A replication study for the association of rs11190870 with curve severity in adolescent idiopathic scoliosis in Japanese

Yohei Takahashi, MD1,2,*, Ikuyo Kou, PhD1, Yoji Ogura, MD1,2, Atsushi Miyake, MD1,2, Kazuki Takeda, MD1,2, Nakajima Masahiro, MS1, Shohei Minami, MD3, Noriaki Kawakami, MD4, Koki Uno, MD5, Manabu Ito, MD6, Ikuho Yonezawa, MD7, Takashi Kaito, MD8, Haruhisa Yanagida, MD9, Kei Watanabe, MD10, Hiroshi Taneichi, MD11, Katsumi Harimaya, MD12, Yuki Taniguchi, MD13, Toshiaki Kotani, MD3, Taichi Tsuji, MD4, Tepppei Suzuki, MD7, Hideki Sudo, MD14, Nobuyuki Fujita, MD2, Mitsuru Yagi, MD2, Kazuhiro Chiba, MD15, Katsuki Kono, MD16, Tsuyoshi Sakuma, MD3, Tsutomu Akazawa, MD17, Kotaro Nishida, MD18, Kenichiro Kukutani, MD18, Hideki Shigematsu, MD19, Takahiro Iida, MD20, Satoshi Demura, MD21, Naobumi Hosogane, MD15, Eiji Okada, MD22, Masaya Nakamura, MD2, Morio Matsumoto, MD2, Kota Watanabe, MD2,*, Shiro Ikegawa, MD1

1Laboratory of Bone and Joint Diseases, RIKEN Center for Integrative Sciences, Tokyo, Japan
2Department of Orthopaedic Surgery, School of Medicine, Keio University, Tokyo, Japan
3Department of Orthopaedic Surgery, Seirei Sakura Citizen Hospital, Sakura, Japan
4Department of Orthopaedic Surgery, Meijo Hospital, Nagoya, Japan
5Department of Orthopaedic Surgery, National Hospital Organization, Kobe Medical Center, Kobe, Japan
6 Department of Orthopaedic Surgery, National Hospital Organization, Hokkaido Medical Center, Hokkaido, Japan

7 Department of Orthopaedic Surgery, Juntendo University School of Medicine, Tokyo, Japan

8 Department of Orthopaedic Surgery, Osaka University Graduate School of Medicine, Osaka, Japan

9 Department of Orthopaedic Surgery, Fukuoka Children's Hospital, Fukuoka, Japan

10 Department of Orthopaedic Surgery, Niigata University Hospital, Niigata, Japan

11 Department of Orthopaedic Surgery, Dokkyo Medical University School of Medicine, Mibu, Japan

12 Department of Orthopaedic Surgery, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan

13 Department of Orthopaedic Surgery, Faculty of Medicine, The University of Tokyo, Tokyo, Japan

14 Department of Advanced Medicine for Spine and Spinal Cord Disorders, Hokkaido University Graduate School of Medicine, Sapporo, Japan

15 Department of Orthopaedic Surgery, National Defense Medical College, Saitama, Japan

16 Department of Orthopaedic Surgery, Kono Orthopaedic Clinic, Tokyo, Japan

17 Department of Orthopaedic Surgery, St. Marianna University School of Medicine, Kawasaki, Japan

18 Department of Orthopaedic Surgery, Kobe University Graduate School of Medicine, Kobe, Japan

19 Department of Orthopaedic Surgery, Nara Medical University, Nara, Japan

20 Department of Orthopaedic Surgery, Dokkyo Medical University Koshigaya Hospital, Koshigaya, Japan

21 Department of Orthopaedic Surgery, Kanazawa University School of Medicine, Kanazawa, Japan

22 Department of Orthopaedic Surgery, Saiseikai Central Hospital, Tokyo, Japan
Correspondence should be addressed to Kota Watanabe:
Department of Orthopaedic Surgery, Keio University School of Medicine
35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan
E-mail: kw197251@keio.jp; phone: +81-3-5363-3475, fax: +81-3-3353-6597

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ABSTRACT

Study design

Case-only study.

Objective

The aim of this study was to confirm the association of rs11190870 with AIS severity in Japanese patients with AIS.

Summary of Background Data

Although the association of rs11190870 with AIS susceptibility is replicated in multiple ethnics, the association of rs11190870 with curve severity is controversial. Since the previous studies are of small, we performed a replication study using far larger number of patients than previous studies.

Methods

1,860 Japanese AIS patients who had reached skeletal maturity or undergone surgical fusion were included in the study. We evaluated the association between rs11190870 and AIS progression for the entire group, and then for patients grouped according to a severe curve (a Cobb angle of 40° or greater) or mild curve (a Cobb angle less than 30°). Because braces could affect the results of the present study, patients in the mild-curve group were divided according to whether or not they had worn a brace. We then evaluated associations between rs11190870 genotype and curve severity in these groups.

Results

The mean Cobb angles were 54.8±12.1° in the severe-curve group and 24.4±4.0° in the mild-curve group. The difference in rs11190870 risk-allele frequency between the severe- and mild-curve groups was evaluated. No significant differences were observed. We then examined the association of
rs11190870 risk-allele frequency between patients in the mild- and severe-curve groups using the $\chi^2$ test for three models, and found a marginal association between rs11190870 and curve severity in the dominant model (P value=0.035, odds ratio=1.51).

**Conclusions**

We found no association between rs11190870 and curve severity using the criteria of previous study. However, we found a marginal association between rs11190870 and curve severity. Large-scale replication studies that consider skeletal maturity and brace history, including replication studies in other ethnic groups, would be helpful for clarifying the association.

**Key Words:** adolescent idiopathic scoliosis; curve severity; replication study; genome-wide association study; skeletal maturity; Risser sign; single nucleotide polymorphism; rs11190870; Japanese

**Level of Evidence:** 4
INTRODUCTION

Adolescent idiopathic scoliosis (AIS) is a structural, lateral, rotated curvature of the spine that arises in otherwise healthy children around the time of puberty. AIS is a common disorder. When defined as a Cobb angle of at least 10°, scoliosis affects an estimated 1–3% of the population, and most cases are AIS. It is crucial for clinicians, patients, and families to understand the risk of curve progression in AIS, since a primary goal of treatment is to prevent the curve from progressing into severe deformity. Genetic factors may be important in the etiology and progression of AIS, and associations between single nucleotide polymorphisms (SNPs) and AIS susceptibility or curve severity have been reported. Researchers recently developed a prediction model for AIS curve progression based on SNP associationsthat were identified by a genome-wide association study (GWAS) in US Caucasians. However, very few of these SNP associations have been replicated in other ethnic groups.

Associations have been reported between SNPs in the ESR1, ESR2, MATN1, IGF1, NTF3, and GPER genes and AIS severity. However, replication studies revealed that these SNPs are not associated with AIS in the Japanese population. Thus, it is desirable to identify genes that are related to AIS severity or progression in the Japanese population.

A GWAS for AIS susceptibility in the Japanese population identified a significant association between AIS susceptibility and a novel SNP, rs11190870, on chromosome 10q24.31. This association was replicated in the Han Chinese (P-value 9.1 × 10^{-10} to 3.3 × 10^{-8}), in US Caucasians (P-value 5.4 × 10^{-9} to 1.8 × 10^{-5}), and in Scandinavians (P-value 7.0 × 10^{-18}). Jiang et al. reported a significant association between rs11190870 and AIS severity in a study population of 314 Chinese AIS patients; however, another study of 234 Chinese AIS patients found no association.

Thus, the present study was conducted to confirm the association of rs11190870 with AIS severity in 1,860 Japanese patients with AIS.
METHODS

Subjects

AIS patients who were diagnosed at 10–18 years of age and had a Cobb angle of 15° or greater were recruited from collaborating hospitals between February 2009 and February 2012. All of the patients underwent clinical and radiologic examination by spine surgeons, who diagnosed the etiology by excluding alternate diagnoses of congenital, juvenile, or adult-onset scoliosis as well as scoliosis secondary to other disorders. Curve severity was determined by the Cobb angle on standing whole-spine postero-anterior radiographs. Since a spinal deformity can progress until skeletal maturity, we set two endpoints for the final Cobb angle: the angle just prior to corrective surgery, or for non-surgical cases, the angle after the patient reached skeletal maturity as determined by a Risser sign of 4 or 523.

We recruited 2,191 Japanese AIS patients, of whom 1,860 patients including 1,763 females and 97 males reached the endpoints and were included in the study. We evaluated the association between rs11190870 and AIS progression for the entire group, and then for patients grouped according to a severe curve (a Cobb angle of 40° or greater) or mild curve (a Cobb angle less than 30°). These criteria were based on the natural history of AIS: a curve with a Cobb angle of 40° or greater has a 70% chance of progressing after skeletal maturity, whereas a curve less than 30° has little chance of progressing24,25. We also examined a group with slight curvature (defined as a Cobb angle less than 20° at skeletal maturity).

The efficacy of treating AIS with bracing was recently reported in a randomized control study26. Because braces could affect the results of the present study, patients in the mild-curve group were divided according to whether or not they had worn a brace. We then evaluated associations between rs11190870 genotype and curve severity in these groups.
Finally, we evaluated associations between rs11190870 genotype and the mean Cobb angle in patients who reached the endpoints, and examined these associations after excluding patients who had worn a brace.

SNP genotyping

Genomic DNA was extracted from peripheral blood leukocytes using standard protocols, and rs11190870 was genotyped using the PCR-based Invader assay. Invader probe sets were designed and synthesized by Third Wave. The Invader assay plates were read with an ABI PRISM 7900HT sequence-detection system.

Statistical analysis

The association between rs11190870 genotype and AIS severity was examined by $\chi^2$ test. The mean Cobb angle for different genotypes was compared by one-way analysis of variance (ANOVA).

The present study was approved by the ethics committees of the participating institutions and by RIKEN, and all patients (or parents of minor patients) provided informed consent for participating in the present study.

RESULTS

Association of rs11190870 genotype with curve severity in groups with a severe or mild curve

Of the 1,860 AIS patients who reached the evaluation endpoints, 326 patients who had a Cobb curve between 30° and 40° were excluded from further analysis. The remaining 1,534 patients were assessed for an association between rs11190870 genotype and curve severity. Of the 940 patients with a severe curve, 680 were treated by correction and fusion surgery. None of the 594 patients with a mild curve were treated surgically. The mean Cobb angles were 54.8±12.1° in the severe-curve group and 24.4±4.0° in the mild-curve group. The difference in rs11190870 risk-allele frequency between the severe- and mild-curve groups was evaluated by the $\chi^2$-test for the allele, recessive, and dominant models. No significant differences were observed in any of these models (Table 1). We further
examined differences in rs11190870 risk-allele frequency between patients with a severe curve and those with a slight curve (a Cobb angle less than 20°). The slight-curve group included 111 patients with a mean Cobb angle of 18.3±1.7°. No significant differences were observed in any of the models (data not shown).

To exclude the effect of wearing a brace, which attenuates curve progression, patients who had worn a brace were excluded from the mild-curve group, leaving 387 patients with a mean Cobb angle of 23.6±4.1°. We then examined the association of rs11190870 risk-allele frequency between patients in the mild-and-severe-curve groups using the χ² test for three models, and found a marginal association between rs11190870 and curve severity in the dominant model (P-value=0.035, odds ratio=1.51) (Table 2).

**Curve-severity analysis by rs11190870 genotype and mean Cobb angle**

We next evaluated the association between the rs11190870 genotype and the Cobb angle in the 1,860 patients who reached one of the endpoints, and found no association between genotype and curve severity (Table 3; the T allele is the risk allele). We also evaluated the association between the rs11190870 genotype and Cobb angle in the 1,043 patients who reached one of the endpoints without having worn a brace, and found no association between the genotype and curve severity (Table 4).

**DISCUSSION**

The association between rs11190870 and curve severity is controversial. Jiang *et al.* found that rs11190870 was associated with curve severity in a study of 314 AIS patients, while Gao *et al.* did not find an association with curve severity in a study of 234 AIS patients. Since statistical analysis is influenced by the number of samples, we examined the association of rs11190870 and curve severity in 1,860 AIS patients, a far larger number of patients than in previous studies.
AIS can progress with age until the skeleton matures. Thus, we analyzed the association of rs11190870 with curve severity in a subgroup of AIS patients who had reached skeletal maturity, defined as Risser 4 or 5. Jiang et al. and Gao et al. considered patients to have reached skeletal maturity if they had reached 16 years of age or Risser 5 \(^{17,18}\). We evaluated the association between rs11190870 and AIS progression in patients grouped by severe or mild curvature. Patients in the mild-curve group had a Cobb angle less than 30°, which carries a 10% risk of curve progression after reaching Risser 4 \(^{25}\). Therefore, we defined skeletal maturity as Risser 4 or 5. Jiang et al. and Gao et al. evaluated associations between rs11190870 genotype and the mean Cobb angle in patients with a Cobb angle of roughly 30° or greater \(^{17,18}\), which has a greater than 30% chance of curve progression, even in patients who have reached 16 years of age \(^{24}\). Our study included AIS patients who had a Risser grade of 4 or 5, indicating skeletal maturity, or who underwent corrective surgery. Comparing the mean Cobb angles of each genotype, especially when the Cobb angle is greater than 30°, has limitations because the curve can still progress even after the patient reaches skeletal maturity \(^{24}\) \((\text{Table 3, 4, 5})\). In this study, we used the Cobb angle obtained just before surgery for patients who underwent corrective surgery. However, many of these cases would have continued to progress if the patient had not had surgery, and we have no idea how far the curve might have progressed.

Because a brace significantly decreases the progression of high-risk curves toward the threshold for corrective surgery \(^{26}\), we further examined associations between rs11190870 and curve severity in mild or severe-curve patients who had not worn a brace, and found a marginal association \((\text{Table 2})\). However, the limitation of this study is that we do not know how much influence the difference in results between the patient who had worn a brace and the patient who had not worn a brace because bracing effect is highly dependent on the compliance of the user \(^{28}\) \((\text{Tables 1, 2})\). We evaluated the same associations using the criteria used by Gao et al., that is, patients who had not used a brace and who had a Risser grade of 5, were at least 16 years old, or had undergone surgical fusion. Using these criteria, we
found no association between rs11190870 and curve severity (data not shown). We were not able to compare the severe-curve and the slight-curve group after removing patients who had worn braces because the number of patients in the slight-curve group was too small for statistical analysis. Thus, large-scale replication studies that consider skeletal maturity and brace history, including replication studies in other ethnic groups, would be helpful for clarifying the association.

Jiang et al. and Gao et al. evaluated associations between rs11190870 and the mean Cobb angle of each genotype, and Jiang et al. found that the SNP was associated with AIS severity in Chinese AIS patients. We were not able to replicate this association, even when we used the same criteria as the study of Jiang et al. (data not shown). However, we found a similar tendency toward higher mean Cobb angles for the TT and TC genotypes than for the CC genotype (T is the risk allele) in patients who reached one of the endpoints and had not worn a brace (Table 5). There was also a tendency toward a higher risk-allele frequency for AIS susceptibility in the severe-curve group than in the groups with a mild curve (Tables 1, 2).

Conversely, we previously found that rs12946942 was associated with AIS severity, but could not demonstrate that the SNP was associated with AIS susceptibility. The genetic factors involved in susceptibility and severity are not necessarily the same; a monozygotic twin study using 32 AIS twin pairs indicated that the curve type involves more genetic factors than does curve severity. Focusing on curve types may lead to the discovery of new genes associated with AIS susceptibility or severity.

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REFERENCES

Table 1 Association of rs11190870 with AIS severity in Japanese patients with AIS.

<table>
<thead>
<tr>
<th>Genotype count*</th>
<th>RAF</th>
<th>P value&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Odds ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe/Mild</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td></td>
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<tr>
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<td>Recessive</td>
<td>Dominant</td>
<td></td>
<td></td>
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<tr>
<td>Allele</td>
<td>Recessive</td>
<td>Dominant</td>
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<td></td>
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<td>Dominant</td>
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<td></td>
</tr>
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<td>9</td>
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*Number of [homozygotes for the risk allele of preceding study]/ [heterozygotes]/ [homozygotes for the other allele]

RAF: risk allele frequency. CI: confidence interval.

<sup>a</sup>P value of χ² tests for allele, recessive and dominant models.
**Table 2** Association of rs11190870 with AIS severity in patients without bracing in the mild-curve group.

<table>
<thead>
<tr>
<th>Genotype count*</th>
<th>RAF</th>
<th>P value(^a)</th>
<th>Odds ratio</th>
<th>95% CI</th>
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</tr>
<tr>
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<td>0.64</td>
<td>0.110</td>
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<td>9</td>
<td>7</td>
<td>0.681</td>
<td>0.64</td>
<td>0.110</td>
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</tbody>
</table>

*Number of [homozygotes for the risk allele of preceding study]/ [heterogygotes]/ [homozygotes for the other allele]

RAF: risk allele frequency. CI: confidence interval.

\(^a\) P value of \(\chi^2\) tests for allele, reccessive and dominant models.
**Table 3** Association of rs11190870 genotype with AIS severity.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. subject</th>
<th>Mean Cobb angle (SD)</th>
<th>P value</th>
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<tbody>
<tr>
<td>TT</td>
<td>818</td>
<td>41.7 (16.5)</td>
<td></td>
</tr>
<tr>
<td>TC</td>
<td>865</td>
<td>42.0 (16.6)</td>
<td>0.13(^a)</td>
</tr>
<tr>
<td>CC</td>
<td>177</td>
<td>39.3 (15.5)</td>
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</tr>
</tbody>
</table>

\(^a\)By one-way ANOVA.
Table 4  Association of rs11190870 genotype with AIS severity in patients who reached one of the endpoints without bracing.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. subject</th>
<th>Mean Cobb angle (SD)</th>
<th>P value</th>
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<tbody>
<tr>
<td>TT</td>
<td>440</td>
<td>41.3 (17.5)</td>
<td></td>
</tr>
<tr>
<td>TC</td>
<td>498</td>
<td>41.0 (17.5)</td>
<td>0.22a</td>
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<tr>
<td>CC</td>
<td>105</td>
<td>38.1 (16.5)</td>
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*a By one-way ANOVA.
Table 5 Association between rs11190870 genotype and AIS severity in patients who reached one of the endpoints without bracing.

<table>
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<th>Study</th>
<th>Genotype</th>
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<tr>
<td></td>
<td>TC</td>
<td>41.0 (17.5)</td>
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<tr>
<td></td>
<td>CC</td>
<td>38.1 (16.5)</td>
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