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BASIC SCIENCE

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A Genetic Variant of *FAM46A* is Associated With the Development of Adolescent Idiopathic Scoliosis in the Chinese Population

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Study Design. A genetic case-control study.

Objective. To replicate recently reported genetic loci associated with adolescent idiopathic scoliosis (AIS) in the Chinese Han population, and to determine the relationship between gene expression and the clinical features of the patients.

Summary of Background Data. A recent study conducted in the Japanese population identified several novel susceptible loci, which might provide new insights into the etiology of AIS. However, the association of these genes with AIS in other populations remains unclear.

Materials and Methods. A total of 1210 AIS and 2500 healthy controls were recruited for the genotyping of 12 susceptibility loci. Paraspinal muscles used for gene expression analysis were obtained from 36 AIS and 36 patients with congenital scoliosis. The difference regarding genotype and allele frequency between patients and controls was analyzed by χ^2 analysis. The *t* test was performed to compare the target gene expression level between controls and AIS patients. Correlation analysis was performed between gene expression and phenotypic data, including Cobb angle, bone mineral density, lean mass, height, and body mass index.

Results. Four SNPs, including rs141903557, rs2467146, rs658839, and rs482012, were successfully validated. Allele C of rs141903557, allele A of rs2467146, allele G of rs658839, and allele T of single nucleotide polymorphism rs482012 showed significantly higher frequency in patients. Allele C of rs141903557, allele A of rs2467146, allele G of rs658839, and allele T of rs482012 could notably increase the risk of AIS patients, with an odds ratio of 1.49, 1.16, 1.11, and 1.25, respectively. Moreover, tissue expression of *FAM46A* was significantly lower in AIS patients as compared with controls. Moreover, *FAM46A* expression was remarkably correlated with bone mineral density of patients.

Conclusion. Four SNPs were successfully validated as novel susceptibility loci associated with AIS in the Chinese population. Moreover, *FAM46A* expression was associated with the phenotype of AIS patients.

Key words: adolescent idiopathic scoliosis, *FAM46A*, gene, etiology

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As the most commonly seen spinal deformity during puberty, the etiology of adolescent idiopathic scoliosis (AIS) remains poorly understood to date.[1] Previous studies have indicated that genetic factors played a crucial role in the pathogenesis of AIS.[2–7] Moreover, recent studies indicated that AIS was influenced by multiple genetic components as well as environmental factors.[6–9] Thus, understanding the genetic underpinnings of AIS could help expand the capacity for detection and treatment.[10,11]

Genome-wide association studies (GWAS) represent a powerful and reliable method for mapping genetic loci of complex traits.[12,13] The first GWAS utilized for the identification of genetic loci of AIS was reported by Sharma *et al*[6] in 2011, which revealed the association of *CHL1* with AIS in the Caucasian population. Subsequently, numerous susceptible genes, including *GRP126*, *LBX1*, *BNC2*, *PAX1*, and *BCL2*, have been reported to be associated with the development of AIS through GWAS.[3,7,9,14,15] Recently,

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Ikuyo *et al*[2] conducted a genome-wide association study consists of 79,211 Japanese individuals and identified 14 novel susceptible loci significantly associated with AIS, which might provide new insights into the etiology.

Previous genetic association studies on complex traits have identified multiple ethnic-specific alleles that may have diverse phenotypic impacts in different populations.[12,16,17] Thus, it is necessary to validate the AIS-associated loci reported by Ikuyo *et al*[2] in other ethnic groups. Our previous work has validated the association between rs1978060 (*TBX1*) and the development of AIS.[18–21] The purpose of the present study was to verify whether the other AIS susceptibility loci identified by Ikuyo *et al*[2] are associated with the development of AIS in the Chinese Han population, and to determine the relationship between gene expression and the clinical features of patients.

MATERIALS AND METHODS

Subjects

A total of 2500 healthy controls and 1210 AIS patients who visited our clinical center between April 2010 and December 2016 were recruited. Standing posteroanterior radiographs of the whole spine were obtained for the evaluation of curve magnitude. MRI and CT examinations were performed to exclude neurological defect, skeletal dysplasia, and congenital vertebral deformity. For the controls, the possibility of scoliosis was ruled out through Adam's Forward Bend Test performed by a senior spine surgeon (Z.Z.).

Genotyping of the Target SNPs

With written informed consent obtained from the families, we collected blood samples from the patients and healthy controls. The commercial kit (QIAGEN, Tokyo, Japan) was used to extract the genomic DNA of blood samples. A total of 12 newly identified susceptibility loci for AIS reported by Ikuyo *et al*,[2] including rs141903557, rs11205303,rs12029076, rs2467146,rs11787412, rs188915802, rs658839, rs160335, rs482012, rs17011903, rs397948882, and rs12149832, were genotyped using TaqMan single nucleotide polymorphism Genotyping Assay, and were read with an ABI PRISM 7900HT sequence detection system (Applied Biosystems, Foster City, CA) as previously described.[16] We randomly selected ten percent of the DNA samples for the validation of the reliability of the genotyping results.

Procedures of Gene Expression Analysis

Under the approval of the local institution review board, muscle samples adjacent to the proximal end vertebra were collected from the subjects during the correction surgery. The cohort of subjects included in the gene expression analysis consisted of 36 AIS patients and 36 congenital scoliosis (CS) patients who were considered as controls. The total RNA was extracted using Trizon reagent (QIAGEN, Tokyo, Japan) and was then transcribed to cDNA by PrimerScript RT reagent kit with gDNA Eraser (TaKaRa, Tokyo, Japan) according to the manufacturer's protocol. Expression levels of the genes which presented significantly different allele frequencies between patients and controls were quantified with real-time polymerase chain reaction (qPCR) using gene-specific primers listed in Table 1. The expression of glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) was used as the endogenous control. The reaction system and thermal condition of qPCR were the same as previously published.[22] All samples were analyzed in triplicate. To confirm the quality of the qPCR products and ensure the reliability of the experimental results, melting curve analysis was performed at the terminal of the reaction.

Statistical Analysis

The Hardy-Weinberg equilibrium test was used for all patients. The Chi-square test was used to compare the differences in allele distribution between controls and AIS patients. The odds ratio (OR) and 95% confidence interval (95% CI) ranges were calculated using the minor allele as a reference. The student *t* test was used to compare the target gene expression between the AIS patients and controls. The Pearson correlation analysis was performed to assess the correlation between the gene expression level and the clinical features of the patients, including age at menarche, Cobb angle, bone mineral contents, bone mineral density, lean mass, height, and body mass index (BMI). The SPSS software (version 22.0; SPSS Inc., Chicago, IL) was utilized for all the statistical analyses, with statistical significance set at a P value of less than 0.05.

RESULTS

Demographic Data of the Participants

For genotyping analysis, the average age of AIS patients and the healthy controls was 13.6 ± 2.1 years and 18.4 ± 4.4 years, respectively. For the patients, the mean curve magnitude was

TABLE 1. Specific Primers for the Amplification of GAPDH, *FAM46A*, and NT5DC1

Gene	Primer sequence	
	Forward	Reverse
GAPDH	5′-GAGTCAACGGATTTGGTCGT-3′	5′-TTGATTTTGGAGGGATCTCG-3′
FAM46A	5′-GTGCCTTGGATAACTCATTGGC-3′	5′-AGTGCATCTAAAGACTCCACCA-3′
NT5DC1	5′-CGACAGCTAAAGAATGCTGGG-3′	5′-TCTGACTTGGTAAGTGGGAGAAG-3′

38.9 ± 3 For the patients, the mean age of menarche was 12.1 ± 2.3 years (range, 10.1–15.4 y), the mean BMI was 17.1 ± 3.3 kg/m^2 (range, 16.3 to 25.1 kg/m^2), and the mean curve magnitude was 38.9 ± 3.5 degrees (range 22 degrees to 71 degrees). The curve pattern included a double major curve in 310 (25.6%) patients, main thoracic curve in 672 (55.5%) patients and major lumbar curves in 228 (18.9%) patients, respectively. For the 36 AIS patients included in the tissue expression analysis, the mean body mass index (BMI) was 19.1 ± 3.4 kg/m^2, the mean age was 13.8 ± 2.7 years, and the mean curve magnitude was 52.3 ± 6.6 degrees (range, 45–62 degrees). For the 36 CS patients who served as controls, the mean age was 14.3 ± 3.1 years, the mean BMI was 20.0 ± 3.2 kg/m^2, and the mean curve magnitude was 49.2 ± 7.3 degrees. The two groups were matched in terms of age ($P = 0.468$), BMI ($P = 0.251$), and curve magnitude ($P = 0.063$).

Association of the SNPs with AIS

A total of 12 SNPs were genotyped for the participants. No significant difference in genotype frequencies was noted from the Hardy-Weinberg equilibrium test. The risk allele frequency of the 12 SNPs are summarized in Table 2. The previously reported polymorphisms of rs188915802 and rs397948882 were both monomorphic in our cohort. Among the other 10 SNPs, 4 SNPs, including rs141903557, rs2467146, rs658839, and rs482012, were successfully validated as the susceptibility loci of AIS. As shown in Table 2, allele C of rs141903557 (2.8% vs. 1.9%, $P = 0.014$), allele A of rs2467146 (72.0% vs. 68.8%, $P = 0.005$), allele G of rs658839 (58.6% vs. 56.0%, $P = 0.036$), and allele T of single nucleotide polymorphism rs482012 (79.7% vs. 75.89%, $P < 0.001$) were significantly associated with the risk of AIS. The ORs were 1.49 (95% CI = 1.09–2.04) for rs141903557, 1.16 (95% CI = 1.04–1.30) for rs2467146, 1.11 (95% CI = 1.01–1.23) for rs658839, and 1.25 (95% CI = 1.11–1.41) for rs482012, respectively. With regard to the allele frequency of the other 6 SNPs, there is no significant difference between AIS patients and healthy controls.

Tissue Expression of the Target Genes in AIS Patients and Controls

As shown in Figure 1, the expressions of *FAM46A* were significantly lower in the AIS patients compared with the controls (0.00146 ± 0.00011 vs. 0.00193 ± 0.00011, $P = 0.003$). No significant difference was observed between the two groups regarding the mRNA expression of *NT5DC1* (0.00346 ± 0.00015 vs. 0.00381 ± 0.00019, $P = 0.16$).

As shown in Figure 2, no significant correlation between the expression of *FAM46A* and the clinical parameters including Cobb angle ($r = 0.118$, $P = 0.493$), height ($r = -0.072$, $P = 0.677$), BMI ($r = 0.039$, $P = 0.819$), or lean mass ($r = -0.010$, $P = 0.952$) was found. Correlation analysis between gene expression and phenotypic data showed that mRNA level of *FAM46A* was slightly positively correlated with bone mineral contents ($r = 0.378$, $P = 0.023$).

DISCUSSION

Recently, by means of GWAS, Ikuyo *et al*[2] revealed several novel susceptibility loci associated with AIS in the Japanese population. In the current study, based on a large cohort of cases and controls, we validated these loci in the Chinese population for the first time and successfully replicated 4 SNPs associated with AIS. The OR values conferred by rs141903557, rs658839, rs2467146, and rs482012 were

TABLE 2. Distribution of the Genotype and Allele Frequency of the SNPs in AIS Patients and Healthy Controls

SNPs	RA	RAF		*P*	OR (95% CI)	
		AIS	Controls		Current study	Ikuyo *et al*
rs141903557 (LOC101928978)	C	0.028	0.019	0.014*	1.49 (1.09-2.04)	1.33 (1.22-1.45)
rs11205303 (*MTMR11*)	C	0.212	0.197	0.139	1.09 (0.98-1.23)	1.17 (1.11-1.23)
rs12029076 (*ARF1*)	G	0.212	0.197	0.131	1.10 (0.97-1.24)	1.18 (1.12-1.24)
rs2467146 (LINC02378/MIR3974)	A	0.720	0.688	0.005*	1.16 (1.04-1.30)	1.15 (1.10-1.20)
rs11787412 (CSMD1)	A	0.394	0.373	0.087	1.09 (0.99-1.21)	1.14 (1.09-1.18)
rs188915802 (*KIF24*)	T	0	0	NA	—	1.66 (1.41-1.96)
rs658839 (BCKDHB/FAM46A)	G	0.586	0.560	0.036*	1.11 (1.01-1.23)	1.14 (1.09-1.19)
rs160335 (*CREB5*)	G	0.652	0.650	0.897	1.00 (0.911.11)	1.13 (1.08-1.18)
rs482012 (NT5DC1)	T	0.797	0.758	< 0.001*	1.25 (1.11-1.41)	1.14 (1.09-1.19)
rs17011903 (*PLXNA2*)	A	0.102	0.100	0.774	1.02 (0.87-1.20)	1.20 (1.13-1.28)
rs397948882 (*AGMO/MEOX2*)	A	0	0	NA	—	1.20 (1.12-1.28)
rs12149832 (*FTO*)	G	0.165	0.171	0.530	0.96 (0.84-1.09)	1.16 (1.10-1.22)

**Statistical significance was assumed at P < 0.05.*
CI indicates confidence interval; OR, odds ratio; RAF, risk-allele frequency.

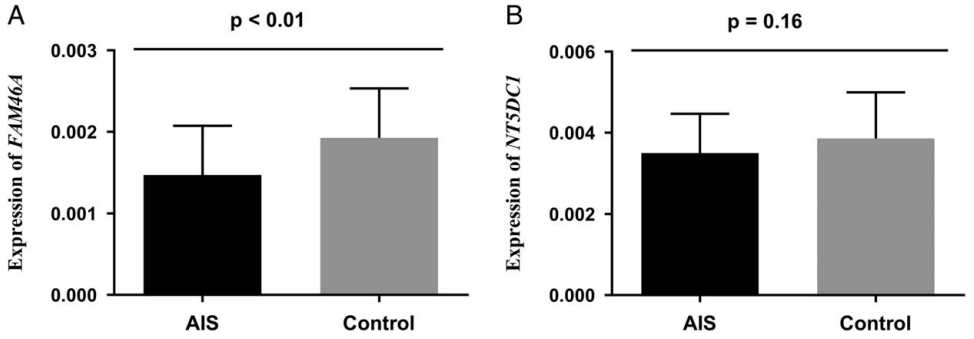

Figure 1. Relative tissue expression of *FAM46A* and *NT5DC1* in AIS and controls. a and b, Expression of *FAM46A* in AIS patients was significantly decreased compared with the controls. b,No significant difference was found between AIS patients and CS patients regarding *NT5DC1* expression. AIS indicates adolescent idiopathic scoliosis; CS, congenital scoliosis.

1.49, 1.11, 1.16, and 1.25, respectively, which were roughly in line with the original results as reported for the Japanese population.[2] Also, it is noteworthy that the OR value of the replicated SNPs was all less than 1.5, which speaks to the role of polygenetic components in the etiology of AIS. Obviously, currently reported SNPs can only explain a small part of the overall variance in AIS. More susceptible genes need to be discovered to further elucidate the genetic background of AIS.

There were 8 susceptible SNPs reported by Ikuyo *et al*,[2] which showed no significant difference between the AIS patients and the healthy controls in the Chinese population. Given the large sample size in this study which provided sufficient statistical power, we do not feel there is concern for a false-negative finding. Lack of replication or discrepant phenotypic impact conferred by the same susceptibility locus is common in genetic association studies, partly due to the existence of genetic heterogeneity among different ethnic groups.[16,23–27] As mentioned by Ikuyo *et al*,[2] several susceptibility loci reported by our previous GWASs were also not successfully replicated in their cohort, suggesting ethnic specificity between the Chinese and Japanese populations. Further investigation of these SNPs with larger sample sizes in other populations is required to validate the implication of these susceptibility loci in the pathogenesis of AIS.

In this study, to explore the potential functional role of the genes which were successfully replicated, we investigated the tissue expression of *FAM46A* and *NT5DC1* in AIS patients. Compared with the control group, a significantly lower expression level of *FAM46A* was observed in AIS patients. *FAM46A* belongs to the family with sequence similarity 46 (*FAM46*), which is involved in various diseases such as metastatic melanoma, lupus nephritis, and multiple myeloma.[28] Although the exact function of

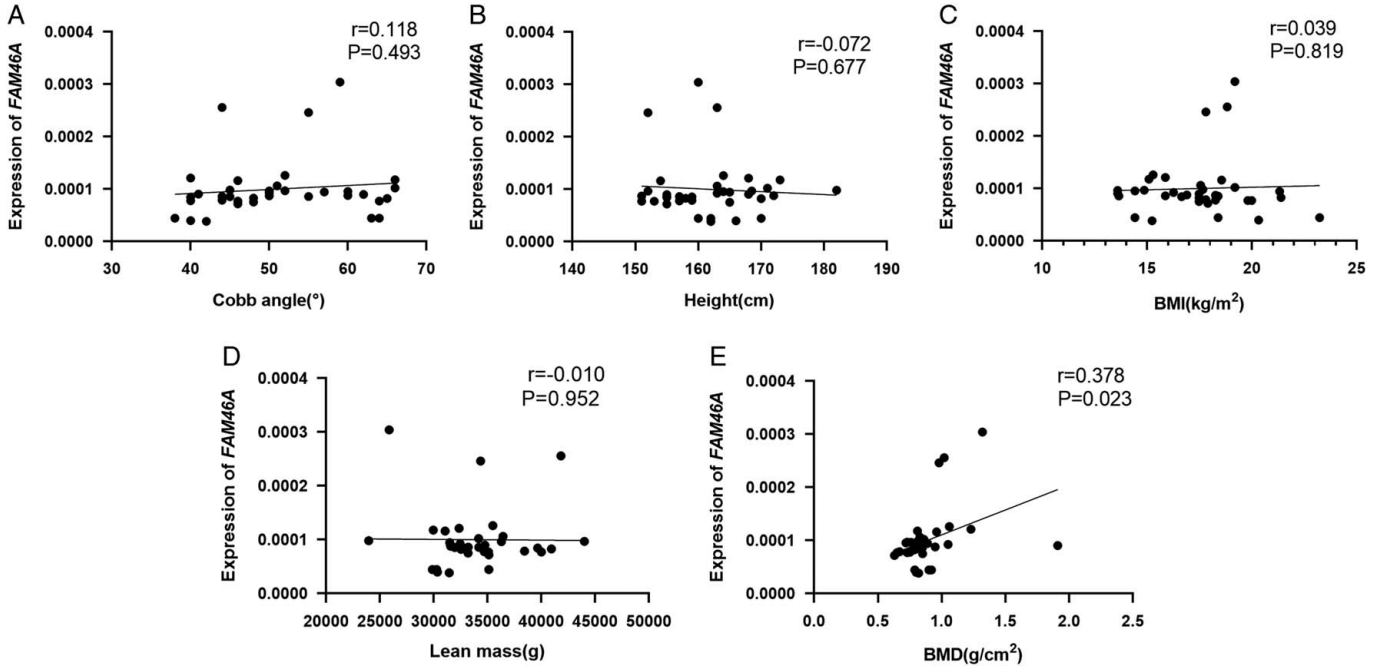

Figure 2. The correlation between the expression of the *FAM46A* gene and clinical features of the patients. There was a remarkable correlation between *FAM46A* expression and BMD ($r = 0.378$, $P = 0.023$). As for Cobb angle ($r = 0.118$, $P = 0.493$), height ($r = -0.072$, $P = 0.677$), BMI ($r = 0.039$, $P = 0.819$) or lean mass ($r = -0.010$, $P = 0.952$), no significant correlation with *FAM46A* expression was found.

FAM46A is as yet unknown, recent studies indicate a strong relationship between *FAM46A* and skeletal dysplasia.[29,30] Another study revealed that *FAM46A* was a genetic marker for total collagen content.[31] Interestingly, we observed that the expression level of *FAM46A* was correlated with the bone mineral density of patients. Low bone mineral density has long been confirmed as a phenotype of AIS patients. Given the decreased expression of *FAM46A* in AIS patients, we speculated this gene might contribute to the development of AIS via bone homeostasis and collagen balance. In future work, construction of animal models and molecular experiments will provide new insight into the role of *FAM46A* in the development of AIS.

Two limitations of our study should be mentioned. First, we only evaluated gene expression in the paraspinal muscle tissue of AIS patients. Expression analysis for more types of AIS tissues, such as vertebrae and spinal ligaments, should be performed in the future. Second, we were unable to obtain paraspinal muscles from healthy age-matched controls. Thereby, age-matched CS patients were recruited as controls for gene expression analysis, despite the fact that they were still not ideal controls, and this may contribute to some confounding. Tissues from spinal trauma patients could be collected to further validate our findings in the future.

CONCLUSION

In this study, four SNPs were successfully validated as novel susceptibility loci associated with AIS in the Chinese population. Moreover, *FAM46A* expression was associated with the phenotype of AIS patients.

➢ Key Points

- ❑ Four SNPs, including rs141903557, rs2467146, rs658839, and rs482012, were successfully validated as novel susceptibility loci of AIS in the Chinese population.
- ❑ FAM46A expression was correlated with the BMD of patients.
- ❑ FAM46A expression was associated with the phenotype of AIS patients.

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