Association of a Single Nucleotide Polymorphism of IL-21 Gene with Asthma in a Chinese Han Population

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Abstract

Background: Autoimmune abnormalities appear to be major predisposing factors for asthma. Rs12508721 and rs2055979 of interleukin-21 (IL-21) gene polymorphisms have been previously found to be associated with autoimmune diseases. This study aimed to assess the role of IL-21 in asthma in a Chinese Han population.

Method: A total of 199 independent asthma patients and 249 unrelated healthy controls were recruited for this case-control association study. Two SNPs (rs12508721 and rs2055979) were genotyped by PCR-RFLP.

Results: The allele T frequency of rs12508721 was significantly higher in asthma patients than in controls (OR=1.58, 95% CI=1.18-2.11, P=0.0021). The effect of dominant model (CC versus CT + TT, OR=0.83, 95CI=1.24-2.69, P=0.0021) was observed. Distributions of allele and genotype frequencies of the SNP rs2055979 showed no significant differences between asthma patients and controls.

Conclusion: Our findings suggest that polymorphism of IL-21 gene plays an important role in susceptibility to asthma in a Chinese Han Population.

Keywords: Asthma; Interleukin-21; Single nucleotide polymorphism

Introduction

Recent decades have brought dramatic increases in the prevalence and severity of allergic asthma; and the worldwide incidence, morbidity, and mortality of allergic asthma are increasing [1]. Asthma control represents the main goal of asthma management and different strategies aim to avoid the long term downsides of inhaled corticosteroids [2]. Although different strategies are broadly administered to patients, a better understanding of pathogenesis is essential to prevent the prognosis.

It has been shown that multi-factorial parameters and many clinical conditions can cause allergic asthma: the pathophysiological features of allergic asthma are thought to result from the aberrant expansion of CD4+ T cells [1,3]; immunosuppressive therapy was shown beneficial to the treatment of allergic asthma, but is not without risks [2,4,5]; immunity related genes such as IL-2, IL-4, IL-13 were shown associated with asthma [6-10]. These findings collectively demonstrate that autoimmune mechanism-mediated damage may play an important role in the pathogenesis.

IL-21, an IL-2 family multifunctional cytokine, is produced by activated CD4+ T cells. IL-21 is a multifunctional cytokine associated with multiple autoimmune diseases, including systemic lupus erythematosus, ulcerative colitis, and DCM [11-16]. We hypothesized that asthma susceptibility was associated with certain polymorphisms in the IL-21 gene. In the present study, we investigated two single nucleotide polymorphisms (SNPs) of IL-21 in asthma patients and controls: rs12508721 (promoter region) and rs2055979 (intron region). The findings allowed evaluating the contribution of these SNPs to asthma risk using available genotyping data in a Chinese Han population.

Subjects and Methods

Study subjects

The present study was approved by the hospital ethics committee and all subjects gave written informed consent to participate. This case–control study enrolled 199 unrelated asthma patients (years, mean ± SD, 43 ± 35.17; gender, male/female, 121/78) from Sichuan Academy of Medical Sciences.
and Sichuan Provincial People’s Hospital between 2012 and 2013. The diagnosis of asthma was made in accordance with the revised criteria: a positive skin prick test reaction to at least 1 aeroallergen (pollen) and a history of shortness of breath and wheezing due to chest tightness. A total of 249 healthy unrelated individuals (years, mean ± SD, 49.94 ± 20.17; gender, male/female, 128/121) from a routine health survey were enrolled as controls. All individuals were Han population living in Sichuan Province of southwestern China. All subjects involved were privy to the study and gave written informed consent.

**PCR amplification and restriction enzyme digestion**

Genomic DNA of each individual was extracted from 200 ul EDTA-anticoagulated peripheral blood samples by a DNA isolation kit from Bioteka (Peking, China), according to the manufacturer’s instructions. Genotyping of the IL-21 gene polymorphisms was carried out using polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP). PCR reaction was performed in a total volume of 25 ul, including 2.5 ul 10 × PCR buffer, 1.5 mmol/L MgCl₂, 0.15 mmol/L dNTPs, 0.5 umol/L each primer, 100 ng of genomic DNA and 2 U of TaqDNA polymerase. PCR products were digested with corresponding restriction enzyme for 8 hours and analyzed by 6% polyacrylamide gels with silver staining. About 10% of the samples were randomly selected to perform the repeated assays and the results were 100% concordant. Both of the primers and restriction enzymes in the genotyping analysis as well as the temperature were listed in Table 1.

**Statistical analyses**

Data were analyzed using SPSS version 13.0 (SPSS Inc., Chicago, IL, USA). Genotype frequencies of rs12508721 and rs2055979 were obtained by direct counting and Hardy–Weinberg equilibrium evaluated by chi-square test. Odds ratio (OR) and 95% confidence intervals (CI) were utilized to assess the effects caused by any differences in alleles, genotypes. Genotypic association tests in a case-control study assuming co-dominant, dominant, recessive or over-dominant genetic models were performed using SNPstats [17].

**Results**

Genotype distribution of rs12508721 and rs2055979 SNPs were determined under the Hardy-Weinberg equilibrium in control and DCM subjects. As shown in Table 2, genotype and allele frequencies were differently distributed among patients and controls for the two SNPs. These data showed that association between the polymorphisms and asthma risk corresponded to co-dominant, dominant, recessive or over-dominant genetic model. The allele T frequency of SNP rs12508721 was 40 % and 31% in asthma patients and healthy controls, respectively (T versus C, OR=1.58, 95% CI=1.18-2.11, P=0.0021). In the co-dominant model, CC versus CT versus TT, OR=1.75, 2.23 respectively (P=0.0066). Moreover, the effect of dominant model (CC versus CT+TT, OR=1.83, 95CI=1.24-2.69, P=0.0021) was observed.

**Table 1:** Primers and enzymes for genotyping IL-21 SNPs.

<table>
<thead>
<tr>
<th>SNPs</th>
<th>Primers (5’-3’)</th>
<th>Annealing Temperature (°C)</th>
<th>PCR products (bp)</th>
<th>Enzyme</th>
<th>Digested PCR products (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs12508721</td>
<td>ggagctgtgttgtttcagaagtagag</td>
<td>64</td>
<td>158</td>
<td>HincII</td>
<td>123 + 25</td>
</tr>
<tr>
<td>rs2055979</td>
<td>aaggtctcaaaggaccgaca</td>
<td>56</td>
<td>213</td>
<td>HphI</td>
<td>162 + 51</td>
</tr>
</tbody>
</table>

**Table 2:** Distribution of the IL-21 SNPs among cases and controls and their associations with asthma risk.

<table>
<thead>
<tr>
<th></th>
<th>rs12508721</th>
<th>rs2055979</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>Genotype</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>Co-dominant</td>
<td>CC</td>
<td>65 (34.7%)</td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>108 (54.3%)</td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>26 (13.1%)</td>
</tr>
<tr>
<td>Dominant</td>
<td>CC</td>
<td>65 (34.7%)</td>
</tr>
<tr>
<td></td>
<td>CT/TT</td>
<td>134 (67.3%)</td>
</tr>
<tr>
<td>Recessive</td>
<td>CC/CT</td>
<td>173 (86.9%)</td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>26 (13.1%)</td>
</tr>
<tr>
<td>Overdominant</td>
<td>CC/TT</td>
<td>91 (45.7%)</td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>108 (54.3%)</td>
</tr>
<tr>
<td>Allele</td>
<td>C</td>
<td>238 (60%)</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>160 (40%)</td>
</tr>
</tbody>
</table>
Discussion

IL-21 is a newly described cytokine that modulates B, T, and natural killer cell responses [18,19]. IL-21 displays multiple actions on a range of lymphohematopoietic lineages, including expansion and differentiation of T helper cell subsets [20]. CD4+ T cells, one subset of T cells, upon activation and expansion, develop into different T helper cell subsets with different cytokine profiles and distinct effect functions [21]. The IL-21 gene, located on chromosome 4q26-q27 approximately 180 kb from the IL-2 gene, is highly expressed in activated CD4+ T cells [22-24]. IL-21 plays a crucial role in immunoglobulin production and regulates B cell differentiation [25-27]. Moreover, it has also been shown to down-regulate IgE production from IL-4 stimulated B cells by the inhibition of germ line C (epsilon) transcription [28-30]. In addition, IL-21 inhibits inducible regulatory T cells’ (Tregs) differentiation and affects CD4+ T cells [31,32]. The effect of IL-21 on immune system makes this cytokine an attractive candidate protein for inflammatory disease studies. Most importantly, IL-21 triggers an increased STAT3 activation without affecting the other STATs including STAT1/2 [33]. It has been shown that defective expression of IL-21 in STAT3-deficient CD4+ T cells resulted in diminished B-cell helper activity in vitro [34,35]. The effect of IL-21 on immune system makes this cytokine an attractive candidate protein for inflammatory disease studies.

In this work, we analyzed allele and genotype frequencies at two SNPs (rs12508721 and rs2055979) of IL-21 gene in a Chinese Han population. Our data indicated that he allele T frequency of rs12508721 was significantly higher in asthma patients than in controls (OR=1.58, 95% CI=1.18-2.11, P=0.0021). The effect of dominant model (CC versus CT + TT, OR=0.83, 95%CI=1.24-2.69, P=0.0021) was observed. However, distributions of allele and genotype frequencies of the SNP rs2055979 showed no differences between asthma patients and controls.

Overall, the present study suggests that IL-21 gene polymorphisms play an important role in susceptibility to asthma in a Chinese Han population. Future studies are needed to further explore the molecular mechanisms involved in the susceptibility to asthma.

References
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