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Association analysis of single nucleotide polymorphisms of proinflammatory cytokine and their receptors genes with rheumatoid arthritis in northwest Chinese Han population

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ABSTRACT

Objective: To analyze the relationship of genetic polymorphisms in IL1β, IL6, TNF-α genes and their receptors genes with rheumatoid arthritis (RA) for northwest Han Chinese. This study also explores whether there are gene–gene interactions among these genetic polymorphisms.
Methods: A total of 452 patients with RA and 373 matched healthy controls were enrolled to carry out a case-control study for 16 SNPs of *IL1B*-511 C > T, *IL1B*-31 T > C, *IL1B*+3954 C > T, *IL1RN* T > C, *IL6*-597 G > A, *IL6*-572 G > C, *IL6*-174 G > C, *IL6R*-183 G > A, *IL6R* exon2 T > A, *IL6R* exon1 A > C, *TNFA*-863 C > A, *TNFA*-857 C > T, *TNFA*-308 G > A, *TNFA*-238 G > A, *TNFR1*-383 A > C and *TNFR2* T676G T > G from seven genes. Genotyping for the SNPs was conducted on the RotorGene 6000 PCR platform using in-house high resolution melting (HRM) approaches. Detection correctness was validated through direct sequencing. Generalized multifactor dimensionality reduction (GMDR) analysis was applied to discover likely gene–gene interaction model among the SNPs.
Results: The results showed that the genotype distributions of *TNFA*-308, *TNFA*-857 and *TNFA*-863 are significantly different between case and control groups ($P = 0.016$, $P = 0.048$ and $P = 0.016$, respectively). Carriers of *TNFA*-857 mutant allele conferred risk to RA (OR = 1.525, 95% CI = 1.157–2.009) while those of *TNFA*-308 and *TNFA*-863 mutant alleles conferred protection to RA (OR = 0.459, 95% CI = 0.286–0.739; OR = 0.490, 95% CI = 0.329–0.732). GMDR analysis for the SNPs indicated that gene–gene interaction existed among *IL1B*-31, *IL1RN*, *IL6*-572, *IL6R*-183, *IL6R*-exon1 and *TNFA*-857. Thirteen of all genotypes of the six SNPs combination were discovered to have significant distribution difference between RA group and the control.
Conclusions: This study demonstrated that PCR-HRM assay is a highly efficient SNP genotyping method especially for the detection of large-scale samples. The SNPs of *TNFA*-308 and *TNFA*-863 are closely associated with RA susceptibility and that gene–gene interactions may occur among the six SNPs of *IL1B*-31, *IL1RN*, *IL6*-572, *IL6R*-183, *IL6R*-exon1 and *TNFA*-857 in RA patients from northwest Chinese Han population, especially these SNPs' combination genotypes CT/TT/CC/GG/AC/CC, CT/TT/GC/AA/AC/CT and CT/CT/CC/GA/AC/CC to show high risk of RA susceptibility in our study.

1. Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disease closely related to proinflammatory cytokines such as IL1β, IL6 and TNF-α, which play fundamental roles in the occurrence and development of RA. The levels of proinflammatory cytokines are found to be higher in synovial and peripheral blood of RA patients. Further studies confirmed that they are involved in the pathogenic processes of RA, especially after successful use of TNF-α and IL1β blockades as a treatment [1,2]. Several association studies on the genetic polymorphisms of the cytokines with RA susceptibility have been performed, but the conclusions from these reports are conflicting and the differences were explained by the collection of subjects are from different races and regions [3–7]. It is now clear that proinflammatory cytokines, and the complex interaction among them, play a key role in the incidence and development of RA [2]. Whether similar interactions exist on the gene level has not yet been explored in previous studies. In this study, we have developed, based on high resolution melting (HRM) technology, PCR genotyping methods for 16 single nucleotide polymorphisms

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(SNPs) from seven genes, and conducted a case-control study for a northwest Chinese Han population. In addition, A generalized multifactor dimensionality reduction (GMDR) analysis was also performed to explores whether there are gene–gene interactions among the 16 SNPs.

2. Materials and methods

2.1. Subjects

A total of 452 northwest Han Chinese RA patients and 373 northwest Han Chinese controls signed informed consents from Lanzhou University Second Hospital from January 2010 to February 2012 to be enrolled in the case-control study. All patients were diagnosed as RA according to Rheumatoid arthritis criteria 2009 released by ACR/EULAR (http://www.medconnect.com.au/tabid/84/ct1/c333948/New-Rheumatoid-Arthritis-Criteria-Released-by-ACREULAR-Panel/Default.aspx) [8]. The inclusion criteria for case were that it must be more than six in quantitative total score including four items of involved joint number, serum indexes, synovitis duration time and acute phase reactant, while the disease course of RA patient must be more than 6 months. The controls were ethnically and geographically matched healthy subjects without RA family history taken from individual check-ups. The study was approved by the Ethics Committee of Lanzhou University Second Hospital.

2.2. Extraction of genomic DNA

Genomic DNA was extracted from 200 μL of venous blood anti-coagulated with EDTA using QuickGene DNA whole blood kit S (Fujifilm, Japan) according to the manufacturer instruction. All DNA samples were detected using the NanoDrop 2000 spectrophotometer (Thermo Scientific, USA) for evaluation of template quality and quantity, and then adjusted to concentrations of 20 ng/μL and stored at −40 °C.

2.3. Primer design

The primer sets for the amplifications of 16 SNPs were designed using the online software Primer 3 (http://primer3plus.com/cgi-bin/dev/primer3plus.cgi) and Beacon designer 7.91 (PREMIER Biosoft, USA). The specificity of primers were checked on Primer-BLAST (http://www.ncbi.nlm.nih.gov/tools/primer-blast). Two-state melting (http://mfold.rna.albany.edu/?q=DINAMelt/Two-state-folding) was used to check the secondary structure of primers. uMELT was used to predict the amplicon melting curve (http://dna.utah.edu/umelt/umelt.html) [9]. The detailed information of these primers was shown in Table 1.

2.4. PCR amplification and HRM genotyping

The 16 SNPs were genotyped on the RotorGene 6000 PCR detection platform (Corbett Life Science, Australia) using in-house PCR-HRM curve analysis assay which has been validated by direct sequencing. HRM is a novel, homogeneous, close-tube, post-PCR method which enables genomic researchers to analyze genetic variations in PCR amplicons just by using HRM fluorescent dye (for example, LcGreen, EvaGreen and Syto9) and direct melting after PCR (http://gene-quantification.com/hrm.html). Genotyping by HRM is a simply, fast and reliable method and wildly used (http://www.dna.utah.edu/Hi-Res/TOP_Hi-Res%20Melting.html). In this study, PCR-HRM was conducted in 15 μL reaction volume which includes 20 ng DNA template, 1.5× Fast EvaGreen® Master Mix for quantitative and high-resolution melting PCR (Biotium, USA), and each primer set ranging from 0.1 M to 0.3 μM based on different amplicons. PCR amplification was performed with initial denaturing at 96 °C for 3 min, followed by 40 cycles of denaturing at 96 °C for 10 s, touchdown annealing from 62 °C to 56 °C for 15 s, and extending at 72 °C for 15 s. After PCR amplification, the samples were heated to 96 °C for 10 s, and then rapidly cooled to 45 °C for 30 s for melting. The HRM was carried out over the range 72–95 °C rising at 0.05 °C s^{-1}, and the genotype of each amplicon was determined using RotorGene 6000 Series Software 1.7. Three samples from each of the 16 SNP genotypes were randomly chosen for sequencing to verify genotyping results.

2.5. Statistical analysis

All data were analyzed using statistical software SPSS 13.0. The relationships of the 16 SNPs with RA susceptibility were assessed using chi-square test for estimation of the odds ratio (OR) and 95% confidence interval for each SNP genotype, and performing binary logistic regression analysis in which healthy condition of subject was defined as dependent variable and their genotypes of 16 SNPs, gender and age were defined as covariates. Gene–gene interaction analysis was performed using DMGR9.0 software [10]. Hardy–Weinberg equilibriums of each SNP in the control group were tested using chi-square test. All the statistics are bilateral probability for inspection, statistical significance was determined as below the conventional level of $p = 0.05$.

Table 1
Sequences and amplicon lengths of 16 SNP primers.

SNP	Allele	Forward primer (5′ → 3′)	Reverse primer (5′ → 3′)	Amplicon
rs16944	*IL1B*-511 C>T	CCCAGCCAAGAAAGGTCAAT	TGAGGGTGTGGGTCTCTACC	100 bp
rs1143627	*IL1B*-31 T>C	TTTCTCAGCCTCCTACTTCTGC	CTTGTGCCTCGAAGAGGTTT	86 bp
rs1143634	*IL1B*+3954 C > T	GGCCTGCCCTTCTGATTTTA	CGTGCACATAAGCCTCGTTA	95 bp
rs4251961	*IL1RN* T > C	CGTGTCATTCATGCTTCCGGTG	CAGGTCTGCAGCCAACCAGTTGTG	140 bp
rs1800797	*IL6*-597 G > A	TGGCAAAAAGGAGTCACACA	TGTGTTCTGGCTCTCCCTGT	112 bp
rs1800796	*IL6*-572 G > C			
rs1800795	*IL6*-174 G > C	GCCTCAATGACGACCTAAGC	GGGGCTGATTGGAAACCTTA	101 bp
rs4845617	*IL6R*-183 G > A	CGCTCTGAGTCATGTGCGAGTG	GGCTCTCTACACACACTGCGAG	112 bp
rs4845374	*IL6R*-exon2 T > A	TCCTCCTATTCCTTTTTCTCCA	GGAATGTGGGCAGTGGTACT	103 bp
rs2228145	*IL6R*-exon1 A > C			
rs1800630	*TNFA*-863 C > A	AGACCTCTGGGGAGATGTGA	CGTCCCCTGTATTCCATACC	159 bp
rs1799724	*TNFA*-857 C > T			
rs1800629	*TNFA*-308 G > A	CCCCAAAAGAAATGGAGGCAATAGG	GTAGGACCCTGGAGGCTGAAC	68 bp
rs361525	*TNFA*-238 G > A	GGGTCCTACACACAAATCAGTCAGT	CCCCTCACACTCCCCATCC	79 bp
rs2234649	*TNFR1*–383 A > C	CTTGGTGTTTGGTTGGGAGT	AGGAAGAGCTGGAGGAGGAG	153 bp
rs1061622	*TNFR2* T676G T > G	CTCCTCCTCCAGCTGTAACG	GTGTTGGGATCGTGTGGAC	139 bp