Single nucleotide polymorphism rs708567 in IL-17RC gene is associated with a susceptibility to and curve severity of adolescent idiopathic scoliosis in a Chinese Han population: a case-control study

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Abstract

Background. Although the pathogenesis of adolescent idiopathic scoliosis (AIS) remains controversial, genetic factors are thought to play a key role in the development of AIS. In a recent genome wide association study, a polymorphism in the interleukin-17 receptor C (IL-17RC) gene was reported to be associated with susceptibility to AIS, implicating IL-17RC as a novel predisposition gene for AIS. However, as this association has not been replicated in other populations, its global applicability is unclear.

Methods. A total of 529 Chinese girls with AIS and 512 healthy, age-matched controls were recruited from June 2007 to December 2009. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis was carried out to detect the genotype of single nucleotide polymorphism (SNP) rs708567 in the IL-17RC gene. Case-control and case-only studies were performed to determine the associations between the IL-17RC gene polymorphism and the susceptibility to and the curve severity of AIS.

Results. The GG genotype and G allele frequency were significantly higher in the AIS patients than that in the controls (χ² test: P = 0.023 and 0.028, respectively). Both the GG genotype and G allele significantly increased the risk of AIS by an OR of 1.550 (95% CI: 1.062 - 2.261) and 1.507 (95% CI: 1.046 - 2.172), respectively. In addition, in a subgroup of skeletally mature AIS patients (n=241), who carrying GG genotype showed a significantly
higher mean maximum Cobb angle than those carrying AG genotype (36.93 ± 13.15° vs. 28.52 ± 7.76°, P = 0.004).

**Conclusion.** This study confirms a significant association between the IL-17RC gene polymorphism and the susceptibility to and the curve severity of AIS in a Chinese Han population, suggesting that the IL-17RC gene is not only an AIS predisposition gene but is also a disease-modifying gene in the Chinese Han population.

**Keywords:** adolescent idiopathic scoliosis, IL-17RC, single nucleotide polymorphism.

**Background**

Adolescent idiopathic scoliosis (AIS) is one of the most common spinal deformities, with a world-wide pediatric prevalence of 2-3%. In recent decades, many etiological factors have been proposed for AIS, such as uncoupled growth of the spinal column, paraspinal muscle dysfunction, hormonal disturbances, and inflammation; however, no single factor can explain all of the clinical characteristics of AIS. Therefore, AIS is generally regarded to be a multi-factorial disorder, and hereditary or genetic factors likely play an important role.

The genetic factors contributing to AIS have been described in many studies. Genome-wide linkage analysis studies have identified variations in chromosomal regions 9q31.2–q34.2, 12p, 17q25.3–qtel, 18q, 19p13 and 19p13.3 as being associated with an increased susceptibility to AIS. Through genetic association studies, several genes, such as tryptophan hydroxylase 1 (TPH1), estrogen receptor α (ESR1), and matrilin-1 gene (MATN1) have been identified as predisposition genes for AIS. Furthermore, a number of genes have also been found to be related to the curve severity of AIS.

In a recent genome wide association study using data from 137 AIS patients of European ancestry, Dormans et al reported that the SNP rs708567 was associated with a susceptibility to AIS. rs708567 is located on chromosome 3p25.3 and occurs in an exon of IL-17RC, which encodes for an integral member of the IL-17R complex. As an essential part of the IL-17 cytokine/IL-17 receptor signaling axis, the IL-17R complex mediates the
signal transduction of the IL-17 cytokine family, which in turn promotes the production of pro-inflammatory cytokines, such as tumor necrosis factor \( \alpha \) (TNF-\( \alpha \)), interleukin-1\( \beta \) (IL-1\( \beta \)), and interleukin-6 (IL-6).\[^{19}\] The findings of Dormans et al\[^{18}\] identified IL-17RC as a novel predisposition gene for AIS, which provides significant information regarding the genetic etiology of AIS.

However, genetic association studies may produce spurious associations due to small sample sizes, population stratification, variability in the control population, and racial and ethnic differences, etc.\[^{20}\] The purpose of this case-control study is to replicate the previously reported association between the IL-17RC gene polymorphism and the susceptibility to AIS and to further investigate the contribution of this polymorphism to the curve severity of AIS within a Chinese Han population. Our results suggest that the IL-17RC gene is not only an AIS predisposition gene but is also a disease-modifying gene in the Chinese Han population.

**MATERIALS AND METHODS**

This study includes two sections, a case-control study and a case-only study. The case-control study evaluated the genetic association between AIS predisposition and the rs708567 within the IL-17RC gene, and the case-only study evaluated the genetic association between rs708567 and curve severity in a subgroup of AIS patients who had reached skeletal maturity. Skeletal maturity was defined as being at least 16 years old and having a Risser sign of 5.\[^{21}\] Because bracing can change the natural history of AIS, patients treated with a brace were excluded from the case-only study.

**Subjects**

A total of 529 Chinese girls with AIS, aged 11 to 18 years, and 512 gender- and age-matched controls were recruited for this study. All patients were examined either as outpatients or inpatients at our scoliosis clinic between June 2007 and December 2009. A diagnosis of AIS was made according to clinical examination and standard up-standing posteroanterior whole-spine radiography. Scoliosis curve severity was measured according to the Cobb method, and patients with a major curvature Cobb angle ranging from 20\(^{\circ}\) to 100\(^{\circ}\) were
included. Patients with a curve less than 20° were excluded because of a possible “false-positive” diagnosis of AIS, and patients with a curve greater than 100° were also excluded because of a possible association with intraspinal and brain stem abnormalities. In addition, those who were diagnosed with congenital scoliosis, neuromuscular diseases, endocrine diseases, skeletal dysplasia, connective tissue abnormalities and scoliosis secondary to some other disorder were also excluded from the subset. The healthy controls were recruited during a regular school health-screening scheme. To rule out any spinal deformity, the Adam forward-bending test[^22^] was carried out by an experienced orthopedic surgeon in all controls. In addition, no subjects showed evidence of bone disease or growth disturbances, systemic illness or other condition known to affect bone metabolism, or history of recent steroid intake.

The study protocols were approved by the University and Hospital Ethics Committee. Informed consent was obtained from all subjects and their parents prior to DNA analysis.

**Blood Sampling and Genotyping**

As described by Dormans et al[^18^], rs708567 in the IL-17RC gene is associated with a susceptibility to AIS. The present study focused on this SNP and carried out genotyping of rs708567 by PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) in the previously described Chinese Han population.

Total genomic DNA was extracted from 5 to 7 mL of ethylenediaminetetraacetic acid anti-coagulated whole blood from each patient using genomic DNA isolation kits (Amersham, Buckinghamshire, UK) according to the manufacturer’s instructions. The DNA samples were frozen and stored until needed. Genotyping was performed using PCR-RFLP. The primers are listed in TABLE 1. Genomic DNA was amplified in a PCR reaction volume of 10 µL, which contained 5 µL of 2×BenchTopTM Taq Master Mix, 0.2 µL of each primer (1.0 µmol/L), 3.6 µL of ddH2O and 1.0 µL of genomic DNA (1 µg). A typical PCR amplification program included 40 amplification cycles that consisted of 30 seconds at 96°C, 45 seconds at the primer-appropriate annealing temperature, and 30 seconds at 72°C. A final
elongation step carried out for 7 minutes at 72°C. For restriction enzyme digestion, the PCR product was incubated with 4 U of HinfI for 4 hours in the presence of the accompanying buffer. The digested PCR products were visualized by electrophoresis in a 3.5% agarose gel. The genotyping results were validated by duplicate genotyping with 10% of the samples (TABLE 1).

**Statistical Analyses**

Power analysis was performed with PASS (version 11; NCSS, Kaysville, UT; http://www.ncss.com/pass.html). The other statistical analyses were carried out using SPSS 13.0 for Windows (SPSS Inc., Chicago, IL). Hardy-Weinberg equilibrium (HWE) was tested by a goodness-of-fit $\chi^2$ test. The case-control difference for the allele and genotype frequencies was analyzed with the $\chi^2$ test. The odds ratios (ORs) and 95% confidence interval (95% CI) ranges were calculated. A one-way analysis of variance was used to compare the mean maximum Cobb angle of the different genotypes in AIS patients. A P-value lower than 0.05 was considered to be statistically significant.

**Result**

A total of 529 Chinese AIS girls with a mean age of 14.54 ± 1.62 years and 512 gender- and age-matched controls with a mean age of 14.36 ± 1.93 years were recruited for this study. The mean Risser sign of the AIS patients and the mean maximum Cobb angle were 3.17 ± 1.89 (range 0 - 5) and 38.30 ± 16.71° (range 20° - 100°), respectively. Our sample size had an 81% power to detect the AIS-relevant SNP within the IL-17RC gene. The genotype frequencies showed no significant deviation from the Hardy-Weinberg equilibrium in either the AIS group or control group. Of these 529 AIS patients, 241 patients (mean maximum Cobb angle of 35.24 ± 12.81°) who reached skeletal maturity were involved in the case-only study.

**Association of SNP rs708567 with a susceptibility to AIS**

The genotype and allele frequencies of SNP rs708567 in the AIS patients and controls are shown in TABLE 2. Overall, the frequency of the GG genotype and the G allele in the AIS
Association of SNP rs708567 with curve severity in AIS

We evaluated the association of SNP rs708567 with the curve severity in a subset of AIS patients who had reached skeletal maturity (n = 241) in a case-only study. Overall, the AIS patients with the GG genotype showed a significantly higher mean maximum Cobb angle (36.01 ± 13.12°) than those with the AG genotype (28.92 ± 7.43°, P = 0.007), indicating that SNP rs708567 is significantly associated with the curve severity of scoliosis (TABLE 3).

Discussion

Previous population studies have reported a significantly higher AIS incidence of 15.8% in first-degree relatives of AIS patients, 36% in dizygotic twins, and 73% in monozygotic twins.\(^{[23,24]}\) In addition, significantly higher incidence of AIS was also found in females than in males with a female-to-male ratio of roughly 10:1.\(^{[25]}\) Given the documented higher incidence of AIS within families and the sexual dimorphism of AIS occurrence, genetics may offer the most possibility to disentangle the etiology of AIS.

A genetic association study is one of the most effective methods for identifying and characterizing the genomic variants that underlie the susceptibility to a multifactorial disease.\(^{[26]}\) In a recent genetic association study, Dormans et al\(^{[18]}\) reported that rs708567 in the IL-17RC gene is associated with a higher susceptibility to AIS in a population with European ancestry. However, the quality of genetic association may be affected by many factors, such as a small sample size, population stratification, racial and ethnic differences etc, and false-positive associations may be produced. For example, Zhang et al\(^{[27]}\) initially reported that SNP rs1256120 in the estrogen receptor β gene (ESR2) was associated with a higher susceptibility to and curve severity from AIS in Chinese females using data from 176 AIS patients and 80 controls. However, Takahashi et al\(^{[28]}\) could not replicate this association.
in a Japanese population using data from 798 AIS patients and 637 controls. The discrepancy between these results indicates that a possible spurious association may exist in a genetic association study because of differences in the genetic background of the population and small sample sizes.\(^{[28]}\) Neale et al\(^{[29]}\) therefore advocated that any positive association findings in gene-based studies should be confirmed in a different population using a larger sample size.

In this study, we investigated the possible association between an IL-17RC gene polymorphism and the susceptibility to AIS in a Chinese Han population. We found that both the GG genotype and the G allele have a significantly higher frequency in AIS patients than in controls (\(P = 0.023\) and \(P = 0.028\), respectively). Our results are consistent with the findings in Dormans et al\(^{[18]}\) confirming that IL-17RC is a novel predisposition gene for AIS.

Genetic polymorphisms associated with scoliosis curve severity have been previously described. Inoue et al\(^{[16]}\) reported that polymorphisms in the estrogen receptor gene are associated with the curve severity of idiopathic scoliosis. Yeung et al\(^{[17]}\) observed that an insulin-like growth factor-I (IGF-I) gene polymorphism affected the curve severity of AIS. Our results show that AIS patients with the GG genotype have a higher mean maximum Cobb angle than those with the AG genotype, indicating that the IL-17RC gene polymorphism is associated with curve severity in AIS patients.

IL-17RC was first characterized as a subunit of the IL-17R complex.\(^{[19]}\) The IL-17R complex mediates the IL-17 effector function through the IL-17 cytokine/IL-17 receptor complex signaling axis, which in turn promotes the production of several pro-inflammatory cytokines, such as tumor necrosis factor \(\alpha\) (TNF-\(\alpha\)), interleukin-1\(\beta\) (IL-1\(\beta\)) and interleukin-6 (IL-6). Increased amounts of TNF-\(\alpha\) and IL-1\(\beta\) have been documented to increase the activity of metalloproteinases (MMPs), which play an important role in disc degeneration.\(^{[30]}\) Regarding the role of IL-17RC in inducing inflammation, SNP rs708567 in the IL-17RC gene may alter the protein product within the intervertebral discs, which may in turn
accelerate disc degeneration and lead to the occurrence and progression of scoliosis. Actually, the hypothesis that defects within the intervertebral discs are an etiological factor for AIS has been proposed in previous research.\textsuperscript{[28]}

Overall, we found a positive association between SNP rs708567 and the susceptibility to and curve severity of AIS in a Chinese Han population. However, we are not certain if rs708567 is a specific causal SNP or a marker of other polymorphisms that play a causative role in the development of AIS. Therefore, further investigation into the possibility of a synergistic interaction between SNP rs708567 and other polymorphisms is necessary to clarify the effect of SNP rs708567 on the susceptibility to AIS. In addition, ethnic differences may result in differences in an individual’s susceptibility to AIS. Although our results are consistent with the report of Dormans et al,\textsuperscript{[18]} this positive finding cannot be generalized to other ethnic groups. Therefore, further evaluations in other ethnic populations may help to identify the full genetic effect of the IL-17RC gene polymorphism on susceptibility to AIS.

**Conclusion**

This study identified a significant association between an IL-17RC gene polymorphism and the susceptibility to and curve severity of AIS in a Chinese Han population, indicating that IL-17RC is not only a predisposition gene but is also a disease modifying gene for AIS with a single thoracic curve. However, further studies are needed to confirm these positive findings in other ethnicities.

**Abbreviations**

AIS, adolescent idiopathic scoliosis; IL-17RC, interleukin-17 receptor C; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; SNP, single nucleotide polymorphism; Ors, odds ratios

**Competing interests**

The authors declare no competing interests.
Authors’ contribution

Song Zhou, Xu-sheng Qiu and Ze-zhang Zhu carried out the genetic studies. Wei-fei Wu recruited patients and were involved in the clinical part of the investigation. Zhen Liu performed the statistical analysis. All authors read and approved the final manuscript.

Yong Qiu conceived the study; participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

Funding

We gratefully recognize the financial support of the basic research special funds of central universities (NO. 021414340027) and the National Natural Science Foundation of China (NO. 81171767).

References


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Table 1. Primers and restriction enzymes for PCR-RFLP analysis

<table>
<thead>
<tr>
<th>SNP</th>
<th>Primers</th>
<th>Annealing Temperature (°C)</th>
<th>PCR Product Size (bp)</th>
<th>Restriction Enzyme</th>
<th>Fragment Size After Enzymatic Cleavage (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs708567</td>
<td>Forward Primer: 5'-AGTAGGGTAGGCCTGGAAGG-3' Reverse Primer: 5'-CACTGGGAAGAGCCTGAAGA-3'</td>
<td>57</td>
<td>210</td>
<td>Hinfl</td>
<td>GG: 210 AG: 161 + 49</td>
</tr>
</tbody>
</table>

*PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; SNP, single-nucleotide polymorphism.

Table 2. Distribution of genotypes and allele frequencies of SNP rs708567 in the IL-17RC gene for AIS patients (n = 529) and controls (n = 512).

<table>
<thead>
<tr>
<th>SNP</th>
<th>Genotypes / Alleles</th>
<th>Group, n (%)</th>
<th>P-value</th>
<th>OR (95% Confidence Interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>AIS</td>
<td>Controls</td>
<td></td>
</tr>
<tr>
<td>rs708567</td>
<td>GG</td>
<td>477 (90.17%)</td>
<td>438 (85.55%)</td>
<td>1.550 (1.062 - 2.261)</td>
</tr>
<tr>
<td></td>
<td>AG</td>
<td>52 (9.83%)</td>
<td>74 (14.45%)</td>
<td>0.645 (0.442 - 0.941)</td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>G</td>
<td>1006 (95.1%)</td>
<td>950 (92.8%)</td>
<td>1.507 (1.046 - 2.172)</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>52 (4.9%)</td>
<td>74 (7.2%)</td>
<td>0.664 (0.460 - 0.956)</td>
</tr>
</tbody>
</table>

OR, Odds Ratio. Data are presented as the no. of individuals and their percentage of the population tested. A value of P < 0.05 ($\chi^2$ test) was considered to be statistically significant.
Table 3. Association of the IL-17RC gene polymorphism and curve severity within AIS patients who had reached skeletal maturity.

<table>
<thead>
<tr>
<th>SNP</th>
<th>Genotype</th>
<th>Number of Subjects</th>
<th>Mean maximum Cobb angle (Mean ± SD)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs708567</td>
<td>GG</td>
<td>215</td>
<td>36.01 ± 13.12</td>
<td>0.007</td>
</tr>
<tr>
<td></td>
<td>AG</td>
<td>26</td>
<td>28.92 ± 7.43</td>
<td></td>
</tr>
</tbody>
</table>

A value of P < 0.05 (one-way analysis of variance) was considered to be statistically significant.