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The association of interleukin-21 polymorphisms with interleukin-21 serum levels and risk of systemic lupus erythematosus

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ABSTRACT

Systemic lupus erythematosus (SLE) is one of the common autoimmune diseases, with complex genetic components. Interleukin-21 (IL-21) is the most recently discovered member of the type-I cytokine family, which has a variety of effects on the immune system, including B cell activation, plasma cell differentiation, and immunoglobulin production. Previous studies have identified that IL-21 was associated with different autoimmune and inflammatory diseases, such as rheumatoid arthritis, multiple sclerosis and SLE. Variations in the DNA sequence in the IL-21 gene may lead to altered IL-21 production and/or activity, and thus this can modulate an individual's susceptibility to SLE. To test this hypothesis, we investigated the association of the IL-21 polymorphisms and its serum levels with the risk of SLE in a Chinese population. We analyzed three single nucleotide polymorphisms of IL-21 gene rs907715 C/T, rs2221903 T/C and rs2055979 C/A in 175 patients with SLE and 190 age- and sex-matched controls, using Snapshot SNP genotyping assays and DNA sequencing method. Soluble IL-21 (sIL-21) levels were measured by ELISA. There were significant differences in the genotype and allele frequencies of IL-21 gene rs2055979 C/A polymorphism between the group of patients with SLE and the control group ($P < 0.05$). sIL-21 levels were increased in patients with SLE compared with controls ($P < 0.01$). Moreover, genotypes carrying the IL-21 rs2055979 A variant allele were associated with increased IL-21 levels compared to the homozygous wild-type genotype in patients with SLE. The rs2055979 C/A polymorphism of IL-21 and its sIL-21 levels were associated with SLE in the Chinese population. Our data suggests that IL-21 gene may play a role in the development of SLE.

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1. Introduction

Many factors have been proposed in the pathogenesis of systemic lupus erythematosus (SLE), such as genetic factors, environmental factors, hormonal action, viruses and dysregulation of cytokine production. The etiology and pathogenetic mechanisms of SLE have not been clearly elucidated. Genetic factors seem to play a significant role in the susceptibility to SLE. However, familial aggregation and a higher rate of concordance for SLE in monozygotic than in dizygotic twins provide strong support for the role of genetic factors in the pathogenesis of this disorder (Costa-Reis and Sullivan, 2013; Grennan et al., 1997; Shai et al., 1999). Subjects who have a first-degree relative with SLE are over six times more likely to develop the disease than those without such relatives (Bengtsson et al., 2002). Association studies have shown a genetic association with cytokine network (Asano et al., 2013; Wang et al., 2013; Warchoł et al., 2011). Moreover, the unbalanced cytokine regulation which contributed to the pathogenesis of SLE development has been investigated. A number of cytokines which were associated with the pathogenesis of SLE including interleukin-12 (IL-12), IL-17, IL-27, IL-10 and tumor necrosis factor-a (TNF-a) have been reported (Duarte et al., 2013; Qiu et al., 2013; Robak et al., 2013; Smiljanovic et al., 2012; Sun et al., 2012).

Interleukin-21 (IL-21) is a novel four helix bundle class I cytokine produced endogenously by activated CD4+ T cells, natural killer T (NKT) cells and T helper (Th) cells (Coquet et al., 2007; Monteleone et al., 2009; Parrish-Novak et al., 2000). IL-21 exerts a variety of effects on the immune system and has an important role in B cell responsiveness, proliferation, plasma cell differentiation, and immunoglobulin production (Kuchen et al., 2007; Ozaki et al., 2004). Furthermore, IL-21 is involved in Th17 cell differentiation (Yang et al., 2008) and modulates the function of both dendritic cells and NK cells (Maeda et al., 2007). More recently, it has been demonstrated that IL-21 not only regulates normal lymphoid development and function, but also has crucial roles in pathological responses, including allergy and autoimmunity (Spolski and Leonard, 2008). Animal studies of SLE have indicated that IL-21 is important in the pathogenesis of murine lupus. SLE patients have higher serum levels of IL-21 than healthy controls. Moreover, association of IL-21 and IL-21R polymorphisms with susceptibility to SLE has

Abbreviations: SLE, systemic lupus erythematosus; CI, confidence interval; OR, odds ratio; SNPs, single nucleotide polymorphisms; IL-21, interleukin-21; LD, linkage disequilibrium; ELISA, enzyme linked immunosorbent assay.

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been reported (Sawalha et al., 2008; Webb et al., 2009). All these evidences indicate that IL-21 may represent a novel target for the treatment of SLE.

The gene encoding IL-21 is located on chromosome 4q26–q27 in humans, which consists of 5 exons spanning approximately 8.44 kb of genomic DNA. Recently, a number of polymorphisms in the gene encoding IL-21 have been identified and a relationship between the IL-21 gene polymorphisms and risk of different autoimmune and inflammatory diseases, such as multiple autoimmune diseases, Graves' disease and inflammatory bowel disease has been reported (Jia et al., 2011; Maiti et al., 2010; Márquez et al., 2009). However, very little data has examined the association between rs907715 C/T, rs2221903 T/C and rs2055979 C/A polymorphisms in IL-21 gene and SLE. Furthermore, the relationship between the IL-21 gene polymorphisms and the plasma level of IL-21 is unknown. In this study, we investigated the relationship of IL-21 gene rs907715 C/T, rs2221903 T/C and rs2055979 C/A polymorphisms and their IL-21 levels were associated with SLE in a Chinese population.

2. Materials and methods

2.1. Study population

Our study was designed as a retrospective study. The study consisted of 175 patients with SLE (21 males and 154 females, aged between 31 and 82 years). All patients with SLE were consecutively selected. They were recruited from the Department of Dermatology, Affiliated Hospital of Youjiang Medical University for Nationalities, Guangxi, China between March 2012 and December 2012. The 190 control subjects were matched to the patients on the basis of age and gender (30 males and 160 females, aged between 29 and 78 years). The control subjects underwent a routine medical check-up in the outpatient clinic of the Department of Internal Medicine, Affiliated Hospital of Youjiang Medical University for Nationalities, Guangxi, China between May 2012 and November 2012. According to the thorough clinical and laboratory evaluation, none of them were found to have any medical condition other than hypertension, autoimmune and inflammatory diseases. All study subjects were Chinese and resided in the same geographic area in China. The study was performed with the approval of the ethics committee of the Affiliated Hospital of Youjiang Medical University for Nationalities, and written informed consent was obtained from all the subjects.

2.2. DNA extraction

Genomic DNA was extracted from EDTA-anticoagulated peripheral blood leukocytes by the salting-out method (John et al., 1991). Briefly, 3 ml of blood was mixed with Triton lysis buffer (0.32 M sucrose, 1% Triton X100, 5 mM $MgCl_2$, H_2O, 10 mM Tris–HCl, pH 7.5). Leucocytes were spun down and washed with H_2O. The pellet was incubated with proteinase K at 56 °C and subsequently salted out at 4 °C using a saturated NaCl solution. Precipitated proteins were removed by centrifugation. The DNA in the supernatant fluid was dissolved in 300 μl H_2O.

2.3. Determination of IL-21 genotype

The IL-21 gene rs907715 C/T, rs2221903 T/C and rs2055979 C/A genotypes were determined by using a Snapshot SNP genotyping assay. The PCR primers were designed based on the GenBank reference sequence (accession no. NC_000004.11) (Table 1). To confirm the genotyping results, PCR-amplified DNA samples were examined by DNA sequencing, and the results were 100% concordant (Figs. 1–3).

Table 1
The primer sequences used for detecting the different IL-21 SNPs.

Reference SNP ID	PCR primers	Sequencing primers
rs907715 C/T	F: 5′-GAGCCACCACACCCAG CTGTA-3′ R: 5′-AAGCAATGGCTTGGTG TTTGGTA-3′ EF: 5′-TTTTTTTTTTTTTTTAAA AAACAGGATTTCC TTGTTTTAACT-3′	F: 5′-AGGATTTCCTTGTT TTAACT-3′ R: 5′-GCATTTATGTGATT ACTAGG-3′
rs2221903 T/C	F: 5′-TGGACACTGACGCCCAT ATTGA-3′ R: 5′-AAGGCAGTTTAGTGGYG ACAGC-3′ ER: 5′-TTTTTTTTTTTTTTTTTT TTGCTCTGCTGCT TGCAGAACA-3′	F: 5′-CAATGGGGTTTTGT TTTCTT-3′ R: 5′-TGTTCTGCAAGCAG CAGAGC-3′
rs2055979 C/A	F: 5′-CAGCCAGGAAACTCTG GAAAGAA-3′ R: 5′-GCTCTGAACCCAAACACT CTCATTT-3′ EF: 5′-TTTTTTTTTTTTTTTTTTTT TTTTTTTTTTTTTT TTTAACTCTAACCATAACAGTT AAAAAGGTG-3′	F: 5′-CATAACAGTTAAAC AAGGTG-3′ R: 5′-ATGAGATGCTAG AAATGTAT-3′

F, forward; R, reverse; E, extension.

2.4. Plasma IL-21 determination

Plasma samples from the patients and healthy controls were separated from venous blood at room temperature, and stored at −70 °C until use. The quantity determination of plasma IL-21 levels was performed by enzyme-linked immunosorbent assay (ELISA) kits (Fermentas, Lithuania), following the manufacturer's protocol. Developed color reaction was measured as OD450 units on an ELISA reader (RT-6000, China). The concentration of plasma IL-21 was determined by using standard curve constructed with the kit's standards over the range of 0–1000 pg/ml.

2.5. Statistical analysis

Genotype and allele frequencies of IL-21 were compared between SLE cases and controls using the χ^2 test and Fisher's exact test when appropriate, and odds ratios (OR) and 95% confidence intervals (CIs) were calculated to assess the relative risk conferred by a particular allele and genotype. Demographic and clinical data between groups were compared by χ^2 test and by Student's *t*-test. Hardy–Weinberg equilibrium was tested for with a goodness of fit χ^2-test with one degree of freedom to compare the observed genotype frequencies among the subjects with the expected genotype frequencies. The linkage disequilibrium (LD) between the polymorphisms was quantified using the Shi's standardized coefficient D′ (|D′|) (Shi and He, 2005). The haplotypes and their frequencies were estimated based on a Bayesian algorithm using the Phase program (Stephens et al., 2001). Statistical significance was assumed at the $P < 0.05$ level. The SPSS statistical software package version 11.5 was used for all of the statistical analysis.

3. Results

3.1. Clinical characteristics of the study participants

There were no statistically significant differences in the age and percentage of males/females between the two study groups. The serum IL-21 levels were significantly higher in the group of patients with SLE than those in the control group [(426.4 ± 275.2 pg/ml, n = 175) vs (378 ± 219.6 pg/ml, n = 190); $P < 0.001$; Fig. 4].

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