VANGL1 is not associated with the susceptibility of adolescent idiopathic scoliosis in the Chinese population

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Abstract

Study Design: A case-control study

Objective: To replicate the association between the VANGL1 gene and the susceptibility of AIS in the Chinese population.

Summary of Background Data: The mutations of VANGL1 gene were recently reported to be associated with adolescent idiopathic scoliosis (AIS) in the Danish population. However, there is a lack of replication in other populations. Further analysis of the functional role of VANGL1 in AIS was warranted.

Methods: A total of 1481 female AIS patients and 1372 age-matched healthy controls were included in this study. SNV sc.407T>A and c.1318T>G were genotyped using allelic-specific multiple ligase detection reactions. SNPs covering VANGL1 gene were selected using Haploview (v2.6). The associations between these SNPs and AIS were investigated through Cochran-Armitage trend test by PLINK (v1.90). Relative mRNA expression of VANGL1 in the paraspinal muscles was analyzed for 30 patients and 24 age-matched controls. The difference of mRNA expression level between the two groups was analyzed with the student t test.

Results: There was no case of mutation for all the subjects. A total of 22 SNPs covering VANGL1 were analyzed. All the SNPs were found to have comparable distribution of genotype and allele frequency in the cases and the controls. Moreover, there was no significant difference regarding the mRNA expression of VANGL1 in the two groups.

Conclusions: VANGL1 gene is not associated with AIS in the Chinese population. Replication studies in other ethnic groups are warranted to further clarify the role of the VANGL1 gene in AIS.

Key Words: VANGL1; Mutation; Adolescent idiopathic scoliosis; Replication

Level of Evidence: 4
Introduction

Adolescent idiopathic scoliosis (AIS) is a complex deformity of the spine that occurs during the pubertal growth.\(^1\) It is commonly believed that genetic factors play an important role in the development of AIS due to the obvious hereditary trend.\(^2\) Previous genome-wide linkage studies in multiplex AIS families discovered a few susceptible regions located in Chromosome 9, 12 and 18.\(^3-5\) In addition, many susceptible genes of AIS have been reported through candidate genetic association studies.\(^6-9\) However, the primary limitation of these association studies lies in that few of them can be successfully replicated in different populations. To address this limitation, the genome-wide association study (GWAS) was then used to investigate the genetic etiology of AIS. Based on the GWAS data conducted in the Caucasian, the Japanese and the Chinese populations, a number of susceptible loci were reported for AIS, including CHL1, LBX1, GPR126, PAX1, BNC2, BCL2, PAX3 and AJAP1.\(^10-15\) Nevertheless, the common variants reported by these studies account for only a limited proportion of the AIS heritability, leaving the genetic architecture poorly understood.

Playing an important role in the familial inherited disease, rare variants have been recently found to be involved in the development of AIS.\(^16-21\) Li et al\(^17\) reported that a novel mutation of AKAP2 was associated with AIS in a three-generation family from the Chinese population. Buchan et al\(^18\) performed the whole-exome sequencing to investigate rare variants associated with AIS. The authors reported that rare variants of FBN1 and FBN2 could remarkably add to the risk of AIS, which were successfully replicated in both European ancestry and Han Chinese population.\(^18\) In the study of Patten et al,\(^19\) rare functional variants in the POC5 were identified to be associated with idiopathic scoliosis (IS) through genetic linkage analyses combined with exome sequencing, which were subsequently replicated in an independent cohort of AIS patients. Rare variant p.Asn786Ser of HSPG2 was reported to be associated with familial IS, and it was also successfully replicated in two independent cohorts.\(^20\)
In a recent study, two rare missense mutations in VANGL1 were identified to be associated with AIS in the Danish population. Since a significant divergence exists between different populations regarding genetic susceptibility of AIS, replication studies of these two rare variants are warranted to validate their roles in the development of AIS. In this study, a large cohort of patients and controls were recruited for the detection of these two reported variants. The primary purpose of this study was to replicate the relationship between VANGL1 and AIS in the Chinese population.

Methods

Subjects

Under the approval of the ethics committees, a cohort of 1481 AIS patients who visited our scoliosis center between June 2011 and April 2016 were included in this study. The inclusion criteria were as follows: 1. female; 2. diagnosed as AIS through clinical and radiological examinations; 3. with curve magnitude more than 40 degrees. MRI examination was performed for each patient to exclude potential neurological defect. The curve magnitude was measured on the standing posteroanterior X-ray films using the Cobb method. 1372 healthy female controls were recruited through a community-based physical examination program. All the controls were excluded to have AIS through Adam’s Forward Bend Test by an experienced spinal surgeon (Q.Y.). Demographic data were recorded for each subject, including age at visit, age at menarche, curve magnitude, and BMI.

Genotyping of rare variation

Blood samples were collected for DNA extraction after informed consent was obtained from the subjects. Genomic DNA was extracted from blood leukocytes following standard protocols (Qiagen K.K., Tokyo, Japan). SNVs c.407T>A and c.1318T>G of VANGL1 were genotyped for all DNA samples using allelic-specific multiple ligase detection reactions (LDR). As shown in Table 1, the primers were designed on the basis of the genomic sequences in the GenBank.
Ten percent of the samples were selected randomly to assess the reliability of the genotyping results, yielding a 100% reproducibility rate.

**RNA extraction and real-time qPCR**

Paraspinal muscle was collected from 30 female AIS patients and 24 age-matched female lumbar disc herniation (LDH) patients during the surgical intervention. LDH patients were included as the normal controls since none of them presented spinal deformity. All the muscle samples were collected at the right side of the lower end vertebral for AIS and at the right side of the L5 level for LDH patients. Total RNA was then isolated from the muscle using a commercial kit (CWBio. Co. Ltd). The mRNA expression of VANGL1 was measured with real-time polymerase chain reaction (PCR) on ABI 7900HT. The gene-specific primers were designed as follows: forward 5’-GACAGAGGAAGTTCAGGATGACA-3’, reverse 5’-GTAGCGTTTGCAATCCAGCC-3’ for VANGL1, and forward 5’-GAGTCAACGGATTTGGTCGT-3’, reverse 5’-TTGATTGTTGGGATCTCG-3’ for the endogenous control gene GAPDH. All amplifications were performed in triplicate.

**Genotyping of common variations in VANGL1**

The GWAS database reported in our previous study was used to investigate the genotyping information of AIS patients and normal controls. Common variants of VANGL1 gene that were encompassed in the Affymetrix Genome Wide Human SNP Array 6.0 (Affymetrix Inc., Santa Clara, California, USA) were indentified through HaploView (v2.6). The genotyping results of each SNP were then calculated accordingly.
Statistical analysis

SPSS version 19.0 (Chicago, USA) was used for the data analysis. Demographic data and mRNA expression level were compared between the cases and the controls using the Student's t test. Specifically, parametric test was applied to the inter-group comparison since all the data followed a Gaussian distribution after being tested for normality. The association of each SNP with AIS was calculated with PLINK version 1.90 (http://pngu.mgh.harvard.edu/~purcell/plink/) through Cochran-Armitage trend test. The Pearson correlation analysis was used to determine the relationship between the VANGL1 expression and the curve severity. Statistical significance was set at p <0.05.

Results

Demographic data

For patients included in the LDR analysis, the mean age was 15.1 ± 3.2 years (range 10.5 - 18.9 years). The mean curve magnitude was 54.3 ± 11.5 degrees (range 25 - 69 degrees). The mean age at menarche was 12.3 ± 1.8 years (range 10.2 - 14.3 years). The mean BMI was 17.6 ± 4.2 kg/m² (range 15.3 - 23.1 kg/m²). 895 patients underwent surgical intervention as they had Cobb angle more than 50 degrees.

Genotyping of variations in VANGL1

The results of LDR analysis showed no case of mutation in SNVs c.407T>A and c.1318T>G of VANGL1. All the subjects were found to have a genotype of TT for both SNVs. The GWAS dataset was composed of 1446 AIS patients and 2080 controls. A total of 22 SNPs covering VANGL1 were analyzed. The allele frequencies were summarized in Table 2. None of these SNPs had significantly different distribution of allele frequency between the cases and the controls.
Tissue expression level of VANGL1

The mRNA expression of VANGL1 in the bilateral paraspinal muscles was successfully detected for 30 patients and 24 controls. The two groups were matched in terms of age (15.1 ± 1.5 years vs. 15.6 ± 1.3 years, p = 0.13). As shown in figure 1, the mRNA expression of VANGL1 was comparable between the two groups (0.000052 ± 0.000024 vs. 0.000045 ± 0.000034, p = 0.37). There was no significant association between the VANGL1 expression and the curve severity (r = 0.085, p = 0.72).

Discussion

Rare variants are believed to partly explain the “missing heritability” of complex diseases. With the development of sequencing techniques, it has become quite feasible to investigate the role of rare variants in the development of AIS through exome sequencing. Unpublished data of Sharma et al suggested that rare variants in VANGL1 may be associated with AIS in non-Hispanic white population. Inspired by the work of Sharma et al, Andersen et al analyzed the coding region of the VANGL1 gene using Sanger sequencing in 157 AIS patients from Danish population. Two rare missense mutations in VANGL1, including c.407T>A and c.1318T>G, were identified to be associated with AIS. In the current study, we performed the genotyping of c.407T>A and c.1318T>G in a large cohort of AIS patients and normal controls, however observing no significant difference regarding the allele frequency of these two variants. Through the GWAS database, we found that there was also a lack of association between the 22 SNPs covering the VANGL1 and AIS. Taken together, the association of the previously reported genetic variations in VANGL1 with AIS was not replicated in the Chinese population.

Lack of replication is not a rare phenomenon in genetic studies, as there is always a high degree of genetic heterogeneity among patients. Previous study showed that few reported susceptibility genes of
AIS could be successfully replicated in different populations. Several factors could lead to such lack of replication, such as the ethnic differences between the different populations and small sample size. In the study of Andersen et al, only 157 patients and 197 controls were included in the sequencing analysis, and the authors did not validate their findings in an independent cohort of patients and controls. In our study, a much larger sample size undoubtedly enhanced the reliability of the results. Herein, it was unlikely for the current replication to have false-negative association due to a lack of statistical power.

VANGL1 encodes an integral membrane protein that participates in a WNT/planar cell polarity (PCP) pathway involved in early axial development. In the study of Andersen et al, the functional analyses in a cell model suggested that mutations are able to abolish the translocation of VANGL1 to the cell membrane. To further investigate the potential functional role of VANGL1 in AIS, we extracted the total RNA from paraspinal muscles of both patients and age-matched controls, but no significant difference regarding the mRNA expression of VANGL1 was observed between the two groups. In addition, there was a paucity of correlation between the mRNA expression level and the curve severity. These results did not support the functional role of VANGL1 in the development of AIS. However, the role of WNT/PCP signaling in AIS cannot be entirely excluded. Hayes et al reported that mutation of PTK7, which encodes a regulator of WNT/PCP signaling, might be associated with AIS. Herein, it may be interesting to investigate the relationship between other genes involved in the WNT/PCP pathway and AIS, which can potentially shed light on the genetic background of the disease.

**Conclusion**

Considering the power calculation and sample size of the present study, we concluded that the VANGL1 gene is not associated with the development of AIS in the Chinese population. Targeted regional sequencing of VANGL1 in more patients may be helpful to identify novel mutations.
associated with AIS. Finally, replication studies in different ethnic groups are warranted to understand the role of the VANGL1 gene in AIS.
Reference


23. Takahashi Y, Matsumoto M, Karasugi T, et al. Replication study of the association between...


AIS patients were found to have similar expression of the VANGL1 as compared with the controls (0.000052 ± 0.000024 vs. 0.000045 ± 0.000034, p = 0.37).
Table 1 Primers for the genotyping assay

<table>
<thead>
<tr>
<th>SNV</th>
<th>Primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>C.407T/A -TA</td>
<td>TTGCCTTCATCCTTTTTACCTCCGAA</td>
</tr>
<tr>
<td>C.407T/A -TT</td>
<td>TTTTTGCTTCATCCTTTTTACCTCCGAT</td>
</tr>
<tr>
<td>C.407T/A -TR</td>
<td>CCTGTGGAGGGATGAGCTGGAGCCT</td>
</tr>
<tr>
<td>C.1318T/G -TG</td>
<td>TTTTGGCTTTTCTCCTTCTGCTTCCAGGCCG</td>
</tr>
<tr>
<td>C.1318T/G -TT</td>
<td>TTTTTTGGCTTTTCTCCTTCTGCTTCCAGGCCCT</td>
</tr>
<tr>
<td>C.1318T/G -TR</td>
<td>TCCTAGAACGGTACCTCAGTCGGGTTT</td>
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Table 2 The allele frequency of SNPs covering VANGL1

<table>
<thead>
<tr>
<th>SNP</th>
<th>MA</th>
<th>Patients (n = 1446)</th>
<th>Controls (n = 2080)</th>
<th>P</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs3010380</td>
<td>G</td>
<td>0.216</td>
<td>0.220</td>
<td>0.73</td>
<td>0.98 (0.86-1.10)</td>
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<tr>
<td>rs41447448</td>
<td>C</td>
<td>0.210</td>
<td>0.216</td>
<td>0.58</td>
<td>0.96 (0.85-1.08)</td>
</tr>
<tr>
<td>rs12034462</td>
<td>T</td>
<td>0.209</td>
<td>0.215</td>
<td>0.58</td>
<td>0.95 (0.85-1.07)</td>
</tr>
<tr>
<td>rs10801911</td>
<td>G</td>
<td>0.131</td>
<td>0.132</td>
<td>0.96</td>
<td>0.99 (0.87-1.11)</td>
</tr>
<tr>
<td>rs4428890</td>
<td>T</td>
<td>0.134</td>
<td>0.126</td>
<td>0.41</td>
<td>1.07 (0.95-1.18)</td>
</tr>
<tr>
<td>rs4839469</td>
<td>A</td>
<td>0.133</td>
<td>0.143</td>
<td>0.29</td>
<td>0.92 (0.80-1.03)</td>
</tr>
<tr>
<td>rs12039617</td>
<td>A</td>
<td>0.377</td>
<td>0.368</td>
<td>0.50</td>
<td>1.04 (0.93-1.15)</td>
</tr>
<tr>
<td>rs4425985</td>
<td>A</td>
<td>0.139</td>
<td>0.124</td>
<td>0.11</td>
<td>1.14 (1.01-1.27)</td>
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<tr>
<td>rs17500488</td>
<td>G</td>
<td>0.177</td>
<td>0.172</td>
<td>0.65</td>
<td>1.03 (0.92-1.14)</td>
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<tr>
<td>rs10801913</td>
<td>A</td>
<td>0.308</td>
<td>0.314</td>
<td>0.65</td>
<td>0.97 (0.86-1.06)</td>
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<td>rs12408340</td>
<td>G</td>
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<td>0.470</td>
<td>0.19</td>
<td>1.07 (0.95-1.18)</td>
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<tr>
<td>rs11586937</td>
<td>A</td>
<td>0.129</td>
<td>0.136</td>
<td>0.45</td>
<td>0.94 (0.84-1.06)</td>
</tr>
<tr>
<td>rs10923156</td>
<td>G</td>
<td>0.426</td>
<td>0.420</td>
<td>0.68</td>
<td>1.02 (0.91-1.13)</td>
</tr>
<tr>
<td>rs4571967</td>
<td>A</td>
<td>0.448</td>
<td>0.455</td>
<td>0.59</td>
<td>0.97 (0.86-1.06)</td>
</tr>
<tr>
<td>SNP</td>
<td>Allele</td>
<td>Odd Ratio</td>
<td>95% CI</td>
<td>p-value</td>
<td></td>
</tr>
<tr>
<td>-----------------</td>
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</tr>
<tr>
<td>rs12568559</td>
<td>T</td>
<td>0.431</td>
<td>0.424</td>
<td>0.57</td>
<td>1.03 (0.92-1.14)</td>
</tr>
<tr>
<td>rs10923176</td>
<td>C</td>
<td>0.273</td>
<td>0.279</td>
<td>0.62</td>
<td>0.97 (0.86-1.06)</td>
</tr>
<tr>
<td>rs10923177</td>
<td>T</td>
<td>0.266</td>
<td>0.268</td>
<td>0.89</td>
<td>0.99 (0.87-1.11)</td>
</tr>
<tr>
<td>rs10754332</td>
<td>C</td>
<td>0.277</td>
<td>0.282</td>
<td>0.71</td>
<td>0.98 (0.86-1.10)</td>
</tr>
<tr>
<td>rs12145661</td>
<td>G</td>
<td>0.280</td>
<td>0.285</td>
<td>0.70</td>
<td>0.98 (0.86-1.10)</td>
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<tr>
<td>rs12121158</td>
<td>C</td>
<td>0.287</td>
<td>0.303</td>
<td>0.19</td>
<td>0.92 (0.80-1.03)</td>
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<tr>
<td>rs17034260</td>
<td>A</td>
<td>0.279</td>
<td>0.289</td>
<td>0.45</td>
<td>0.95 (0.85-1.07)</td>
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<tr>
<td>rs12402684</td>
<td>C</td>
<td>0.131</td>
<td>0.147</td>
<td>0.09</td>
<td>0.87 (0.75-1.01)</td>
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