Rare variant of HSPG2 is not involved in the development of adolescent idiopathic scoliosis: evidence from a large-scale replication study

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Abstract

Background: Rare variants of HSPG2 have recently been reported to function as a potential contributor to the susceptibility of adolescent idiopathic scoliosis (AIS) in the Caucasians. A replication study in the different population is warranted to validate the role of HSPG2 in AIS. The aim of this study was to determine the association between HSPG2 and AIS in the Chinese patients and to further investigate its influence on the phenotype of the patients.

Methods: SNVs p.Asn786Ser of HSPG2 was genotyped in 1752 patients and 1584 normal controls using multiple ligase detection reactions. The mRNA expression of HSPG2 in the paraspinal muscles was quantified for 90 patients and 26 controls. The The Student’s t test was used to analyze the inter-group comparison of the HSPG2 expression. The relationship between the HSPG2 expression and the curve magnitude of the patients was analyzed by the Pearson correlation analysis.

Results: No case of mutation in the reported SNV p.Asn786Ser of HSPG2 was found in our cohort. The mRNA expression of HSPG2 in patients was comparable with that in the controls (0.0016 ± 0.0013 vs. 0.0019 ± 0.0012, p = 0.29). 42 patients with curve magnitude > 60 degrees were assigned to the severe curve group. The other 58 patients were assigned to the moderate curve group. These two groups were found to have comparable HSPG2 expression (0.0015 ± 0.0011 vs. 0.0017 ± 0.0014, p = 0.57). And there was no remarkable correlation between the expression level of HSPG2 and the curve severity (r = 0.131, p = 0.71).

Conclusions: HSPG2 gene was not associated with the susceptibility or the phenotypes of AIS in the Chinese population. The whole HSPG2 gene can be sequenced in more AIS patients to identify potentially causative mutations.

Keywords: HSPG2, Rare variation, Adolescent idiopathic scoliosis, Replication

Background

Adolescent idiopathic scoliosis (AIS) is a complex spinal deformity that affects millions of children worldwide [1]. To benefit the prognosis and therapeutic strategy for patients with AIS, it is crucial to have a clear understanding of the etiopathogenesis of this disease. For the past half century, numerous studies have been carried out to investigate the etiology of AIS while the conclusions remain obscure [2]. Traditionally, AIS is considered as a multifactorial disorder that involves interaction between different factors, including genetic susceptibility, growth disturbance, hormones and metabolic dysfunction, abnormal central nervous system and proprioception impairment [3–7]. Among these factors, previous familial studies of AIS have strongly implied the genetic background of AIS [8, 9]. But to date, the exact inheritance mode of AIS remains unclear.

Confined by the low efficiency to define the genetic markers, whole-genome linkage analysis was firstly used in the genetic research of AIS which could only identify large chromosome regions potentially containing disease-related genes [10, 11]. Gradually, it became feasible to analyze SNPs through TaqMan real-time quantitative PCR assay,
which was a high-throughput method to differentiate the genotype of target variants. Since then, multiple susceptible genes of AIS have been discovered through candidate genetic association studies [12–17]. To be noted, however, few of these genes could be successfully replicated in different populations, which greatly weakened the reliability of such candidate susceptible genes in AIS [18, 19]. Commonly, ethic differences and small sample size of the patients were considered to underlie the discrepancy between the original and the replication studies [18, 19]. In addition, the speculation based on which those candidate genes was selected was mostly lack of essential scientific evidence, thus inevitably leading to the failed replication.

To overcome the above-mentioned limitations of candidate genetic association studies, a much powerful tool, genome-wide genotyping chip, was utilized to identify the susceptible gene of AIS [20–26]. Sharma et al. [26] performed the first genome-wide association study (GWAS) in the Caucasians and reported that CHL1 was associated with AIS. In the following decade, more susceptible loci of AIS were discovered in the Caucasian, the Japanese and the Chinese populations through GWAS, including LBX1, GPR126, BNC2, PAX1, LBX1-AS1, BCL2, AJAP1, PAX3, TNIK, MEIS1, and MAGI1 [20–25]. However, only a small proportion of the heritability of AIS can be explained by these common variants.

With the advances of sequencing techniques, more research teams have begun to analyze the role of rare variants in AIS using whole exome sequencing (WES) in recent years [27–29]. With a low minor allele frequency in normal population, rare variants are usually expected to have larger impact on the inheritance of complex disease than common variants. Patten et al. [27] performed the first WES in AIS and reported 3 rare variants of the POC5 functionally associated with AIS. Subsequently, Buchan et al. [28] reported that rare variants in FBN1 and FBN2 could contribute to both risk and severity of AIS. Haller et al. [30] found that rare variants across extracellular matrix genes contributed strongly to the risk of AIS in patients with European ancestry. Overall, identification and validation of rare variants associated with AIS is a promising method to explain the missing heritability of this complex disease.

In a recent study, rare variants of HSPG2 were reported to function as a potential contributor to the susceptibility of AIS in the Caucasians [31]. Obviously a replication study in different population is warranted to validate the role of HSPG2 in AIS. This study aimed to determine the association between HSPG2 and AIS in the Chinese patients and to further investigate its influence on the phenotype of the patients.

### Methods
#### Subjects
Under the approval of the local institutional review board, a cohort of 1752 female AIS patients and 1584 controls were included in the current study. All the patients were excluded to have neurolological defect through MRI examination. All the controls were excluded to have scoliosis through Adam’s Forward Bend Test using the standard criteria performed by a senior spine surgeon (Z.Z.). Demographic data were collected for each participant, including initial age, curve pattern and curve magnitude measured by the Cobb angle method on the standing posteroanterior X-ray films.

#### Genotyping of the rare variant
All the subjects signed the informed consent for the collection of blood samples. Genomic DNA was then extracted with the commercial kit (Qiagen K.K., Tokyo, Japan). Single nucleotide variant (SNV) p.Asn786Ser (rs143736974) of HSPG2 was genotyped for all participants. Allelic-specific multiple ligase detection reactions (LDR) was used for genotyping assay as previously reported, with the primer shown in Table 1. Fifteen percent of the samples were randomly selected for Sanger sequencing to ensure the reproducibility of the LDR results.

#### Genotyping of common variations in HSPG2
Common variants covering HSPG2 gene were identified through the Ensemble database (http://www.ensembl.org/index.html). GWAS database that was composed of 1446 AIS patients and 2080 controls was processed with the PLINK (version 1.90, http://zzz.bwh.harvard.edu/plink/tutorial.shtml) to determine the genotyping results of these common variants.

#### RNA extraction and real-time qPCR
Ninety-eight female AIS patients were included in the expression analysis of HSPG2. Twenty-eight female lumbar disc herniation (LDH) patients with no presence of spinal disc herniation (LDH) were re-
deformity were included as the controls. The paraspinal muscle was collected from each subject during the surgery. For AIS patients, we collected the muscle sample from both the concave side and convex side at the apex of the curve. Total RNA was then extracted from the muscle tissue using a commercial kit (CWBio. Co. Ltd). The mRNA expression of HSPG2 was quantified with real-time polymerase chain reaction (PCR) on ABI 7900HT, with GAPDH used as the endogenous control. All amplifications were performed in triplicate. The relative expression of HSPG2 was normalized using the ΔΔCt method. The primers of HSPG2 and GAPDH were shown in Table 1.

Statistical analysis
SPSS version 17.0 (SPSS Inc., Chicago, USA) was used for data analysis. Cochran-Armitage trend test was used to calculate the association between common variants and AIS. Patients included expression analysis were classified into different subgroups according to the curve pattern or the curve severity. The Student’s t test was used to analyze the inter-group comparison of the baseline characteristics and mRNA expression of HSPG2. The relationship between the HSPG2 expression and the curve magnitude of the patients was analyzed by the Pearson correlation analysis. The statistical was set at $p < 0.05$.

Results
Demographic data
The mean age of patients included in the LDR analysis was 15.3 ± 3.4 years (range 10.5–18.8 years). The mean curve magnitude was 54.3 ± 11.2 degrees (range 27–72 degrees). One thousand eighteen patients had curve magnitude more than 50 degrees and thus underwent posterior spinal correction surgery. The mean age of the healthy controls was 18.9 ± 3.2 years (range 16.5–22.8 years).

Genotyping of SNVs
No case of mutation in the reported SNV p.Asn786Ser of HSPG2 was found in our cohort. All the 3336 subjects were found to have genotype TT. A total of 16 SNPs covering HSPG2 were analyzed, with the distribution of minor allele frequency summarized in Table 2. All the SNPs were found to have comparable allele frequency between the patients and the controls.

mRNA expression level of HSPG2
A total of 98 patients and 28 controls were included for expression analysis. Eight patients and 2 controls were excluded from the analysis due to degradation of total RNA. The expression level of HSPG2 was successfully detected in the paraspinal muscles of 90 patients and 26 controls. There was no significant difference regarding the mean age of the two groups (15.2 ± 1.1 years vs. 15.5 ± 1.2 years, $p = 0.48$). The mRNA expression of HSPG2 in patients was comparable with that in the controls (0.0016 ± 0.0013 vs. 0.0019 ± 0.0012, $p = 0.29$).

Table 2 The allele frequency of 16 SNPs covering HSPG2

| SNP       | MA (n = 1446) | MAF       | Controls (n = 2080) | p            | OR (95% CI)   |
|-----------|---------------|-----------|---------------------|--------------|---------------|
| rs4654770 | T             | 0.170     | 0.169               | 0.9262       | 1.01 (0.90–1.12) |
| rs12040859| T             | 0.190     | 0.192               | 0.8902       | 0.99 (0.87–1.11) |
| rs17459139| T             | 0.165     | 0.167               | 0.8384       | 0.99 (0.87–1.11) |
| rs7556412 | G             | 0.179     | 0.195               | 0.1463       | 0.90 (0.81–0.99) |
| rs12043008| G             | 0.179     | 0.175               | 0.7164       | 1.03 (0.92–1.14) |
| rs10799718| A             | 0.385     | 0.371               | 0.278        | 1.06 (0.94–1.17) |
| rs11810496| G             | 0.393     | 0.390               | 0.8497       | 1.01 (0.90–1.12) |
| rs2445142 | G             | 0.395     | 0.381               | 0.3187       | 1.06 (0.94–1.17) |
| rs878949  | T             | 0.148     | 0.151               | 0.8051       | 0.99 (0.87–1.11) |
| rs2501257 | A             | 0.395     | 0.386               | 0.5053       | 1.04 (0.94–1.16) |
| rs6680566 | C             | 0.394     | 0.388               | 0.6465       | 1.03 (0.92–1.14) |
| rs6698486 | G             | 0.347     | 0.341               | 0.6487       | 1.03 (0.92–1.14) |
| rs9426783 | C             | 0.443     | 0.436               | 0.62         | 1.03 (0.92–1.14) |
| rs4654773 | T             | 0.440     | 0.433               | 0.5781       | 1.03 (0.92–1.14) |
| rs16826053| C             | 0.199     | 0.189               | 0.383        | 1.06 (0.94–1.17) |
| rs10917067| T             | 0.107     | 0.116               | 0.2869       | 0.91 (0.82–1.01) |

MA indicates minor allele, MAF indicates minor allele frequency, OR indicates odds ratio, CI indicates confidential interval.
Relationship between HSPG2 and phenotype of the patients
For the 90 patients included in the expression analysis, 42 patients had curve magnitude more than 60 degrees, thus assigned to the severe curve group. Forty-eight patients with curve magnitude less than 60 degrees were assigned to the moderate curve group. These two groups classified by curve severity were found to have comparable $HSPG2$ expression ($0.0015 \pm 0.0011$ vs. $0.0017 \pm 0.0014$, $p = 0.57$). And there was no remarkable correlation between the expression level of $HSPG2$ and the curve severity ($r = 0.131$, $p = 0.71$), either.

As for the curve pattern, 67 patients had single thoracic curve and the other 23 patients had double major curve. There was no significant difference of $HSPG2$ expression between the curve pattern groups ($0.0017 \pm 0.0012$ vs. $0.0014 \pm 0.0013$, $p = 0.31$).

Discussion
Genetic findings of AIS have been greatly hindered by its clinical and genetic heterogeneity. Previous association studies have revealed many disease-related genes, most of which however appeared inconclusive as indicated by the following replication studies [18, 32, 33]. Carried out in different populations, replication studies have failed to validate the reported associations of ER-$\alpha$, ER-$\beta$, MTNR1b, MATN1, TPH1, IGF1, MMP3, IL6 and TGF-$\beta$ with AIS [18, 19, 32, 34]. To the best of our knowledge, LBX1 and PAX1 are the only two susceptible genes that could be successfully validated in Caucasians and Asians with a minimum sample size of over 3000 patients [20, 35]. The statistical power regarding the associations between these genes and AIS has reached the genome-wide significance that was defined as a $p$ value of less than $5.0 \times 10^{-8}$. Obviously, a large sample size and a rigorous setting of statistical power are key factors to ensure reliable and reproducible findings in genetic studies.

Another difficulty of AIS genetic research lies in that the reported common variants can only explain a small amount of AIS heritability. All the susceptible variants reported by previous GWASs of AIS had an OR value between 1 and 2, leaving over 90% of the AIS heritability unexplained [20–26]. Since low frequency variants with large effect sizes could be involved in the missing heritability, the role of rare variants in the development of AIS has recently become a widely investigated topic [27–32]. Baschal et al. [31] performed WES in a four-generation idiopathic scoliosis (IS) family and identified the SNV p.Asn786Ser of $HSPG2$ gene as a novel causative variant of IS. Enrichment of this mutation was then validated in two independent cohorts composed of 241 IS patients [31]. To validate the association of $HSPG2$ with the development of AIS, we performed the genotyping of p.Asn786Ser in a large cohort consisted of 1752 patients and 1584 controls. No case of mutation was found in this cohort as all the subjects presented the same genotype TT. As for common variants covering $HSPG2$, none of the 16 SNPs had remarkably different allele frequency between the patients and the controls. Moreover, we observed comparable $HSPG2$ expression in the paraspinal muscles between the patients and the controls. To summarize, our findings did not support the association of $HSPG2$ with the susceptibility of AIS in Chinese population.

$HSPG2$ gene, which has 94 exons, encodes perlecan which binds to basement membrane proteins such as collagen and laminin and to cell surface receptors [36]. Homozygous $HSPG2$ mutations could lead to a more severe phenotype in human disease, such as Schwartz-Jampel syndrome Type 1 and Dyssegmental Dysplasia, Silverman-Handmaker Type [37, 38]. Schwartz-Jampel syndrome is reported to be associated with kyphoscoliosis [39]. Baschal et al. [31] therefore speculated that haploinsufficiency of $HSPG2$ may be associated with a progressive IS and acted as a potential contributor to the phenotype. Aiming to clarify the relationship between $HSPG2$ and phenotypes of the patients, we classified patients into different subgroups according to the curve pattern or the curve severity. We found that $HSPG2$ expression was not associated with either the curve severity or the curve pattern. Herein, it appeared that $HSPG2$ had no effect on the phenotype of the AIS patients.

It is unlikely for the current study to have false-negative results as our sample size is large enough to detect the potential mutation. The susceptibility genes of AIS may play important roles in the diagnosis and therapy of AIS in the future. We believe that the role of $HSPG2$ in the development of AIS still needs further investigation. The primary limitation of our study lies in that we did not sequence all the exons of $HSPG2$. It cannot be excluded that enrichment of other mutations of $HSPG2$ could play a role in AIS. Besides, the function of p.Asn786Ser should be investigated through in-vivo cellular experiment to clarify its role in AIS.

Conclusions
$HSPG2$ gene was not associated with the susceptibility or the phenotypes of AIS in the Chinese population. In future study, functional studies of p.Asn786Ser is warranted to clarify whether this variant can regulate the expression of $HSPG2$. The whole $HSPG2$ gene can be sequenced in more AIS patients to identify potentially causative mutations.
Abbreviations
AIS: Adolescent idiopathic scoliosis; GWAS: Genome-wide association study; HSPG2: Human heparan sulfate proteoglycan 2; LDR: Ligase detection reactions; SNV: Single nucleotide variant; WES: Whole exome sequencing

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Availability of data and materials
The raw data is available from the corresponding author upon reasonable request.

Authors’ contributions
XC and XL performed the study. XB and SF participated in the experiment and data collection/interpretation for the study. QY and ZZ conceived of the study. Written informed consent was obtained from the parents of the participants in this study that are under 18 years old.

Ethics approval and consent to participate
Approved by the Institutional Review Board (IRB)/Independent Ethics Committee (IEC) of Nanjing Drum Tower Hospital (The Affiliated Drum Tower Hospital of Nanjing University Medical School) at Zhongshan Road 321, Nanjing 210008, China. All subjects provided informed consent to take part in the study. Written informed consent was obtained from the parents of the participants in this study that are under 18 years old.

Consent for publication
Not applicable.

Competing interests
The corresponding author Zezhang Zhu is a member of the Editorial Board of BMC Musculoskeletal Disorders.

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References
1. Murray DW, Bulstrode CJ. The development of adolescent idiopathic scoliosis. Eur Spine J. 1996;5(4):251–7.
2. Kouwenhoven JW, Castelein RM. The pathogenesis of adolescent idiopathic scoliosis; review of the literature. Spine. 2008;33(26):2898–908.
3. Xu L, Qiu X, Sun X, Mao S, Liu Z, Qiao J, Qiu Y. Potential genetic markers predicting the outcome of brace treatment in patients with adolescent idiopathic scoliosis. Eur Spine J. 2011;20(10):1757–64.
4. Wei-Jun W, Xu S, Zhi-Wei W, Xu-Sheng Q, Zhen L, Yong Q. Abnormal anthropometric measurements and growth pattern in male adolescent idiopathic scoliosis. Eur Spine J. 2012;21(1):177–83.
5. Wang S, Qiu Y, Ma Z, Xia C, Zhu F, Zhu Z. Expression of Runx2 and type X collagen in vertebral growth plate of patients with adolescent idiopathic scoliosis. Connect Tissue Res. 2010;51(3):188–96.
6. Guoy MA, Agnani O, Peyrodu L, Samantha D, Donze C, Catanarzi JF. Cervicocephalic relocation test to evaluate cervical proprioception in adolescent idiopathic scoliosis. Eur Spine J. 2016;25(10):3135–6.
7. Sun X, Qiu Y, Zhu Z. Variations of the position of the cerebellar tonsil in adolescent idiopathic scoliosis with severe curves: a MRI study. Stud Health Technol Inform. 2006;123:565–70.
8. Mocquereau JA, Milhars R, Dolan L, Stevens J, Beck J, Wang K, Weinstein SL, Sheffield V. Allelic variants of human melanotin 1A receptor in patients with familial adolescent idiopathic scoliosis. Spine. 2003;28(17):2005–8 discussion 2009.
9. Miller NH, Mims B, Child A, Milewicz DM, Sponseller P, Blanton SH. Genetic analysis of structural elastic fiber and collagen genes in familial adolescent idiopathic scoliosis. J Orthop Res. 1996;14(6):994–9.
10. Miller NH. Genetics of familial idiopathic scoliosis. Clin Orthop Relat Res. 2007;465:26–10.
11. Ruchti NH, Justice CM, Marosy B, Doheny KF, Pugh E, Zhang J, Dietz HC 3rd, Wilson AF. Identification of candidate regions for familial idiopathic scoliosis. Spine. 2005;30(10):1181–7.
12. Xu L, Xia C, Sun W, Qin X, Qiu Y, Zhu Z. Genetic polymorphism of NUCKS1 is associated with the susceptibility of adolescent idiopathic scoliosis. Spine. 2017;42(21):1629–34.
13. Xu L, Huang S, Qin X, Mao S, Qiao J, Qian BP, Qiu Y, Zhu Z. Investigation of the SNVs markers in a DNA-based prognostic test revealing new predisposition genes for adolescent idiopathic scoliosis. Spine. 2015;40(14):1086–91.
14. Mao S, Xu L, Zhu Z, Qian B, Qiu Y, Ji L, Qiu Y. Association between genetic determinants of peak height velocity during puberty and predisposition to adolescent idiopathic scoliosis. Spine. 2013;38(12):1034–9.
15. Ioune M, Minami S, Nakata Y, Kihara H, Otsuka Y, Isobe K, Takaso M, Tokunaga M, Nishikawa S, Morita T, et al. Association between estrogen receptor gene polymorphisms and curve severity of idiopathic scoliosis. Spine. 2002;27(21):2357–62.
16. Qiu XS, Tang NL, Yeung HY, Qiu Y, Qin L, Lee KM, Cheng JC. The role of melanotin receptor 1B gene (MTNR1B) in adolescent idiopathic scoliosis—a genetic association study. J Biomed Sci. 2006;13:233–8.
17. Ryzhkiv I, Borzilov EE, Chumsov MI, Ataman AV, Dedkov AA, Polonikov AV. Transforming growth factor beta 1 is a novel susceptibility gene for adolescent idiopathic scoliosis. Spine. 2013;38(12):6699–704.
18. Takahashi Y, Matsumoto M, Kurasugi T, Watanabe K, Chiba K, Kawakami N. Tsuji T, Uno K, Suki T, To M, et al. Lack of association between adolescent idiopathic scoliosis and previously reported single nucleotide polymorphisms in MATN1, MTNR1B, TPH1, and GIPF in a Japanese population. J Orthop Res. 2011;29(7):1055–61.
19. Liu Z, Tang NL, Gao XB, Liu WJ, Qiu XS, Cheng JC, Qiu Y, Li Y, et al. Lack of association between the promoter polymorphisms of MMP-3 and Y6 genes and adolescent idiopathic scoliosis; a case-control study in a Chinese Han population. Spine. 2015;40(18):1701–5.
20. Zhu Z, Xu L, Leung-Sang Tang N, Qin X, Feng Z, Sun W, Zhu W, Shi B, Liu P, Mao S, et al. Genome-wide association study identifies novel susceptible loci and highlights Wnt/beta-catenin pathway in the development of adolescent idiopathic scoliosis. Hum Mol Genet. 2017;26(8):1577–83.
21. Zhu Z, Tang NL, Xu L, Qin X, Mao S, Song Y, Liu L, Li F, Liu P, Yi Y, et al. Genome-wide association study identifies new susceptibility loci for adolescent idiopathic scoliosis in Chinese girls.Nat Commun. 2015;6:8355.
22. Sharma S, Londono D, Eckalbar WL, Gao X, Zhang D, Mauldin K, Kou I, Takahashi A, Matsumoto M, Kamiya N, et al. A PAX1 enhancer locus is associated with susceptibility to idiopathic scoliosis in females. Nat Commun. 2015;6:6642.
23. Ogura Y, Kou I, Miura S, Takahashi A, Xu L, Takeida K, Takahashi Y, Kono K, Kawakami N, Uno K, et al. A functional SNP in INB2 is associated with adolescent idiopathic scoliosis. Am J Hum Genet. 2015;97(2):337–42.
24. Kou I, Takahashi Y, Johnson TA, Takahashi A, Guo L, Dai J, Qiu X, Sharma S, Takamoto A, Ogura Y, et al. Genetic variants in GRPR are associated with adolescent idiopathic scoliosis. Nat Genet. 2013;45(6):676–9.
25. Takahashi Y, Kou I, Takahashi A, Johnson TA, Kono K, Kawakami N, Uno K, Ito M, Minami S, Yanagida H, et al. A genome-wide association study identifies common variants near LBD1 associated with adolescent idiopathic scoliosis in females. Nat Commun. 2015;6:6642.
26. Buchan JG, Alvarado DM, Hailer GE, Cruchaga C, Harms MB, Zhang T, Willing MC, Gagne DK, BVravenan AC, Miller NH, et al. Rare variants in FBNI and FBNI are associated with severe adolescent idiopathic scoliosis. Hum Mol Genet. 2014;23(19):5271–82.
27. Justice CM, Bishop K, Carrington B, Mullikin JC, Swindle K, Marosy B, Sood R, Miller NH, Wilson AF. Evaluation of IRX genes and conserved noncoding
elements in a region on 5p13.3 linked to families with familial idiopathic scoliosis and kyphosis. G3 (Bethesda, Md). 2016;6(6):1707–12.
30. Haller G, Alvarado D, McCall K, Yang P, Cruchaga C, Harms M, Goate A, Willing M, Morcuende JA, Baschal E, et al. A polygenic burden of rare variants across extracellular matrix genes among individuals with adolescent idiopathic scoliosis. Hum Mol Genet. 2016;25(1):202–9.
31. Baschal EE, Wethey CI, Swindle K, Baschal RM, Gowen K, Tang NL, Alvarado DM, Haller GE, Dobbs MB, Taylor MR, et al. Exome sequencing identifies a rare HSPG2 variant associated with familial idiopathic scoliosis. G3. 2014;5(2):167–74.
32. Xu L, Xia C, Zhu W, Feng Z, Qin X, Sun W, Qiu Y, Zhu Z. Lack of association between AKAP2 and the susceptibility of adolescent idiopathic scoliosis in the Chinese population. BMC Musculoskelet Disord. 2017;18(1):368.
33. Qiu XS, Lv F, Zhu ZZ, Qian BP, Wang B, Yu Y, Qiu Y. Lack of association between the CHL1 gene and adolescent idiopathic scoliosis susceptibility in Han Chinese: a case-control study. BMC Musculoskelet Disord. 2014;15:38.
34. Xu L, Sun W, Qin X, Qiu Y, Zhu Z. The TGFBI gene is associated with curve severity but not with the development of adolescent idiopathic scoliosis: a replication study in the Chinese population. BMC Musculoskelet Disord. 2016;17:15.
35. Nada D, Julien C, Samuels ME, Moreau A. A replication study for association of LBX1 locus with adolescent idiopathic scoliosis in French-Canadian population. Spine. 2017;43(3):172–8
36. Costell M, Gustafsson E, Aszodi A, Morgelin M, Bloch W, Hunziker E, Addicks K, Timpl R, Fassler R, Perlecan maintains the integrity of cartilage and some basement membranes. J Cell Biol. 1999;147(5):1109–22.
37. Stum M, Davoine CS, Vicart S, Guillot-Noel L, Topaloglu H, Carod-Artal FJ, Kayserili H, Hentati F, Merlini L, Urtizberea JA, et al. Spectrum of HSPG2 (Perlecan) mutations in patients with Schwartz-Jampel syndrome. Hum Mutat. 2006;27(11):1082–91.
38. Ladhani NN, Chitayat D, Nezarati MM, Laureane MC, Keating S, Silver RJ, Unger S, Velsher L, Sirkin W, Toi A, et al. Dyssegmental dysplasia, Silverman-Handmaker type: prenatal ultrasound findings and molecular analysis. Prenat Diagn. 2013;33(11):1039–43.
39. Mukaihara K, Godai K, Yamada T, Hasegawa-Moriyama M, Kanmura Y. Successful airway management using a MultiViewScope handle with a stylet scope in a patient with Schwartz-Jampel syndrome. JA Clin Rep. 2016;2(1):36.