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IL10 polymorphisms associated with Behçet's disease in Chinese Han

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ABSTRACT

Objective: IL-10 is a potent anti-inflammatory cytokine that plays important roles in the pathogenesis of Behçet's disease (BD). Two genome-wide association studies have identified *IL10* as a potential risk factor for BD. Here, we investigated the association between *IL10* polymorphisms and BD in Chinese Han.
Methods: 407 BD patients and 679 healthy controls were enrolled, and genotyped by Sequenom MassArray system (Sequenom iPLEX assay, San Diego, CA).
Results: The frequency of risk allele of rs1800871 was notably higher in BD patients than in controls (71.9% vs. 66.2%, OR: 1.30, 95%CI: 1.08–1.58, $p_c = 0.024$). Similarly, rs1518111, which showed strong linkage disequilibrium ($r^2 = 1$) with allele rs1800871, was also associated with BD ($p_c = 0.026$). Rs3021094 was in association with BD in a dominant model ($p_c = 0.035$), and the haplotype (GACC) formed by rs1518111, rs3021094, rs3790622, and rs1800871 was associated with BD ($p_c = 0.023$). Results obtained from meta-analysis combined with our data showed that rs1800871 and rs1518111 were associated with BD.
Conclusion: IL10 may be the susceptibility gene for BD in Chinese Han population.

1. Introduction

Behçet's disease (BD) is a chronic auto-inflammatory disorder classified as a systemic vasculitis and characterized by recurrent aphthous ulceration, genital ulcers, ocular inflammation, and skin lesions. BD frequently involves the joints, central nervous system, and gastrointestinal tract [1]. It occurs most frequently in the Far East and the Mediterranean basin, a region consistent with the Old Silk road [2]. The highest prevalence of BD is reported in Turkey (80–420 cases per 100,000 population), therein Caucasian prevalence is significantly lower (0.27–7.5 cases per 100,000 inhabitants) [3,4]. The prevalence of BD in China is unclear, as, to date, no large-scaled epidemiological investigations have been reported.

BD is a complicated disease. Its etiology involves the intricate interplay of putative environmental triggers and genetic predispositions [5]. It has been reported that the sibling recurrence risk ratio (λs), a common indicator for familial aggregation, was between 11.4 and 52.5 for BD patients, suggesting that genetic factors play central roles in the pathogenesis of BD [6]. *HLA-B*51* shared the strongest association with BD across different ethnicities [7], yet it contributed to less than 19% of the genetic susceptibility in siblings of BD [8], which indicated that the effects of non-major histocompatibility complex genes should not be ignored. Two genome-wide association studies (GWAS) undertaken in Turkey and Japan revealed that *HLA-B*51*, *HLA-A*26*, *IL10*, and *IL23R-IL12RB2* were susceptibility loci for BD [9,10], while GWAS undertaken in China and Korea showed that *STAT4* and *GIMAP* were BD risk genes [11,12]. A more recent GWAS also found new risk loci in *CCR1* and *KLRC4* [13].

IL-10 is a pleiotropic cytokine exerting anti-inflammatory effects that are mediated by the down-regulation of the Th1 cytokines TNF-α, IL-1, IL-8, and IFN-γ [14]. In addition, as much as 75% of the inter-individual variability in human IL-10 expression has been attributed to genetic variations [15]. The gene coding for IL-10 is mapped to chr.1q32.2, and the single nucleotide polymorphisms (SNPs) of *IL10* are recognized as risk factors for multiple autoimmune and inflammation diseases, including systemic lupus erythematosus (SLE), type 1 diabetes (T1DM), rheumatoid arthritis

Abbreviations: BD, Behçet's disease; SLE, systemic lupus rythematosus; RA, rheumatoid arthritis; UC, ulcerative colitis; GWAS, genome-wide association studies; SNP, single nucleotide polymorphism; LD, linkage disequilibrium.

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(RA), ulcerative colitis (UC), systemic sclerosis (SSc), Churg-Strauss syndrome (CSS), and juvenile idiopathic arthritis (JIA) [16–19]. While GWAS and candidate gene association studies have identified *IL10* as a risk locus for BD in Turkish, Japanese and Iranian populations [9,10,20], studies have never been conducted in the Chinese Han population. Considering the significant ethnic differences and population heterogeneity, it remains important to determine whether *IL10* SNPs are associated with BD in Chinese Han.

2. Materials and methods

2.1. BD patients and controls

BD patients, totaling 407, and 679 healthy controls were enrolled. All subjects were self-reported Han Chinese and unrelated to one another. The patients all satisfied the International Study Group Criteria of BD [21] and were recruited from the Peking Union Medical College Hospital from October 2011 to November 2012. Patients with other autoimmune diseases including SLE, RA, T1D or primary Sjögren's syndrome (pSS) were excluded. Healthy controls were enrolled during their physical examination from the same hospital. These participants did not have any autoimmune disorders or family history of BD. The study was approved by the local ethics committee of Peking Union Medical College Hospital, and all subjects provided written informed consent of their participations in the study.

2.2. Selection of SNPs

We determined the physical position of the *IL10* gene using the UCSC browser (http://genome.ucsc.edu/)[22]. As the position information in Hapmap was hg18 [Mar.2006, (NCBI36/hg18)], we used chr1:205,007,571–205,012,462 as the position for the *IL10* gene (hg19 chr1:206,940,948–206,945,839). Considering the important roles of the promoter and regulatory regions, we expanded the region to 7 kb upstream and downstream (chr1:205,000,571–205,019,462). We set the following parameters as follows: pairwise modes of analysis, LD values of $r^2 \geqslant 0.8$ and minor allele frequency (MAF) values $\geqslant 0.05$. We then selected tagging SNPs of *IL10* gene from HapMap CHB data (Hapmap Data Rel 27 PhaseII + III, Feb 09, on NCBI 36 assembly, dbSNP126) by Haploview v4.2 [23,24]. There were a total of eight common SNPs in *IL10*, four were selected as tagger SNPs (rs3021094, rs3790622, rs1800871 and rs2945417).

Although rs1518111 shared strong linkage disequilibrium (LD) with rs1800871 ($r^2 = 1$), it was significantly associated with BD as indicated by the GWAS [9]. As such, we included rs1518111 in this study. Each SNP is detailed in Supplementary Table 1.

2.3. Genotyping

A 2 ml blood sample in ethylenediaminetetraacetic acid (EDTA) anticoagulant tube was collected from each participant. DNA was isolated from peripheral white blood cells using Bioteke kits (China) following the manufacturer's instructions. DNA was genotyped by the Sequenom MassArray system (Sequenom iPLEX assay, CA), according to the manufacturer's instructions. The primers for the multiplex polymerase chain reaction andthe locus-specific extension of SNPs were designed by the MassArray Assay Design 3.0 (Sequenom, CA). The final products were then desalted and spotted on a 384-element SpectroCHIP array (Sequenom, CA). Allele detection was performed by matrix-assisted laser desorption ionization–time-of-flight mass spectrometry. The resultant mass spectrograms and genotype data were analyzed by MassArray Typer (Sequenom, CA).

2.4. Statistical analysis

The Hardy–Weinberg equilibrium (HWE) was analyzed in each SNP by chi-square test. SNPs which deviated from HWE ($p < 0.05$) in controls were excluded from subsequent analyses. Data analysis was conducted using PLINK 1.07 [25]. Significance was set at 0.05 (two-tailed). The odds ratio (OR) and 95% confidence interval (95%CI) were calculated. Bonferroni adjustment was performed for multiple comparisons. Haplotype analysis was conducted by Haploview v4.2 [23].

2.5. Meta analysis

Meta analysis was performed by STATA software, version 12.0, (Stata Corp., TX). The associations between rs1800871, rs1518111, and BD were estimated by crude OR and 95%CI. Inter-study heterogeneity was assessed by *Q*-test (p value [p_{het}] < 0.10 was regarded as statistically significant heterogeneity) and I^2 statistics [26]. If no significance between study heterogeneity was detected, a fixed-effects model was used. Otherwise, a random-effects model was used.

Studies relating associations between *IL10* SNPs and BD were carefully retrieved by searching both PubMed and Embase databases in February 2013. Studies fulfilling the following inclusion criteria were included in the meta-analysis: (1) case-control studies; (2) studies relating an association between *IL10* SNPs (rs1518111 and rs1800871) and BD; (3) studies with allele distributions in both cases and controls available for estimating an OR with 95%CI; (4) independent studies without repeat reports on the same populations or subpopulations.

3. Results

3.1. Characteristics of participants

A total of 407 BD and 679 controls were genotyped, with a call rate more than 99% except for rs2945417 (70%). Rs2945417 was then excluded from further analysis due to the low call rate. The average age of participants was 38.02 ± 12.44 years and 38.81 ± 10.45 years for the BD and healthy control subjects, respectively. The gender ratio (male/female) in the BD group was 1.24, while that for controls was 1.16 (Table 1). The distribution of genotype frequencies of the four remaining SNPs in the control group did not show any deviation from HWE ($p > 0.05$). Power analysis revealed more than 80% power ($\alpha = 0.05$) for detecting association with an OR of 1.00–1.56 for both heterozygotes and homozygotes, based on an assumption of 0.25% BD prevalence in a Chinese population and 0.51 for the risk allele frequency, similar to the allele frequencies of the tested SNPs in Asian studies [27].

Table 1
Clinical data for BD patients and controls.

Characteristic	BD patients	Controls
Number of subjects	407	679
Sex ratio(male/female)	1.24	1.16
Average age	38.02 ± 12.44	38.81 ± 10.45
Clinical symptoms, No/total (%)	–	–
Oral aphthous ulcers	401/407 (98.5)	–
Genital ulcers	310/407 (76.2)	–
Skin manifestations	239/407 (58.7)	–
Ocular manifestations	116/407 (28.5)	–