

# Lack of association of two polymorphisms of *IRF5* with Behcet's disease

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**Purpose:** Interferon regulation factor 5 (*IRF5*) is a member of the *IRF* family of transcription factors that control the transactivation of type I interferon system-related genes as well as the expression of several other genes involved in immune response. Here, we investigated its association with Behcet's disease (BD) in a well defined group of Chinese Han patients.

**Methods:** A total of 152 unrelated Chinese patients with BD and 149 healthy blood donors were genotyped for *IRF5* rs2280714 and rs752637 polymorphisms. Genomic DNA was isolated from peripheral blood mononuclear cells. Genotyping of each single nucleotide polymorphism (SNPs) was performed by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP). Allele and genotype frequencies of *IRF5* rs2280714 and rs752637 polymorphisms were compared between patients and controls using a two-sided  $\chi^2$  test.

**Results:** The results showed no significant difference concerning the frequency of the allele of rs2280714 and rs752637 polymorphisms between BD patients and the normal controls ( $p=0.647$  and  $p=0.105$ , respectively). The frequencies of the genotype of rs2280714 and rs752637 were not different between BD patients and the normal controls ( $p=0.233$  and  $p=0.266$ , respectively). Clinical manifestation stratification analysis did not show any association of *IRF5* polymorphisms with BD patients ( $p>0.05$ ).

**Conclusions:** Our study revealed that the rs2280714 and rs752637 SNPs were not associated with the susceptibility to BD. There was no association between the two polymorphisms of *IRF5* and any extraocular clinical manifestations in BD.

Behcet's disease (BD) is a chronic autoimmune disease in which both genetic and environmental factors are involved. It is a common uveitis entity in China, Japan, and the Middle East countries [1]. BD is an autoimmune disease that results from damage to blood vessels throughout the body [2]. However, what triggers this reaction is unclear.

The family of interferon regulatory factors (*IRF*) had been implicated in providing a principal basis for host resistance against pathogens [3]. The family also plays a role in the regulation of the development of the immune system and the production of pro-inflammatory cytokine [4-7]. Studies have demonstrated that *IRF1* is associated with Behcet's disease [8] whereas *IRF5* has been found to be associated with certain autoimmune diseases such as systemic lupus erythematosus (SLE) [9,10], inflammatory bowel disease (IBD) [11], rheumatoid arthritis (RA) [12], and multiple sclerosis (MS) [13]. Whether there is an association of *IRF5* with BD is not yet clear. Here, we examined the association of *IRF5* single polymorphisms (rs2280714 and rs752637) with BD in a Chinese Han population.

## METHODS

**Patients and normal controls:** A total of 152 unrelated Chinese patients with BD according to the international study group criteria [14] and 149 age-, sex-, and ethnically-matched healthy controls were included in this study. Samples from patients and controls were genotyped for *IRF5* rs2280714 and rs752637 polymorphisms after they all gave their informed consents. All study subjects were recruited from Zhongshan Ophthalmic Center (Sun Yat-sen University, Guangzhou, P.R. China), the First Affiliated Hospital, Chongqing Medical University (Chongqing, P.R. China), and Daping Hospital (the Third Military Medical University, Chongqing, P.R. China). The study was approved by the local ethics committees of the Third Military Medical University and Chongqing Medical University.

**Genomic DNA extraction and PCR:** Blood samples were collected in ethylenediamine tetra acetic acid (EDTA) tubes and kept at  $-70$  °C until required [15]. Genomic DNA was extracted from peripheral blood mononuclear cells, which was performed by conventional salting of cellular protein, an alcohol precipitation using standard proteinase K digestion, and phenol chloroform extraction procedure [16]. We amplified the target DNA in *IRF5* by polymerase chain reaction (PCR) using the relevant primers. The primers of the two remaining sites were designed using Primer Premier 5.0 software (Premier Biosoft International, Palo Alto, CA). The

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TABLE 1. THE PRIMERS AND ENZYMES USED FOR PCR-RFLP GENOTYPING.

| SNP       | Forward primer sequence         | Reverse primer sequence       | Enzyme |
|-----------|---------------------------------|-------------------------------|--------|
| rs2280714 | 5'-CGTGGTCACATTGGTGATGCTC-3'    | 5'-GGCTCTTCTCTCCAAGGCAGACA-3' | BSMFI  |
| rs752637  | 5'-AAGGTGCCAGAAAGAAGCTTCTTAC-3' | 5'-GCACCTGGGAAATCACCCCTTTT-3' | BCEAI  |

The details of the primers and enzymes used for PCR-RFLP genotyping.

TABLE 2. GENOTYPE AND ALLELE DISTRIBUTIONS OF *IRF5* rs2280714 AND rs752637 POLYMORPHISMS IN BD PATIENTS AND CONTROLS.

| <i>IRF5</i> SNPs | Genotype allele | Patients   | Normal controls | p value | OR (95%CI)          |
|------------------|-----------------|------------|-----------------|---------|---------------------|
| rs2280714        | AA              | 51 (56.1)  | 60 (54.9)       | 0.233   |                     |
|                  | AG              | 74 (66.7)  | 58 (65.3)       | 0.233   |                     |
|                  | GG              | 27 (29.3)  | 31 (28.7)       | 0.233   |                     |
|                  | A               | 176 (57.9) | 178 (59.7)      | 0.647   | 0.927 (0.670–1.283) |
|                  | G               | 128 (42.1) | 120 (40.3)      | 0.647   | 1.079 (0.780–1.493) |
| rs752637         | AA              | 64 (59.0)  | 49 (54.0)       | 0.266   |                     |
|                  | AG              | 68 (68.4)  | 63 (62.6)       | 0.266   |                     |
|                  | GG              | 20 (24.5)  | 27 (22.5)       | 0.266   |                     |
|                  | A               | 196 (64.5) | 161 (57.9)      | 0.105   | 1.319 (0.944–1.843) |
|                  | G               | 108 (35.5) | 117 (42.1)      | 0.105   | 0.758 (0.543–1.059) |

BD=Behcet's disease, OR=odds ratio, 95% CI=95% confidence interval. The genotype and allele distributions of *IRF5* rs2280714 and rs752637 polymorphisms in BD patients and controls. No significant differences of the genotype and allele distributions were found between patients and controls.

details of the primers and enzymes used for PCR-RFLP genotyping are presented in Table 1.

Each PCR was performed in 10 µl of solution containing 5 µl premix Taq (Takara Biotechnology Co. Ltd, Dalian, P.R. China), 10 pmol primers, and 0.2 µg of genomic DNA. The cycling profile was: initial denaturation at 94 °C for 4 min, 45 cycles consisting of 94 °C for 5 s, proper annealing temperature 63 °C for 30 s, 72 °C for 30 s, and a final extension for 4 min at 72 °C.

**Genotyping:** Genotyping of each single nucleotide polymorphism (SNP) was performed by PCR restriction fragment length polymorphism (PCR-RFLP). PCR products of rs2280714 and rs752637 were respectively digested with 2 µl of BSMFI, BCEAI restriction enzymes (Fermentas, MBI, Vilnius, Lithuania) in a 10 µl reaction volume overnight. Digestion products were visualized on agarose of 2%–3% concentration and stained with Goldview™ (SBS Genetech, Beijing, P.R. China). BSMFI digestion of 130 bp PCR product of AA genotype gives two fragments, the GG gives one fragment, and the AG gives three fragments. BCEAI digestion of 120 bp PCR product of AA genotype gives two fragments, the GG gives one fragment, and the AG gives three fragments. To confirm the accuracy of the method employed, randomly selected subjects (20% of all samples) were analyzed by direct sequencing (Invitrogen Biotechnology Co., Guangzhou, P.R. China). An appropriate control was included in each typing run.

**Statistical analysis:** Allele and genotype frequencies of *IRF5* rs2280714 and rs752637 polymorphisms were compared between patients and controls. Statistical analysis was performed with the SPSS version 10.0 for Windows (SPSS

Inc., Chicago, IL). We evaluated the frequency of genotypes and alleles in this study using the  $\chi^2$  test. The association of rs2280714 and rs752637 with the extraocular findings was evaluated by logistic regression and statistical significance was taken when  $p < 0.05$ .

## RESULTS

The average age of the BD patients was 32.7±6.4 years and that of the healthy controls was 33.8±9.3 years. No statistical difference in the distribution of age was observed between BD patients and controls ( $p > 0.05$ ).

One hundred and fifty-two patients and one hundred forty-nine normal controls were genotyped for two *IRF5* SNPs. It is worthwhile to point out that some genotypes were missing for controls for rs752637 SNPs due to insufficient amount of DNA in some samples. The results showed that there was no deviation from the Hardy–Weinberg equilibrium (HWE). The genotype and allele frequencies of the rs2280714 SNPs in BD patients were not different from those in normal controls ( $p = 0.233$  and  $p = 0.647$ , respectively; Table 2). A similar result was found for rs752637 SNP versus normal controls ( $p = 0.266$  and  $p = 0.105$ , respectively; Table 2). Stratification analysis according to oral ulcer, genital ulcer, hypopyon, skin lesions, and arthritis did not show any association of *IRF5* polymorphisms with these parameters (Table 3;  $p > 0.05$ ).

## DISCUSSION

It has been demonstrated that both genetic and environmental factors are involved in the development of BD [17]. In this study, we investigated the association of *IRF5* polymorphisms

TABLE 3. THE CLINICAL MANIFESTATIONS OF BD PATIENTS.

| Clinical manifestations | N   | Percentage |
|-------------------------|-----|------------|
| Uveitis                 | 152 | 1%         |
| Oral ulcer              | 127 | 0.836%     |
| Genital ulcer           | 64  | 0.421%     |
| Hypopyon                | 41  | 0.27%      |
| Skin lesions            | 84  | 0.553%     |
| Arthritis               | 43  | 0.283%     |

N=number of patients; BD=Behcet's disease. This table summarizes the proportion of clinical manifestations in relation to the BD patients

with BD in the Chinese Han population. Our study showed that [rs2280714](#) and [rs752637](#) were not associated with BD. There was no association between the polymorphisms of *IRF5* with any extraocular clinical manifestations.

BD is a common multisystem inflammation disorder characterized by recurrent oral and genital ulceration, multiple skin lesions and uveitis [18,19]. It has a distinctive geographical distribution. *IRF5* has been shown to be strongly associated with certain autoimmune diseases in different ethnic populations [10,11,20,21] and to play a critical role in the pathogenesis of autoimmunity [22]. Therefore, this gene has been considered as a general susceptible gene for autoimmune diseases. However, the genetic factors associated with this disease in Chinese Han patients have been scarcely studied. In this study, we focused on the association of two SNPs of *IRF5* with BD in the Chinese patients. As population stratification may lead to a false-positive result in a case-control study, we strictly selected the BD patients and controls with Chinese Han nationality. To validate the result of genotyping by PCR-RFLP, 20% of the samples were directly sequenced.

Unfortunately, in this study, we did not find any association of [rs2280714](#) and [rs752637](#) SNPs with BD. A similar result was observed after stratification analysis based on extraocular features. These results are not consistent with those seen in other autoimmune diseases reported in European and some other Asian populations [10,12,20,21]. This could be explained by the fact that the etiology and pathogenesis of BD may be different from those of the other autoimmune diseases [2,18,19]. The other possibility could be that Chinese Han people may have a different genetic background from other ethnic populations [2,19]. Additionally, association of some other *IRF5* SNPs should be tested in BD.

Lee and coworkers found the association of *IRF1* polymorphism with Behcet's disease in Koreans [8]. Here, we failed to find the association of *IRF5* with BD. The inconsistent result can be explained by the different immune function of IRF1 from IRF5. Both of them interact with MyD88, an adaptor for multiple innate immune receptors, but induce a distinctively different set of pro-inflammatory cytokines. These results suggest that IRF1 and IRF5 play

different roles in the production of pro-inflammatory cytokine and the regulation of the development immune system, that is to say, different mechanisms may be induced by IRF1 than by IRF5 in the development of BD disease.

In conclusion, our study revealed that the [rs2280714](#) and [rs752637](#) SNPs were not associated with the susceptibility to BD. There was no association between the two polymorphisms of *IRF5* with any clinical manifestations. Further studies are needed to investigate the association of other SNPs in *IRF5* with BD.

#### ACKNOWLEDGMENTS

This work was supported by the Project of Chongqing Key Laboratory of Ophthalmology (CSTC, 2008CA5003) and was finished in the Chongqing Key Laboratory of Ophthalmology.

#### REFERENCES

- Keino H, Okada AA. Behcet's disease: global epidemiology of an Old Silk Road disease. *Br J Ophthalmol* 2007; 91:1573-4. [PMID: 18024803]
- Yang PZ. Concern on the changes of clinical pattern and etiological factors in uveitis in China. *Zhonghua Yan Ke Za Zhi* 2008; 44:865-6. [PMID: 19176110]
- Sato M, Taniguchi T, Tanaka N. The interferon system and interferon regulatory factor transcription factors—studies from gene knockout mice. *Cytokine Growth Factor Rev* 2001; 12:133-42. [PMID: 11325597]
- Yanai H, Chen HM, Inuzuka T, Kondo S, Mak TW, Takaoka A, Honda K, Taniguchi T. Role of IFN regulatory factor 5 transcription factor in antiviral immunity and tumor suppression. *Proc Natl Acad Sci USA* 2007; 104:3402-7. [PMID: 17360658]
- Schoenemeyer A, Barnes BJ, Mancl ME, Latz E, Goutagny N, Pitha PM, Fitzgerald KA, Golenbock DT. The interferon regulatory factor, IRF5, is a central mediator of toll-like receptor 7 signaling. *J Biol Chem* 2005; 280:17005-12. [PMID: 15695821]
- Barnes BJ, Kellum MJ, Pinder KE, Frisancho JA, Pitha PM. Interferon regulatory factor 5, a novel mediator of cell cycle arrest and cell death. *Cancer Res* 2003; 63:6424-31. [PMID: 14559832]
- Barnes BJ, Kellum MJ, Field AE, Pitha PM. Multiple regulatory domains of IRF-5 control activation, cellular localization, and induction of chemokines that mediate recruitment of T

- lymphocytes. *Mol Cell Biol* 2002; 22:5721-40. [PMID: 12138184]
8. Lee YJ, Kang SW, Song JK, Baek HJ, Choi HJ, Bae YD, Ryu HJ, Lee EY, Lee EB, Song YW. Associations between interferon regulatory factor-1 polymorphisms and Behcet's disease. *Hum Immunol* 2007; 68:770-8. [PMID: 17869652]
  9. Kelly JA, Kelley JM, Kaufman KM, Kilpatrick J, Bruner GR, Merrill JT, James JA, Frank SG, Reams E, Brown EE, Gibson AW, Marion MC, Langefeld CD, Li QZ, Karp DR, Wakeland EK, Petri M, Ramsey-Goldman R, Reveille JD, Vilá LM, Alarcón GS, Kimberly RP, Harley JB, Edberg JC. Interferon regulatory factor-5 is genetically associated with systemic lupus erythematosus in African Americans. *Genes Immun* 2008; 9:187-94. [PMID: 18288123]
  10. Kawasaki A, Kyogoku C, Ohashi J, Miyashita R, Hikami K, Kusaoi M, Tokunaga K, Takasaki Y, Hashimoto H, Behrens TW, Tsuchiya N. Association of IRF5 polymorphisms with systemic lupus erythematosus in a Japanese population: support for a crucial role of intron 1 polymorphisms. *Arthritis Rheum* 2008; 58:826-34. [PMID: 18311811]
  11. Dideberg V, Kristjansdottir G, Milani L, Libiouille C, Sigurdsson S, Louis E, Wiman AC, Vermeire S, Rutgeerts P, Belaiche J, Franchimont D, Van Gossum A, Bours V, Syvänen AC. An insertion-deletion polymorphism in the interferon regulatory Factor 5 (IRF5) gene confers risk of inflammatory bowel diseases. *Hum Mol Genet* 2007; 16:3008-16. [PMID: 17881657]
  12. Rueda B, Reddy MV, González-Gay MA, Balsa A, Pascual-Salcedo D, Petersson IF, Eimon A, Pairs S, Scherbarth HR, Pons-Estel BA, González-Escribano MF, Alarcón-Riquelme ME, Martín J. Analysis of IRF5 gene functional polymorphisms in rheumatoid arthritis. *Arthritis Rheum* 2006; 54:3815-9. [PMID: 17133578]
  13. Kristjansdottir G, Sandling JK, Bonetti A, Roos IM, Milani L, Wang C, Gustafsdottir SM, Sigurdsson S, Lundmark A, Tienari PJ, Koivisto K, Elovaara I, Pirttilä T, Reunanen M, Peltonen L, Saarela J, Hillert J, Olsson T, Landegren U, Alcina A, Fernández O, Leyva L, Guerrero M, Lucas M, Izquierdo G, Matesanz F, Syvänen AC. Interferon regulatory factor 5 (IRF5) gene variants are associated with multiple sclerosis in three distinct populations. *J Med Genet* 2008; 45:362-9. [PMID: 18285424]
  14. Mignogna MD, Fedele S, Lo Russo L. International diagnostic criteria and delay of diagnosis in Behcet's disease. *J Rheumatol* 2000; 27:2725. [PMID: 11093466]
  15. 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]
  16. Hao S, Li PF, Zhao JL, Hu YL, Hou YY. 17beta-estradiol suppresses cytotoxicity and proliferative capacity of murine splenic NK1.1+ cells. *Cell Mol Immunol* 2008; 5:357-64. [PMID: 18954559]
  17. Salvarani C, Pipitone N, Catanoso MG, Cimino L, Tumiati B, Macchioni P, Bajocchi G, Olivieri I, Boiardi L. Epidemiology and clinical course of Behcet's disease in the Reggio Emilia area of Northern Italy: a seventeen-year population-based study. *Arthritis Rheum* 2007; 57:171-8. [PMID: 17266063]
  18. 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]
  19. 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]
  20. Siu HO, Yang W, Lau CS, Chan TM, Wong RW, Wong WH, Lau YL, Alarcon-Riquelme ME. Association of a haplotype of IRF5 gene with systemic lupus erythematosus in Chinese. *J Rheumatol* 2008; 35:360-2. [PMID: 18260169]
  21. Maalej A, Hamad MB, Rebaï A, Teixeira VH, Bahloul Z, Marzouk S, Farid NR, Ayadi H, Cornelis F, Petit-Teixeira E. Association of IRF5 gene polymorphisms with rheumatoid arthritis in a Tunisian population. *Scand J Rheumatol* 2008; 37:414-8. [PMID: 18752149]
  22. Taniguchi T, Ogasawara K, Takaoka A, Tanaka N. IRF family of transcription factors as regulators of host defense. *Annu Rev Immunol* 2001; 19:623-55. [PMID: 11244049]