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BRIEF COMMUNICATION

A multiethnic meta-analysis defined the association of rs12946942 with severe adolescent idiopathic scoliosis

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Abstract

Adolescent idiopathic scoliosis (AIS) is the most common type of scoliosis. Controlling its curve progression is the most important clinical task. Although recent genome-wide association studies (GWASs) identified several susceptibility loci associated with the development of AIS, the etiology of curve progression has been still unknown. Our previous GWAS has identified that rs12946942 showed significant association with severe AIS. To confirm the association, we conducted an international meta-analysis using four cohorts with different ethnicity. We analyzed 2272 severe AIS cases and 13,859 controls in total, and found the replication of significant association of rs12946942 (combined $P = 7.23\times10^{-13}$; odds ratio = 1.36, 95% confidence interval = 1.25−1.49). In silico analyses suggested that *SOX9* is the most likely susceptibility gene for AIS curve progression in the locus.

Introduction

Adolescent idiopathic scoliosis (AIS) is defined as a structural lateral spinal curvature of at least 10° of the Cobb angle that occurs between the age of 10 and the end of pubertal growth spurt [1]. The prevalence of AIS in the at-risk population (from the age of 10−16) is approximately 2−3%, while that of more than 30° is 0.1−0.3% [1, 2]. Recent Japanese epidemiological study showed that the prevalence of more than 20° which is a treatment threshold

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was 0.31% [2]. The etiology of AIS is still unclear, but twin studies and heritability strongly suggest that genetic factors play an important role in its pathogenesis [3, 4]. The genetic factors for AIS have been investigated extensively through genome-wide association studies (GWASs). Our GWASs previously identified several loci associated with the development of AIS [5–9].

While genetic factors for the development of AIS have been investigated well, the etiology of curve progression has been still unknown. Early detection of progression risk is important to establish the treatment strategy of AIS. There were many candidate gene approaches to identify the genes associated with the curve severity and progression; single nucleotide polymorphisms (SNPs) of *TGFB1*, *NTF3*, *GPER*, *TIMP2*, *ESR1*, *ESR2*, *MATN1*, and *IGF1* were associated with the curve severity [10–17]; however, *NTF3*, *GPER*, and *TIMP2* were not replicated in Japanese cohorts [5, 18–20].

We previously conducted a GWAS that focused on the cases with severity curve and identified a common variant rs12946942 (risk allele: T) on chromosome 17q24.3 associated with severe AIS [21]. rs12946942 did not show any significant association in GWAS for AIS susceptibility. In the previous GWAS, rs12946942 showed significant association in Japanese in the recessive model ($P = 4.00\times10^{-8}$). Combined P value with a Chinese replication study improved to 6.43×10^{-12} in the recessive model [21].

The multiethnic studies can increase the reliability of the association not to mention the increase of the statistical power. In this study, to confirm the association of rs12946942 with severe AIS, we conducted a meta-analysis using multiethnic cohorts of ~17,000 subjects. The result provided the convincing association of rs12946942 with AIS severity worldwide. Moreover, in silico analyses suggested that *SOX9* is the most promising candidate for the susceptibility gene at this locus.

Materials and methods

Subjects and genotyping

Informed consent was obtained from all subjects participating in this study. The ethics committee of RIKEN approved this study. All experiments were performed in accordance with relevant guidelines and regulations. The datasets generated during the current study are available from the corresponding authors on reasonable request. Severe AIS was defined as Cobb's angle greater than 40° at the time the subject was recruited in this study according to the previously described criteria [21]. The subjects in the Japanese and Nanjing-Chinese cohorts were recruited and genotyped as previously described [21]. The details of additional studies: i.e., Hong Kong and Scandinavia studies were described as below.

Hong Kong study

AIS subjects were recruited from the Duchess of Kent Children's Hospital in Hong Kong. The inclusion criteria were as previously described [22]. Control subjects were randomly selected from the subjects recruited for the Genetic Study of Degenerative Disc Disease project [23]. All were confirmed not to have AIS by Magnetic Resonance Imaging (MRI) examination of the spine. Genomic DNA was extracted from peripheral blood lymphocytes using standard procedures. The PCR-based invader assay (Third Wave Technologies, WI, USA) was used for genotyping.

Scandinavia study

AIS subjects were recruited from six hospitals in Sweden and one in Denmark as described previously to the Scoliosis and Genetics in Scandinavia (ScoliGeneS) study [24–27]. Individuals with a history or clinical sign of a nonidiopathic scoliosis and with neural abnormalities in an MRI of the spine were excluded. All control subjects were females and recruited from the Osteoporosis Prospective Risk Assessment cohort and PEAK-25 cohort [28, 29]. Dual-energy X-ray absorptiometry (DXA) scan was performed in both cohorts and subjects showing any sign of a curved spine on DXA were excluded. Genomic DNA was extracted from blood or saliva using the QIAamp 96 DNA Blood Kit and the Autopure LS system (Qiagen). iPLEX Gold chemistry and the MassARRAY system (Agena Bioscience) were used for genotyping. Genotype calls were checked by two persons individually using the MassARRAY Typer v4.0 Software (Agena Bioscience).

Statistical analysis

The association of rs12946942 with severe AIS in each study was evaluated by the Cochrane−Armitage trend test. Data from the four studies were combined using the inverse variance method assuming a fixed effects model in the METAL software package (http://csg.sph.umich.edu//abecasis/Metal/). The heterogeneity among studies was tested using Cochran's Q test based upon inverse variance weights using METAL.

Results and discussion

We conducted the meta-analysis of rs12946942 using four cohorts (Table 1). We confirmed that rs12946942 showed no deviation from Hardy−Weinberg equilibrium in neither case nor control in each cohort ($P < 1\times10^{-6}$) (Table S1). Two of them (Japanese and Nanjing) were previously

Table 1 Association of rs12946942 with severe adolescent idiopathic scoliosis

Population	Study	Sample number		RAF		*P* value*	OR (95% CI)	P_{het}
		Case	Control	Case	Control			
Japanese	Japan	822	11,294	0.258	0.211	9.72E-06	1.30 (1.16−1.45)	
Chinese	Nanjing	571	326	0.392	0.288	1.78E-05	1.59 (1.30−1.96)	
	Hong Kong	183	442	0.276	0.270	8.40E-01	1.03 (0.78−1.35)	
	Combined					1.58E-04	1.37 (1.16−1.61)	0.01
Caucasian	Scandinavia	696	1797	0.114	0.075	1.04E-05	1.58 (1.29−1.94)	
All	Combined	2272	13,859			7.23E-13	1.36 (1.25−1.48)	0.03

RAF risk allele (rs12946942-T) frequency, *OR* odds ratio, *CI* confidence interval, P_{het} *P* value for Cochran's Q test for heterogeneity

*The *P* values were calculated from the Cochran−Armitage trend test for each stage and combined *P* values were calculated by the inverse variance method

reported [21] and the other two were recruited for this study that included the cohorts of Hong Kong (case 183 and control 442) and Scandinavia (case 696 and control 1797). Finally, 2272 cases and 13,859 controls were included in the meta-analysis. We first conducted the meta-analysis in the recessive model, because rs12946942 only showed significant association in the recessive model in the previous GWAS [21]. We found that rs12946942 showed significant association: combined $P = 1.98\times10^{-10}$; odds ratio (OR) = 1.98; 95% confidence interval (CI) = 1.60−2.44. rs12946942 showed more significant association by the Cochrane−Armitage trend test: combined $P = 7.23\times10^{-13}$; OR = 1.36; 95% CI = 1.25−1.49 (Table 1). ORs were > 1 in all four cohorts; however, the Forest plot showed a little difference between the cohorts (Fig. 1) and significant heterogeneity of the cohorts was found (combined *P* heterogeneity in all = 0.03; *P* heterogeneity in Chinese population = 0.01) (Table 1). It might be caused by the Hong Kong cohort, because the analysis excluded the cohort did not show significant heterogeneity (combined *P* heterogeneity = 0.1). Moreover, rs12946942 showed more significant association in the meta-analysis excluded the Hong Kong cohort than that in all: combined $P = 8.16\times10^{-14}$; OR = 1.40; 95% CI = 1.28−1.54. We also identified the significant association by the random effect model (combined $P = 5.00\times10^{-4}$; OR = 1.37; 95% CI = 1.15−1.63).

rs12946942 is located in the intergenic region on chromosome 17q24.3 and there are no genes in the linkage disequilibrium (LD) block ($r^2 > 0.8$) represented by rs12946942 [21]. The closest genes are *SOX9* and *KCNJ2* that are 0.8−1 Mb away from rs12946942. Neither *SOX9* nor *KCNJ2* was contained in the LD block of rs12946942 (Fig. 2). We evaluated the topologically associating domains (TADs) using H1-mesenchymal stem cell lines and H1-neural progenitor cell lines to identify the candidate susceptibility gene in the locus. Hi-C data [30] (http://promoter.bx.psu.edu/hi-c/view.php) revealed that *SOX9* was included in the TAD, but *KCNJ2* was not (Fig. 2a, b).

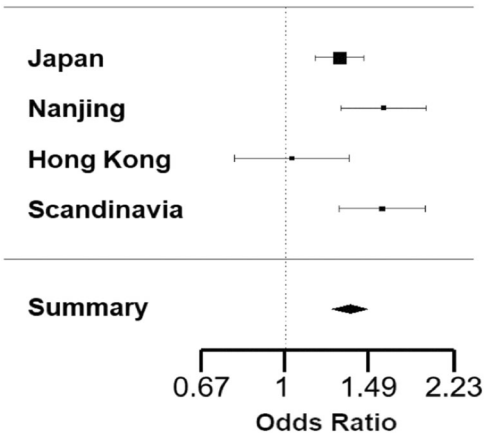

Fig. 1 The forest plot for the association of rs12946942 with severe AIS. The odds ratios and 95% confidence intervals were estimated by the fixed-effect model using METAL. The contributing effect from each study is shown by a square with its confidence interval indicated by a horizontal line. Summary: the combined meta-analysis estimate. AIS adolescent idiopathic scoliosis

We then evaluated the expression level of *SOX9* in many tissues and cells including fibroblast, spinal cord, and skeletal muscle which are considered as the tissues associated with AIS according to the expression quantitative trait loci (eQTL) data from the Genotype-Tissue Expression (GTEx) project [31]. The lower *SOX9* expression level was associated with risk allele (T allele) of rs12946942 ($P = 0.038$ in skin) (Fig. S1).

In our previous GWAS study, we identified the common variant, rs12946942 that showed a significant association with severe AIS in Japanese and the association was replicated in a Chinese cohort. However, the association was significant only in the recessive model, probably because of the limited number of subjects. In the present study, we conducted a meta-analysis for the genetic association of rs12946942 with severe AIS using more than 16,000 subjects from four independent multiethnic cohorts that included non-East Asian populations and identified

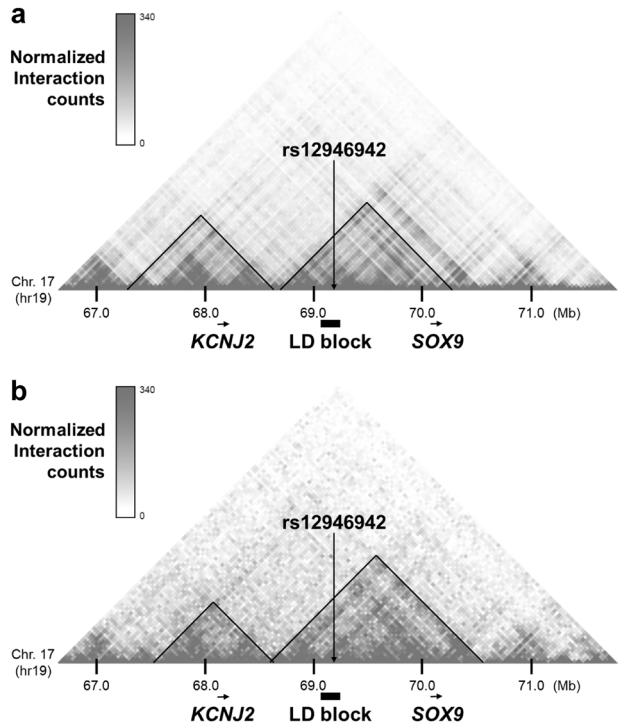

Fig. 2 The Hi-C interaction around the severe AIS-associated region on chromosome 17q24.3. Hi-C interactions in **a** H1-mesenchymal stem cell and **b** H1-neural progenitor cell were generated by using the Interactive Hi-C Data Browser. The linkage disequilibrium (LD) block (bold line) does not contain any genes. *SOX9* only lies within the topologically associated domain (black triangle) that contains the LD block of SNPs associated with severe AIS. AIS adolescent idiopathic scoliosis

significant association of rs12946942 with severe AIS in other models than the recessive model. rs12946942 is the first SNP that has convincing evidence of association with AIS curve severity worldwide [21].

rs12946942 is located in the intergenic region far away from the closest genes, *SOX9* and *KCNJ2*. It is well known that *SOX9* is absolutely necessary for chondrocyte specification and early differentiation and that its heterozygous loss of function mutations cause campomelic dysplasia that presents severe kyphoscoliosis [32–34]. Therefore, it is reasonable to consider that decreased *SOX9* expression by the risk allele of rs12946942 would lead to scoliosis. On the other hand, *KCNJ2* encodes an inwardly rectifying potassium channel which is expressed in neuronal and muscle tissues and its heterozygous mutations cause Andersen and Bartter syndrome that also presents scoliosis [35, 36]. Thus, both *SOX9* and *KCNJ2* are good candidate genes for AIS. Because no genes, including *SOX9* and *KCNJ2* were contained in the LD block of rs12946942, we evaluated the TADs around rs12946942 and found that the TAD contained *SOX9* but not *KCNJ2*. Therefore, we considered *SOX9* as the best candidate gene and rs12946942 might affect the *SOX9* expression of as a long-range locus control element. The existent of such element(s) controlling *SOX9* has been reported [37]. The eQTL data for *SOX9* and rs12946942 are not convincing. Further in silico and in vitro analysis is necessary to clarify the association of rs12946942 with *SOX9*.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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