STAT4 rs7574865 polymorphism and the susceptibility of systemic sclerosis (SSc).

Yunxia Lei, Xiao Zhang*, Guangfeng Zhang, Haobo Lin, Yuan Feng, Ting Xu

Department of Rheumatology, Guangdong Academy of Medical Sciences, Guangdong General Hospital, Guang Zhou, Guangdong, PR China

Abstract

Previous study suggested that signal transducers and activators of transcription-4 (STAT4) rs7574865 polymorphism may play an important role in susceptibility to Systemic Sclerosis (SSc) in Chinese. However, no further study was conducted to confirm this result. Thus, we did a case-control study. 174 SSc patients and 202 controls were enrolled in this study. When the STAT4 rs7574865 polymorphism allele GG genotype was used as the reference, the TT genotype was associated with the risk for SSc (OR=2.79, 95% CI 1.22-6.76, P=0.01). Further analysis indicated that the T allele frequency was significantly higher in SSc patient group than the control group (OR=1.55, 95% CI 1.02-2.32, P=0.01). In conclusion, this study suggested that STAT4 rs7574865 polymorphism was associated with SSc risk in a Chinese Han population.

Keywords: Systemic sclerosis, STAT4, Polymorphism, Association.

Introduction

Systemic Sclerosis (SSc) is an autoimmune disease characterized by widespread vasculopathy, immune system activation and fibrosis of the skin and of the internal organs [1]. Patients can be classified into 2 subsets based on the distribution of skin changes: diffused cutaneous SSc (dcSSc) and limited cutaneous SSc (lcSSc) [2]. The underlying mechanisms of SSc are poorly understood.

Signal transducers and activators of transcription-4 (STAT4) are primarily activated by IL-12 to promote cytotoxic responses and T helper 1 cell differentiation [3]. STAT4 protein comprises an N-terminal domain that plays an important role in phosphorylation and nuclear translocation, as well as a four-stranded helical coiled coil that is implicated in protein-protein interaction and nuclear import and export [4]. Yi et al. suggested that STAT4 rs7574865 polymorphism may play an important role in susceptibility to SSc [5]. However, no further study was conducted to confirm this result. Thus, we did a case-control study to assess the association between STAT4 rs7574865 polymorphism and SSc risk.

Methods

Study subjects

174 SSc patients and 202 controls were enrolled from GuangDong General Hospital from January 2011 to March 2017. Controls were selected based on a physical examination in the same region during the same period as patient recruitment. The protocol was approved by the Ethics Review Committee of GuangDong General Hospital, and signed informed consent was obtained from all subjects. All participants were Han Chinese.

Genotyping method

The blood samples were collected from each enrolled subjects. The genomic DNA was extracted from peripheral blood cells using the Nucleon Bacc kit (TianGen Biotech Co., Ltd., Beijing, China). The target DNA sequences were amplified using multiplex polymerase chain reaction (PCR) with specific primer sequences as follows: forward: 5'-AGTATGAAAAGTTGGTGAC-3', reverse: 5'-AATCCCCTGAAATTCCACTG-3'. Polymerase chain reaction was performed in a total volume of 20 μl reaction mixture containing 50 ng of genomic DNA, 0.2 μl TransStartTaq polymerase (TransGen Biotech, Beijing, China), 0.5 μl dNTP, 0.5 μl each primer, 2 μl 10X Buffer and 15.3 μl ddH2O. The polymerase chain reaction profile consisted of an initial melting step at 95°C for 5 min followed by 40 cycles of 95°C for 45 s, 58°C for 45 s and 72°C for 30 s, and an additional extension 72°C for 5 min in a thermal cycler (Bio-Rad). Purified products were sequenced on an ABI Prism 3730xl sequencer (Applied Biosystems, Foster City, CA, USA) using BigDye Terminator Sequencing Standards.

Statistical analysis

Chi-square (χ2) test was used to examine the deviation in genotype frequencies from Hardy-Weinberg Equilibrium.
(HWE) in controls. The association of STAT4 rs7574865 polymorphism and SSc risk was calculated using Odds Ratios (ORs) and 95% Confidence Intervals (CIs). The analysis was performed using SPSS 17.0 (SPSS Inc., Chicago, IL, USA). P value less than 0.05 was considered statistically significant.

Results
In this study, there were 174 SSc patients enrolled, and their clinical features were listed in Table 1. The mean age of these patients was 47.5 ± 11.8 y. In this case-control study, the distributions of age and gender were consistently investigated among all cases and controls (P>0.05). The genotype frequency of STAT4 rs7574865 polymorphism in the controls was coincident with HWE (P>0.05).

The STAT4 rs7574865 polymorphism allele was associated with the risk of SSc in terms of the frequency of allele comparison (Table 2). When the STAT4 rs7574865 polymorphism allele GG genotype was used as the reference, the TT genotype was associated with the risk for SSc (OR=2.79, 95% CI 1.22-6.76, P=0.01). Further analysis indicated that the T allele frequency was significantly higher in SSc patient group than the control group (OR=1.55, 95% CI 1.02-2.32, P=0.01).

Table 1. Characteristics of the cases and controls.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Case (n=174)</th>
<th>Control (n=202)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>47.5 ± 11.8</td>
<td>47.2 ± 12.1</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Female (%)</td>
<td>86%</td>
<td>84%</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Antinuclear Antibody (ANA)</td>
<td>92%</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Anticentromere Antibody (ACA)</td>
<td>89%</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Anti-Scl-70</td>
<td>45%</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Renal</td>
<td>2%</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Pulmonary</td>
<td>50%</td>
<td>NA</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Frequencies of STAT4 rs7574865 polymorphism in cases and controls.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Case (n=174)</th>
<th>Control (n=202)</th>
<th>OR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG</td>
<td>99</td>
<td>134</td>
<td>1 (Reference)</td>
<td></td>
</tr>
<tr>
<td>TG</td>
<td>56</td>
<td>58</td>
<td>1.32 (0.84-2.07)</td>
<td>0.21</td>
</tr>
<tr>
<td>TT</td>
<td>19</td>
<td>10</td>
<td>2.79 (1.22-6.76)</td>
<td>0.01</td>
</tr>
<tr>
<td>G</td>
<td>254</td>
<td>326</td>
<td>1 (Reference)</td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>94</td>
<td>78</td>
<td>1.55 (1.02-2.32)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Discussion
In this study, STAT4 rs7574865 polymorphism was found to have an associate with SSc risk in the Han Chinese population. When the STAT4 rs7574865 polymorphism allele GG genotype was used as the reference, the TT genotype was associated with the risk for SSc. Further analysis indicated that the T allele frequency was significantly higher in SSc patient group than the control group.

Lamana et al. found that the presence of the rs7574865 T allele enhances STAT4 mRNA transcription and protein expression [6]. Yi et al. suggested that STAT4 rs7574865 polymorphism was associated with diabetes risk [7]. Liu et al. indicated that STAT4 rs7574865 G/T SNP was significantly associated with increased AS susceptibility and severity in Chinese Han Population [8]. Fan et al. suggested that STAT4 rs7574865 G/T polymorphism as susceptibility factors for juvenile idiopathic arthritis [9]. However, Zhu et al. did not support that STAT4 variants contribute to IBD susceptibility in the Chinese Han population [10]. Migita et al. showed a positive association between a STAT4 polymorphism and type-1 autoimmune hepatitis [11]. Joshi et al. showed that STAT4 is involved in PBC susceptibility [12].

Some limitations should be addressed. First, the sample size of our case-control study was relatively small. Second, we did not investigate other STAT4 polymorphisms in this study. Third, we did not perform subgroup analysis since we did not have sufficient data.

In conclusion, this study suggested that STAT4 rs7574865 polymorphism was associated with SSc risk in a Chinese Han population.

Conflicts of Interest
None

References


*Correspondence to
Xiao Zhang
Department of Rheumatology
GuangDong Academy of Medical Sciences
GuangDong General Hospital
PR China