorders. In AS, peptide handling via HLA-B27 may lead to disease by a mechanism that is different from the HLA-B51–mediated peptide handling that may result in the development of BD. As mentioned above, the two SNPs influenced *ERAP1* expression. When comparing genotype effects using nonstimulated PBMCs, no effects were observed. Lipopolysaccharide stimulation raised the *ERAP1* expression regardless of the genotype investigated, whereby the rs1065407 AA genotype and rs10050860 CC genotype showed a higher *ERAP1* expression compared with the other genotypes. We studied the effect of *ERAP1* genotype on its mRNA expression in healthy controls to rule out confounding effects in patients due to the degree of inflammation and/or immunosuppressive treatment. Earlier studies demonstrated that LPS and IFN synergistically induced ERAP1 secretion from the endoplasmic reticulum in the murine macrophage cell line RAW264.7 via a Toll-like receptor–mediated signaling pathway and that the secreted ERAP1 directly enhanced the phagocytic activity of both RAW264.7 cells and murine peritoneal macrophages.[37,38] These authors noted an increased expression of *ERAP1* induced by LPS as well. Previous studies also found that peptide handling by ERAP1 might play a role in the pathogenesis of immune disorders such as AS.[34] Cancer studies revealed that the absence or downregulated expression of *ERAP1* is closely related to metastasis and invasion of lymph nodes in ovarian carcinoma.[39] Others have shown that *ERAP1* downregulation and partial HLA class I loss are associated with decreased survival in cervical carcinoma, with *ERAP1* loss being an independent predictor for survival.[40] Abrogation of endoplasmic reticulum aminopeptidase associated with antigen progressing (*ERAPP*) in mice induces a conformational change in the MHC class I complexes resulting in the stimulation of both innate and adaptive immune responses and the rejection of a murine lymphoma that is otherwise refractory to immune elimination.[41] Knockout of the *ERAAP* gene elicited a $CD8^+$ T-cell response specific for a tumor-associated antigen that is normally destroyed by ERAAP.[42] These studies support the function of *ERAP1* in antigen presentation and suggest that proper trimming and binding to HLA class I may play a role in the pathogenesis of BD. Moreover, we found that *ERAP1* expression in healthy controls was significantly higher than BD patients, which also supports the above-mentioned hypothesis. *ERAP1* SNPs may lead to alterations in *ERAP1* expression and/or function, leading to decreased trimming of relevant epitopes or altered substrate specificity. Expression within the normal range and proper functioning of *ERAP1* are probably necessary to maintain immune tolerance.

There are a number of limitations in our study that we would like to mention. Although we tried to match the controls for sex, 84% of cases were male whereas only 56% of controls were male. The association found is small because 91% of healthy Chinese are rs1065407 AA carriers as compared with 83% of the BD patients. Similar differences are found for rs10050860 CC (92% in controls versus 86% in BD). Of interest is the observation that the genotype frequency of rs10050860 CC is much lower in a European population (62%) as compared with Chinese Han (92%).[21] The association of *ERAP1* with BD is only one factor among many immune response–related genes that are now emerging to play a role in BD.[43] Further research is needed to elucidate the relative contributions of each of them. It is known that BD is a systemic disease and that the patients recruited from an ophthalmology department may represent a subpopulation of this disease. Therefore, the susceptible SNPs identified in our study may only be associated with uveitis in BD and it is therefore necessary to expand our studies and also investigate the association of *ERAP1* with BD in Han Chinese patients from other medical departments. We showed that active BD patients had a lower *ERAP1* expression compared with controls but further longitudinal studies are needed to examine the effect of treatment and disease activity on *ERAP1* expression during the course of the disease. Given that the examination of HLA-B51 was not performed in all BD patients and healthy controls, we did not assess the role of *HLA-B51* in our study and did not investigate whether there is an interaction with *ERAP1* polymorphisms and *HLA-B51*. This deserves further study and would support the role of *ERAP1* in the presentation of antigens via HLA-B51. In conclusion, our study showed that rs1065407 and rs10050860 polymorphisms in the *ERAP1* gene contribute to the genetic susceptibility of BD. In addition, our study suggested that the gene variants identified may affect the genetic predisposition by modulating the expression of *ERAP1*.

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