

Materials and Methods

Subjects

There were 798 subjects (400 psoriasis patients and 398 healthy controls) recruited in this study. 400 patients with psoriasis vulgaris, confirmed by dermatologists, were recruited from the Second Affiliated Hospital of Harbin Medical University. Psoriasis area and severity index (PASI) scores, which have become evaluating indicators of psoriasis levels, were calculated according to the degree of erythema, desquamation, and the infiltration degree of lesions, together with the surface extension [12]. A PASI score over 12 was designated as severe. All patients were subdivided into 2 groups: level 1 (PASI \leq 12, n = 316) and level 2 (PASI $>$ 12, n = 84). In terms of onset age, 292 patients were considered to belong to the early-onset group since their age of onset was before or at 40 years; the other 108 patients were in the late-onset group as their age of onset was above 40 years. The ethnicity-matched control group, consisting of 398 healthy individuals with no history of psoriasis or any other autoimmune/inflammatory disease, was from outpatients of the same hospital who came for health examinations. The patients and controls were matched for age and gender. Detailed participant information was shown in a previous study and Table 1 [13]. All individuals were of northeastern Han Chinese ancestry and living in northeastern China. The study was approved by the Clinical Research Ethics Committee of Harbin Medical University. All subjects signed an informed consent form prior to entering the study.

SNP Genotyping

Genomic DNA was extracted from peripheral blood anticoagulated in acid-citrate dextrose using a QIAamp DNA Blood Mini Kit (Qiagen, Hamburg, Germany).

Eight SNPs (single-nucleotide polymorphisms), including rs2293152, rs3816769, rs4796793 and rs744166 in STAT3, rs7574865 and rs3024866 in STAT4, and rs324011 and rs3024974 in STAT6, were genotyped using the Multiplex SNaPshot method [14]. Polymerase chain reactions (PCRs) contained 10–50 ng of DNA, 1 \times GC-I buffer (Takara, Japan), 3.0 mmol/L Mg²⁺, 0.3 mmol/L dNTPs, 1 unit HotStarTaq polymerase (Qiagen, Germany) and 0.08 μ mol/L of each primer in a 10- μ l reaction volume. The following touchdown PCR program was used: denaturation at 95°C for 2 min, followed by 11 cycles of 94°C for 20 s, annealing at 65°C for 40 s (decreasing by 0.5°C per cycle), and extension at 72°C for 90 s. This was followed by 24 cycles of denaturation at

94°C for 20 s, annealing at 59°C for 30 s, and extension at 72°C for 90 s and a final extension at 72°C for 2 min. The PCR products were purified by treatment with 2 units exonuclease I (USB Corporation, USA) and 5 units shrimp alkaline phosphatase (USB Corporation, USA) at 37°C for 1 h followed by incubation at 75°C for 15 min. The extension reaction contained 2 × ABI Prism SNaPshot Multiplex ready reaction mix (Applied Biosystems, USA), 0.5 μmol/L of each primer, and 2 μl of each PCR product in a 10-μl reaction volume and was carried out as recommended (Applied Biosystems, USA). The extension PCR products were purified using 1 unit of shrimp alkaline phosphatase and then analyzed using an ABI 3730xl Genetic Analyzer. SNP calling was carried out using GeneMapper™ software v.4.1 (Applied Biosystems, USA), and 5% of the samples were randomly selected and re-genotyped to validate the study results. The concordance rate between duplicate genotyping was 100% without discrepancies in the randomly selected samples (40 samples).

Primer 3 V4.0.0 (<http://primer3.ut.ee/>) was used to design primers to amplify a differently sized fragment for each SNP within a multiplex PCR. Extension primers, again differing in length within a multiplex, were chosen from the sequence immediately up- or downstream of each SNP. The primers for PCR and SNaPshot are listed in Table 2.

Haplotype Analysis

The haplotype block was constructed from the genotypes of the 8 polymorphisms using Haploview 4.1 software (<http://www.broad.mit.edu/mpg/haploview>).

Statistical Analysis

Hardy-Weinberg equilibrium for each SNP was tested using the χ^2 test with expected frequencies derived from the allele frequencies. The χ^2 test was used to compare the frequency distributions of the alleles, genotypes, and haplotypes between patients and controls. The relative risk associated with minor alleles, estimated as an odds ratio with a 95% confidence interval, was calculated. Two-sided tests with statistical significance set at $p < 0.05$ were performed using SPSS 17.0 software for Windows (SPSS Inc., Chicago, IL, USA).