

RESEARCH ARTICLE

A Genetic Predictive Model Estimating the Risk of Developing Adolescent Idiopathic Scoliosis

Leilei Xu^{1,2}, Zhichong Wu^{1,2}, Chao Xia^{1,2}, Nelson Tang^{2,3}, Jack C.Y. Cheng^{2,3}, Yong Qiu^{1,2} and ZeZhang Zhu^{1,2,*}

¹Department of Spine Surgery, The Affiliated Drum Tower Hospital of Nanjing University Medical School, Zhongshan Road 321, Nanjing 210008, China; ²Joint Scoliosis Research Center of The Chinese University of Hong Kong and Nanjing University, Nanjing, China; ³SH Ho Scoliosis Research Laboratory, Department of Orthopaedics and Traumatology, The Chinese University of Hong Kong, Shatin, Hong Kong

Abstract: Background: Previous GWASs have revealed several susceptible variants associated with adolescent idiopathic scoliosis (AIS). Risk prediction based on these variants can potentially improve disease prognosis. We aimed to evaluate the combined effects of genetic factors on the development of AIS and to further develop a genetic predictive model.

Methods: A total of 914 AIS patients and 1441 normal controls were included in the discovery stage, which was followed by the replication stage composed of 871 patients and 1239 controls. Genotyping assay was performed to analyze 10 previously reported susceptible variants, including rs678741 of *LBX1*, rs241215 of *AJAPI*, rs13398147 of *PAX3*, rs16934784 of *BNC2*, rs2050157 of *GPR126*, rs2180439 of *PAX1*, rs4940576 of *BCL2*, rs7593846 of *MEIS1*, rs7633294 of *MAGII* and rs9810566 of *TNIK*. Logistic regression analysis was performed to generate a risk predictive model. The predicted risk score was calculated for each participant in the replication stage.

Results: The association of the 10 variants with AIS was successfully validated. The established model could explain approximately 7.9% of the overall variance. In the replication stage, patients were found to have a remarkably higher risk score as compared to the controls (44.2 ± 14.4 vs. 33.9 ± 12.5 , $p < 0.001$). There was a remarkably higher proportion of the risk score *i.e.* >40 in the patients than in the controls (59% vs. 28.9%, $p < 0.001$).

Conclusion: Risk predictive model based on the previously reported genetic variants has a remarkable discriminative power. More clinical and genetic factors need to be studied, to further improve the probability to predict the onset of AIS.

ARTICLE HISTORY

Received: May 15, 2019

Revised: June 18, 2019

Accepted: July 06, 2019

DOI:

10.2174/1389202920666190730132411

Keywords: Predictive model, susceptible variants, adolescent idiopathic scoliosis, onset, prognosis, replication, level of evidence Level IV.

1. INTRODUCTION

As a complex disease, the genetic background of adolescent idiopathic scoliosis (AIS) has been extensively investigated [1-7]. Many variants associated with AIS were reported through genetic association studies [8-14]. The combined effects of genetic variants may have a better prediction of the complex disease. Herein, early detection and risk prediction based on a panel of genetic markers could potentially improve the prognosis of AIS by allowing gene-based treatment or spurring them to modify lifestyle habits.

Ward *et al.* [15] developed the first genetic diagnostic kit for AIS that was registered as "ScoliScore". It consisted of 53 genetic markers, and a numeric risk can be calculated to

determine the probability of curve progression. Recently, a large-scale replication study of ScoliScore was performed in the Chinese population to validate its reliability [16]. However, none of the SNPs were replicated, indicating that ScoliScore might not be applicable to the Chinese AIS patients [16]. Similarly, lack of validation of ScoliScore was also reported in 3 other studies performed in the Japanese, the Caucasian and the Canadian population [17-19]. Apparently, to ensure the reliability of the diagnostic kit, the association of genetic markers with AIS should be well-refined before being embedded in the predictive model.

Previous GWASs have revealed several susceptible variants associated with AIS [8-11, 14, 20]. Zhu *et al.* [12, 14] reported 6 novel genes associated with AIS in the Chinese population, including *PAX3*, *AJAPI*, *TNIK*, *MAGII*, *MEIS1* and *BCL2*. GWAS performed in Japanese population revealed that *LBX1*, *GPR126* and *BNC2* were the susceptible genes of AIS [9, 10, 20]. Sharma *et al.* [11] reported that *PAX1* was involved in the development of AIS in both Cau-

*Address correspondence to this author at the Department of Spine Surgery, The Affiliated Drum Tower Hospital of Nanjing University Medical School, Zhongshan Road 321, Nanjing 210008, China; and Joint Scoliosis Research Center of The Chinese University of Hong Kong and Nanjing University, Nanjing, China; Tel: (86)025-83105121; Fax: (86)025-83105121; E-mail: zezhangzhu@126.com

casians and Japanese. It is noteworthy that all the 4 genes have been successfully replicated in the Chinese population [12, 21-23]. It is thus plausible to predict the risk of AIS based on a combination of these 10 genetic variants mentioned above. This study aimed to evaluate the independent and combined effects of previously reported GWAS variants on the development of AIS and to develop a genetic predictive model.

2. METHODS

2.1. Subjects

Under the approval of a local Institutional Review Board, the current two-stage case-control study comprised of 1785 female patients and 2680 healthy controls from the Chinese Han population. Scoliosis patients who visited our clinic between April 2012 and January 2017 were retrospectively reviewed. The inclusion criteria were as follows: 1. Diagnosed with AIS through clinical and radiological examinations; 2. Aged between 10 and 16 yrs. Patients having scoliosis secondary to known etiology, including congenital scoliosis, neuromuscular scoliosis, scoliosis secondary to skeletal dysplasia, or connective tissue abnormalities were excluded from this study. Healthy subjects undergoing a physical examination prior to admission to high school were recruited as normal controls. A total of 914 patients and 1441 normal controls were assigned to the discovery stage, which was followed by the replication stage comprising of 871 patients and 1239 controls. The age was recorded for each subject. The curve severity was recorded as the Cobb angle measured at the latest visit. Guardians of all the participants signed an informed consent.

2.2. Genotyping of the Target SNP

The extraction of blood and DNA was approved by the local Institutional Review Board. Informed consent was obtained from the subjects or their guardians. Genomic DNA was extracted under standard protocols (Qiagen K.K., Tokyo, Japan). Genotyping assay was performed to analyze 10 previously reported susceptible variants, including rs678741 of *LBX1*, rs241215 of *AJAPI*, rs13398147 of *PAX3*, rs16934784 of *BNC2*, rs2050157 of *GPR126*, rs2180439 of *PAX1*, rs4940576 of *BCL2*, rs7593846 of *MEIS1*, rs7633294 of *MAGII* and rs9810566 of *TNIK*. The results were read with an ABI PRISM 7900HT sequence detection system (Applied Biosystems, Foster City, CA) as previously described [16]. Twenty percent of the samples were randomly selected to validate the reproducibility of the genotyping results.

2.3. Development and Replication of the Predictive Model

A predictive regression model was developed according to the independent contribution of genetic variants to the risk of AIS. The model was replicated in a different cohort of patients and controls. A predicted risk score was calculated for each participant, theoretically distributed between 0 and 1. For the convenience of understanding, the risk score was subsequently transformed by multiplying with 100.

2.4. Statistical Analysis

The Hardy-Weinberg Equilibrium (HWE) test was performed for all the subjects. The student t-test was used to compare the demographic data between the cases and the controls. The differences of genotype and allele distributions between the cases and the controls were compared using the Chi-square test. Odds ratio (OR) was calculated using the minor allele as a reference. Logistic regression analysis was performed to generate a risk predictive model. Sensitivity and specificity were calculated to maximize the sum of the 2 values using receiver operating characteristic (ROC) curves. The average risk score was compared between the cases and the controls with the student t-test. The SPSS software (version 16.0, Chicago, IL) was used for statistical analyses. Statistical significance was set at a p-value of less than 0.05.

3. RESULTS

3.1. Demographic Data

The two groups were matched in terms of age. The mean Cobb angle of the patients was 35.4 ± 18.3 degrees (range 25 - 73 degrees) and the mean age was 12.3 ± 1.9 years (range 10.3 - 16 years).

3.2. Genetic Association Analysis

All the 10 SNPs were successfully genotyped for all the participants. HWE test showed no significant difference in genotype frequencies in both patients and normal controls. All the SNPs were found to have significant differences in terms of allele frequency and genotype frequency between the two groups (Table 1).

3.3. Establishment of the Predictive Model

The results of the logistic regression analysis are shown in Table 2. All the 10 SNPs were enrolled in the final model with statistical significance. The model was therefore established using the following formula:

$$p = 1/[1 + e^{(-(-2.186 - 0.239 * rs241215 + 0.178 * rs7593846 + 0.405 * rs13398147 + 0.222 * rs7633294 + 0.311 * rs9810566 + 0.199 * rs2050157 + 0.310 * rs16934784 + 0.388 * rs678741 + 0.326 * rs4940576 + 0.251 * rs2180439))}]$$

The Hosmer-Lemeshow test showed no significant deviation between the observed and predicted values ($p = 0.26$). The value of Cox & Snell R Square was 0.079, indicating that the predictive model can explain approximately 7.9% of the overall variance. As shown in Table 3, with a cut-off set at 0.5, the predictive model yielded 85.9% sensitivity and 31.3% specificity.

3.4. Replication of the Predictive Model

For the replication population composed of 871 patients and 1239 controls, the mean risk score was averaged 44.2 ± 14.4 (range, 8.2 - 79.1) for the patients and 33.9 ± 12.5 (range, 11.0 - 83.7) for the controls. As shown in Fig. 1, patients were found to have a remarkably higher risk score as compared to the controls ($p < 0.001$).

As summarized in Table 4, there were significantly different distributions of the risk score between the patients and the controls. There was a remarkably higher proportion of

Table 1. Association of the 10 variants with AIS in Chinese.

SNPs	MA	Genotype ^a		P	MAF		P	Odds Ratio (95% CI) ^b
		Patients	Controls		Patients	Controls		
rs241215	T	62/351/498	133/584/613	0.0001	0.261	0.320	2.2 x 10 ⁻⁵	0.75 (0.66 - 0.86)
rs7593846	G	112/432/353	107/636/589	0.0009	0.366	0.319	0.001	1.23 (1.09 - 1.39)
rs13398147	T	66/353/483	53/428/849	7.7 x 10 ⁻⁷	0.269	0.201	1.0 x 10 ⁻⁷	1.46 (1.27 - 1.49)
rs7633294	G	127/429/348	141/560/603	0.0007	0.378	0.323	0.0001	1.27 (1.12 - 1.44)
rs9810566	A	176/411/321	176/592/551	0.0001	0.420	0.358	2.6 x 10 ⁻⁵	1.30 (1.15 - 1.47)
rs2050157	T	139/442/325	173/576/581	0.001	0.397	0.347	0.0006	1.24 (1.10 - 1.41)
rs16934784	G	60/375/476	70/436/824	2.8 x 10 ⁻⁵	0.272	0.217	2.1 x 10 ⁻⁵	1.35 (1.18 - 1.55)
rs678741	T	255/452/201	248/684/400	3.4 x 10 ⁻⁸	0.529	0.443	1.1 x 10 ⁻⁸	1.42 (1.26 - 1.60)
rs4940576	A	209/447/255	206/636/487	6.3 x 10 ⁻⁷	0.475	0.394	8.6 x 10 ⁻⁸	1.39 (1.23 - 1.57)
rs2180439	G	188/429/293	197/639/497	0.0005	0.442	0.388	0.0002	1.25 (1.11 - 1.42)

^aThe three values in the 'genotype' column indicate the numbers of homozygotes with respect to the minor allele, heterozygotes and homozygotes with respect to the major allele, respectively.

^bCalculated with the minor allele as reference.

MA, Minor Allele; MAF, Minor Allele Frequency; CI, Confidential Interval.

the risk score *i.e.* >40 in the patients than in the controls (59% vs. 28.9%, $p < 0.001$). As shown in Fig. 2, the ROC analysis indicated that a score of 43 was the most discriminative cut-off point. The area under the curve (AUC) was 0.705 for the replication population.

Table 2. Results of logistic regression analysis.

SNPs ^a	Regression Coefficient	p	Odds Ratio (95% CI) ^b
rs241215	-0.239	0.0005	0.78 (0.69 - 0.90)
rs7593846	0.178	0.008	1.19 (1.05 - 1.36)
rs13398147	0.405	3.6 x 10 ⁻⁸	1.49 (1.30 - 1.73)
rs7633294	0.222	0.0006	1.25 (1.10 - 1.42)
rs9810566	0.311	7.1 x 10 ⁻⁷	1.37 (1.21 - 1.54)
rs2050157	0.199	0.001	1.22 (1.08 - 1.39)
rs16934784	0.310	2.3 x 10 ⁻⁵	1.36 (1.18 - 1.57)
rs678741	0.388	1.1 x 10 ⁻⁹	1.47 (1.30 - 1.67)
rs4940576	0.326	2.3 x 10 ⁻⁷	1.39 (1.22 - 1.57)
rs2180439	0.251	7.3 x 10 ⁻⁵	1.29 (1.14 - 1.45)

^aGenotypes of homozygotes with respect to the minor allele, heterozygotes and homozygotes with respect to the major allele were coded as 2, 1 and 0, respectively.

^bCI=Confidence Interval.

Table 3. The sensitivity, specificity and accuracy of the regression model.

Cut-off Point	Sensitivity	Specificity	Accuracy
0.1	1%	100%	38.9%
0.2	10%	97.3%	42.5%
0.3	37.7%	81.5%	54.7%
0.4	64.1%	57.8%	61.6%
0.5	85.9%	31.3%	64.7%
0.6	96.7%	15%	65.1%
0.7	99.3%	3.7%	62.2%
0.8	100%	0%	61.4%

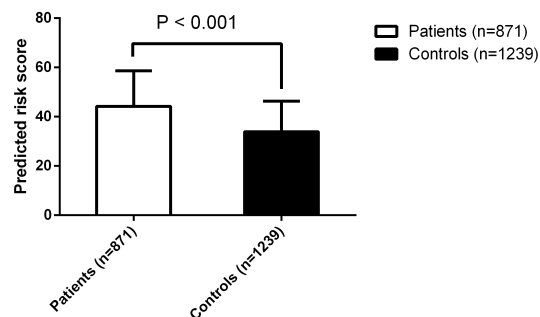


Fig. (1). Comparison of the risk score between the patients and the controls. The predicted risk score was calculated for 871 patients and 1239 controls based on the genotype of the ten genetic variants. Patients were found to have remarkably higher risk score as compared with the controls (44.2 ± 14.4 vs. 33.9 ± 12.5 , $p < 0.001$).

Table 4. The distribution of individual risk score in the replication cohort.

Risk Score	Replication Population		p ^a
	Patients (n = 871)	Controls (n = 1239)	
0 - 20	24 (2.8%)	158 (12.8%)	<0.001
20 - 40	333 (38.2%)	723 (58.3%)	-
40 - 60	373 (42.8%)	323 (26.1%)	-
60 - 80	136 (15.6%)	35 (2.8%)	-
80 - 100	5 (0.6%)	0 (0%)	-

^a calculated by Chi-square test.

4. DISCUSSION

In the current study, a predictive model for AIS was constructed on the basis of 10 susceptible genes discovered through GWAS. Our results suggested that the mathematic formula may serve as a helpful tool to identify the individuals at high risk of AIS prior to the onset of the spinal curve. To the best of our knowledge, this is the first predictive model developed for AIS in the Chinese population. As expected, we demonstrated independent significant associations between AIS and known genetic polymorphisms as reported by previous GWASs. The results of allele frequencies and the ORs for each variant were comparable with those of the previously published papers [12, 14, 21-23].

Targeting high-risk individuals could facilitate more frequent surveillance and active clinical conservative interven-

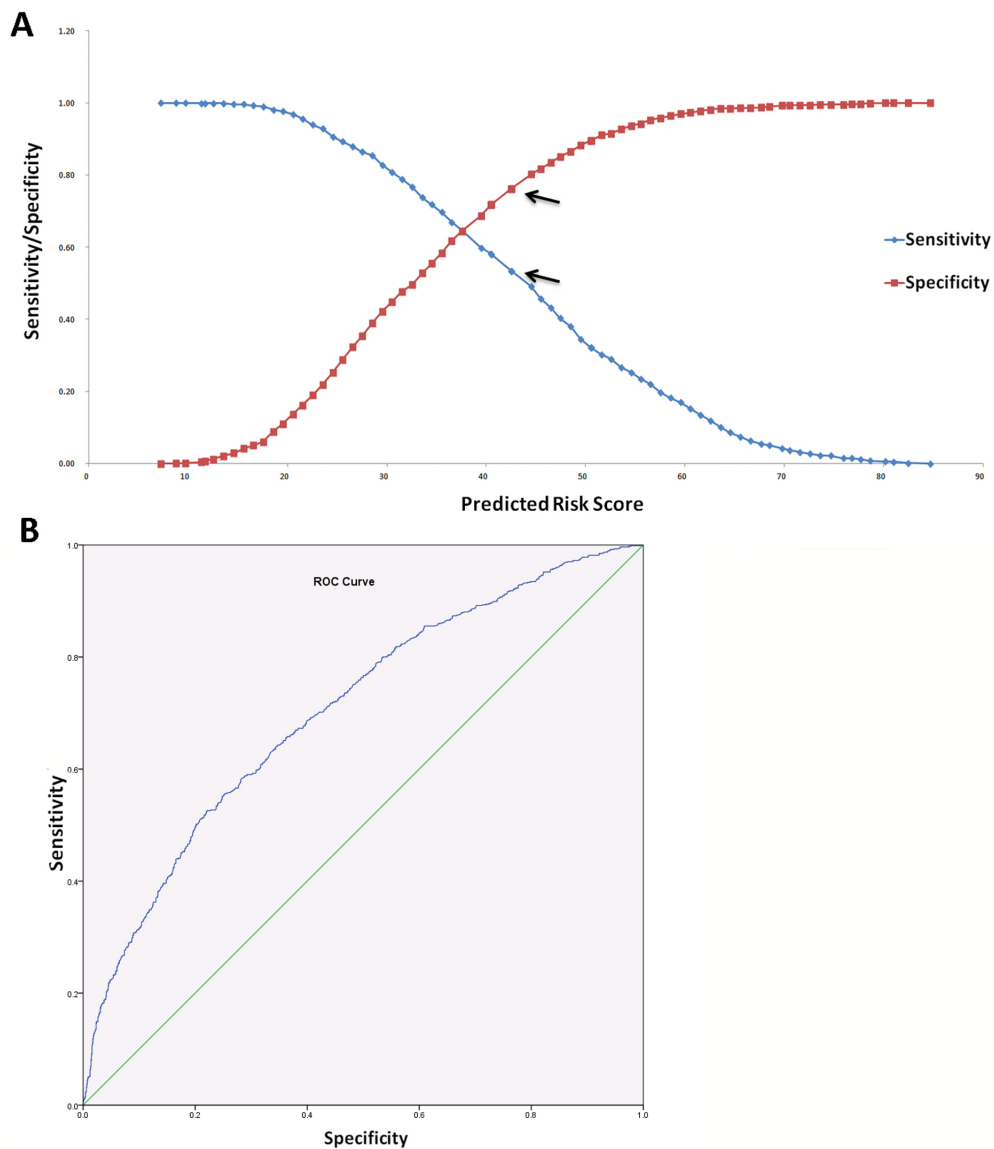


Fig. (2). Receiver operating characteristic curve of the predicted risk score in replication populations. (A) The best cut-off value of the risk score was 43. As indicated by the arrow, the corresponding sensitivity and specificity at the score of 43 was 53% and 76.2%, respectively; **(B)** The area under the ROC curve was 0.705. (A higher resolution / colour version of this figure is available in the electronic copy of the article).

tions. Moreover, patients would benefit from a more targeted therapy for a healthy lifestyle. Overall, our predictive model can explain 7.9% of the whole variance of AIS. With a cut-off value set at 0.5, the predictive model yielded 85.9% sensitivity and 31.3% specificity. As shown in the replication stage, most of the patients had a risk score of more than 40, with 15% of them exceeding 60. By contrast, only 2.8% of the controls had a risk score of more than 60. Apparently, a risk score of more than 60 was indicative of an extremely high risk of developing AIS. For children with such a high risk score, especially those with a family history of AIS, a close observation during growth spurt until skeletal maturity seems necessary.

Commonly, the AUC is recognized as the measure of the discriminatory power of the diagnostic model. Predictive models with an AUC above 0.75 are considered as clinically useful, and models with an AUC above 0.90 are considered as excellent [24]. Herein, it appears that the current risk model has a remarkable discrimination power but is still insufficient for clinical diagnostic use. We speculated that insufficient discrimination power was mostly attributed to the relatively small number of genetic markers and the complexity of the disease. Similar to the current study, a regression model was created in previous research to evaluate the risk of bracing failure in AIS patients [25]. With two genetic markers and one clinical parameter included in the model, the sensitivity and specificity were 92.3% and 41.7%, respectively. To further improve the prediction model of AIS, more genetic factors need to be studied and included in the model.

In this study, we described a formula facilitating the calculation of individual risk score of AIS, which was successfully replicated in an independent cohort. To be noted, however, the risk prediction resulted from this model is currently applicable only to the Chinese population. Also, this formula needs to be modified continuously with new predictors included in the model. Therefore, it should be carefully interpreted when extended to other populations in the current form.

The present study has several limitations. First, further validation of our model in different population is required to validate its reliability. Second, as only 10 SNPs were eligible to be included, identification of more AIS-associated loci in the Chinese population is required in order to strengthen the accuracy of the predictive model. Third, some confounding factors were not taken into consideration. We predicted the risk of AIS onset using genetic variants only. Other factors such as family history of idiopathic scoliosis, abnormal bone mineral density, abnormal body mass index, rapid growth velocity and unknown environmental factors can be associated with the development of AIS. In future studies, more factors need to be included in the predictive model to improve its sensitivity and specificity.

CONCLUSION

The present study established a novel predictive model concerning the risk of AIS onset in the Chinese population. Larger sample size, more influential factors and validation of the model in other populations will allow for the development of a more powerful prediction tool for the risk of AIS.

LIST OF ABBREVIATIONS

AIS	=	Adolescent Idiopathic Scoliosis
HWE	=	Hardy-Weinberg Equilibrium
OR	=	Odds Ratio
ROC	=	Receiver Operating Characteristic
AUC	=	Area Under the Curve
SNPs	=	Single-Nucleotide Polymorphisms

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The extraction of blood and DNA was approved by the local Institutional Review Board, Nanjing Drum Tower Hospital, China, (Approval no. NJDT-180321).

HUMAN AND ANIMAL RIGHTS

No animals were used for studies that are the basis of this research. All the experimental procedure on humans were in accordance with the ethical standards of the committee responsible for human experimentation (institutional and national), and with the Helsinki Declaration of 1975, as revised in 2013.

CONSENT FOR PUBLICATION

Informed consent was obtained from the subjects or their guardians.

AVAILABILITY OF DATA AND MATERIALS

The data supporting the findings of the article is available from the corresponding author (Dr. ZeZhang Zhu) on request.

FUNDING

This work was supported by the Natural Science Foundation of China (No. 81661168013, No. 81772304, & No. 81871747) and Joint Research Scheme sponsored by the National Natural Science Foundation of China and the Research Grants Council of the Hong Kong Special Administrative Region (N_CUHK416/16).

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Declared none.

REFERENCES

- [1] Miller, N.H.; Justice, C.M.; Marosy, B.; Doheny, K.F.; Pugh, E.; Zhang, J.; Dietz, H.C.; Wilson, A.F. Identification of candidate regions for familial idiopathic scoliosis. *Spine*, **2005**, *30*(10), 1181-1187. [http://dx.doi.org/10.1097/01.brs.0000162282.46160.0a] [PMID: 15897833]
- [2] Kouwenhoven, J.W.; Castelein, R.M. The pathogenesis of adolescent idiopathic scoliosis: Review of the literature. *Spine*, **2008**, *33*(26), 2898-2908. [http://dx.doi.org/10.1097/BRS.0b013e3181891751] [PMID: 19092622]
- [3] Wu, J.; Qiu, Y.; Zhang, L.; Sun, Q.; Qiu, X.; He, Y. Association of estrogen receptor gene polymorphisms with susceptibility to adolescent idiopathic scoliosis. *Spine*, **2006**, *31*(10), 1131-1136. [http://dx.doi.org/10.1097/01.brs.0000216603.91330.6f] [PMID: 16648749]

- [4] Chen, Z.; Tang, N.L.; Cao, X.; Qiao, D.; Yi, L.; Cheng, J.C.; Qiu, Y. Promoter polymorphism of matrilin-1 gene predisposes to adolescent idiopathic scoliosis in a Chinese population. *Eur. J. Hum. Genet.*, **2009**, *17*(4), 525-532. [http://dx.doi.org/10.1038/ejhg.2008.203] [PMID: 18985072]
- [5] Baschal, E.E.; Wethey, C.I.; Swindle, K.; Baschal, R.M.; Gowan, K.; Tang, N.L.; Alvarado, D.M.; Haller, G.E.; Dobbs, M.B.; Taylor, M.R.; Gurnett, C.A.; Jones, K.L.; Miller, N.H. Exome sequencing identifies a rare HSPG2 variant associated with familial idiopathic scoliosis. *G3 (Bethesda)*, **2014**, *5*(2), 167-174. [http://dx.doi.org/10.1534/g3.114.015669] [PMID: 25504735]
- [6] Buchan, J.G.; Alvarado, D.M.; Haller, G.E.; Cruchaga, C.; Harms, M.B.; Zhang, T.; Willing, M.C.; Grange, D.K.; Braverman, A.C.; Miller, N.H.; Morcuende, J.A.; Tang, N.L.; Lam, T.P.; Ng, B.K.; Cheng, J.C.; Dobbs, M.B.; Gurnett, C.A. Rare variants in FBN1 and FBN2 are associated with severe adolescent idiopathic scoliosis. *Hum. Mol. Genet.*, **2014**, *23*(19), 5271-5282. [http://dx.doi.org/10.1093/hmg/ddu224] [PMID: 24833718]
- [7] Patten, S.A.; Margaritte-Jeannin, P.; Bernard, J.C.; Alix, E.; Labalme, A.; Besson, A.; Girard, S.L.; Fendri, K.; Fraisse, N.; Biot, B.; Poizat, C.; Campan-Fournier, A.; Abelin-Genevois, K.; Cunin, V.; Zauter, C.; Liao, M.; Lamy, R.; Lesca, G.; Menassa, R.; Marcaillou, C.; Letexier, M.; Sanlaville, D.; Berard, J.; Rouleau, G.A.; Clerget-Darpoux, F.; Drapeau, P.; Moldovan, F.; Edery, P. Functional variants of POC5 identified in patients with idiopathic scoliosis. *J. Clin. Invest.*, **2015**, *125*(3), 1124-1128. [http://dx.doi.org/10.1172/JCI77262] [PMID: 25642776]
- [8] Sharma, S.; Gao, X.; Londono, D.; Devroy, S.E.; Mauldin, K.N.; Frankel, J.T.; Brandon, J.M.; Zhang, D.; Li, Q.Z.; Dobbs, M.B.; Gurnett, C.A.; Grant, S.F.; Hakonarson, H.; Dormans, J.P.; Herring, J.A.; Gordon, D.; Wise, C.A. Genome-wide association studies of adolescent idiopathic scoliosis suggest candidate susceptibility genes. *Hum. Mol. Genet.*, **2011**, *20*(7), 1456-1466. [http://dx.doi.org/10.1093/hmg/ddq571] [PMID: 21216876]
- [9] Kou, I.; Takahashi, Y.; Johnson, T.A.; Takahashi, A.; Guo, L.; Dai, J.; Qiu, X.; Sharma, S.; Takimoto, A.; Ogura, Y.; Jiang, H.; Yan, H.; Kono, K.; Kawakami, N.; Uno, K.; Ito, M.; Minami, S.; Yanagida, H.; Taneichi, H.; Hosono, N.; Tsuji, T.; Suzuki, T.; Sudo, H.; Kotani, T.; Yonezawa, I.; Londono, D.; Gordon, D.; Herring, J.A.; Watanabe, K.; Chiba, K.; Kamatani, N.; Jiang, Q.; Hiraki, Y.; Kubo, M.; Toyama, Y.; Tsunoda, T.; Wise, C.A.; Qiu, Y.; Shukunami, C.; Matsumoto, M.; Ikegawa, S. Genetic variants in GPR126 are associated with adolescent idiopathic scoliosis. *Nat. Genet.*, **2013**, *45*(6), 676-679. [http://dx.doi.org/10.1038/ng.2639] [PMID: 23666238]
- [10] Ogura, Y.; Kou, I.; Miura, S.; Takahashi, A.; Xu, L.; Takeda, K.; Takahashi, Y.; Kono, K.; Kawakami, N.; Uno, K.; Ito, M.; Minami, S.; Yonezawa, I.; Yanagida, H.; Taneichi, H.; Zhu, Z.; Tsuji, T.; Suzuki, T.; Sudo, H.; Kotani, T.; Watanabe, K.; Hosogane, N.; Okada, E.; Iida, A.; Nakajima, M.; Sudo, A.; Chiba, K.; Hiraki, Y.; Toyama, Y.; Qiu, Y.; Shukunami, C.; Kamatani, N.; Kubo, M.; Matsumoto, M.; Ikegawa, S. A functional SNP in BNC2 is associated with adolescent idiopathic scoliosis. *Am. J. Hum. Genet.*, **2015**, *97*(2), 337-342. [http://dx.doi.org/10.1016/j.ajhg.2015.06.012] [PMID: 26211971]
- [11] Sharma, S.; Londono, D.; Eckalbar, W.L.; Gao, X.; Zhang, D.; Mauldin, K.; Kou, I.; Takahashi, A.; Matsumoto, M.; Kamiya, N.; Murphy, K.K.; Cornelia, R.; Herring, J.A.; Burns, D.; Ahituv, N.; Ikegawa, S.; Gordon, D.; Wise, C.A. A PAX1 enhancer locus is associated with susceptibility to idiopathic scoliosis in females. *Nat. Commun.*, **2015**, *6*, 6452. [http://dx.doi.org/10.1038/ncomms7452] [PMID: 25784220]
- [12] Zhu, Z.; Tang, N.L.; Xu, L.; Qin, X.; Mao, S.; Song, Y.; Liu, L.; Li, F.; Liu, P.; Yi, L.; Chang, J.; Jiang, L.; Ng, B.K.; Shi, B.; Zhang, W.; Qiao, J.; Sun, X.; Qiu, X.; Wang, Z.; Wang, F.; Xie, D.; Chen, L.; Chen, Z.; Jin, M.; Han, X.; Hu, Z.; Zhang, Z.; Liu, Z.; Zhu, F.; Qian, B.P.; Yu, Y.; Wang, B.; Lee, K.M.; Lee, W.Y.W.; Lam, T.P.; Qiu, Y.; Cheng, J.C. Genome-wide association study identifies new susceptibility loci for adolescent idiopathic scoliosis in Chinese girls. *Nat. Commun.*, **2015**, *6*, 8355. [http://dx.doi.org/10.1038/ncomms9355] [PMID: 26394188]
- [13] Nada, D.; Julien, C.; Samuels, M.E.; Moreau, A. A replication study for association of LBX1 locus with adolescent idiopathic scoliosis in French-Canadian population. *Spine*, **2017**, *43*(10), Doi: 10.1097/BRS.0000000000002280. [PMID: 28604496]
- [14] Zhu, Z.; Xu, L.; Leung-Sang Tang, N.; Qin, X.; Feng, Z.; Sun, W.; Zhu, W.; Shi, B.; Liu, P.; Mao, S.; Qiao, J.; Liu, Z.; Sun, X.; Li, F.; Chun-Yiu Cheng, J.; Qiu, Y. 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), 1577-1583. [http://dx.doi.org/10.1093/hmg/ddx045] [PMID: 28334814]
- [15] Ward, K.; Ogilvie, J.W.; Singleton, M.V.; Chettier, R.; Engler, G.; Nelson, L.M. Validation of DNA-based prognostic testing to predict spinal curve progression in adolescent idiopathic scoliosis. *Spine*, **2010**, *35*(25), E1455-E1464. [http://dx.doi.org/10.1097/BRS.0b013e3181ed2de1] [PMID: 21102273]
- [16] Xu, L.; Qin, X.; Sun, W.; Qiao, J.; Qiu, Y.; Zhu, Z. Replication of association between 53 single-nucleotide polymorphisms in a DNA-based diagnostic test and AIS progression in Chinese han population. *Spine*, **2016**, *41*(4), 306-310. [http://dx.doi.org/10.1097/BRS.0000000000001203] [PMID: 26579958]
- [17] Roye, B.D.; Wright, M.L.; Matsumoto, H.; Yorgova, P.; McCalla, D.; Hyman, J.E.; Roye, D.P.; Shah, S.A.; Vitale, M.G. An independent evaluation of the validity of a DNA-based prognostic test for adolescent idiopathic scoliosis. *J. Bone Joint Surg. Am.*, **2015**, *97*(24), 1994-1998. [http://dx.doi.org/10.2106/JBJS.O.00217] [PMID: 26677232]
- [18] Tang, Q.L.; Julien, C.; Eveleigh, R.; Bourque, G.; Franco, A.; Labelle, H.; Grimard, G.; Parent, S.; Ouellet, J.; Mac-Thiong, J.M.; Gorman, K.F.; Moreau, A. A replication study for association of 53 single nucleotide polymorphisms in ScolioScore test with adolescent idiopathic scoliosis in French-Canadian population. *Spine*, **2015**, *40*(8), 537-543. [http://dx.doi.org/10.1097/BRS.0000000000000807] [PMID: 25646748]
- [19] Ogura, Y.; Takahashi, Y.; Kou, I.; Nakajima, M.; Kono, K.; Kawakami, N.; Uno, K.; Ito, M.; Minami, S.; Yanagida, H.; Taneichi, H.; Yonezawa, I.; Tsuji, T.; Suzuki, T.; Sudo, H.; Kotani, T.; Watanabe, K.; Chiba, K.; Toyama, Y.; Matsumoto, M.; Ikegawa, S. A replication study for association of 53 single nucleotide polymorphisms in a scoliosis prognostic test with progression of adolescent idiopathic scoliosis in Japanese. *Spine*, **2013**, *38*(16), 1375-1379. [http://dx.doi.org/10.1097/BRS.0b013e3182947d21] [PMID: 23591653]
- [20] Takahashi, Y.; Kou, I.; Takahashi, A.; Johnson, T.A.; Kono, K.; Kawakami, N.; Uno, K.; Ito, M.; Minami, S.; Yanagida, H.; Taneichi, H.; Tsuji, T.; Suzuki, T.; Sudo, H.; Kotani, T.; Watanabe, K.; Chiba, K.; Hosono, N.; Kamatani, N.; Tsunoda, T.; Toyama, Y.; Kubo, M.; Matsumoto, M.; Ikegawa, S. A genome-wide association study identifies common variants near LBX1 associated with adolescent idiopathic scoliosis. *Nat. Genet.*, **2011**, *43*(12), 1237-1240. [http://dx.doi.org/10.1038/ng.974] [PMID: 22019779]
- [21] Xu, L.; Sheng, F.; Xia, C.; Qin, X.; Tang, N.L.; Qiu, Y.; Cheng, J.C.; Zhu, Z. Genetic variant of PAX1 gene is functionally associated with adolescent idiopathic scoliosis in the Chinese population. *Spine*, **2018**, *43*(7), 492-496. [http://dx.doi.org/10.1097/BRS.0000000000002475] [PMID: 29095406]
- [22] Xu, L.; Xia, C.; Qin, X.; Sun, W.; Tang, N.L.; Qiu, Y.; Cheng, J.C.; Zhu, Z. Genetic variant of BNC2 gene is functionally associated with adolescent idiopathic scoliosis in Chinese population. *Mol. Genet. Genomics*, **2017**, *292*(4), 789-794. [http://dx.doi.org/10.1007/s00438-017-1315-3] [PMID: 28342042]
- [23] Qin, X.; Xu, L.; Xia, C.; Zhu, W.; Sun, W.; Liu, Z.; Qiu, Y.; Zhu, Z. Genetic variant of GPR126 gene is functionally associated with adolescent idiopathic scoliosis in Chinese population. *Spine*, **2017**, *42*(19), E1098-E1103. [http://dx.doi.org/10.1097/BRS.0000000000002123] [PMID: 28198779]
- [24] Gupta, M.; Moily, N.S.; Kaur, H.; Jajodia, A.; Jain, S.; Kukreti, R. Identifying a predictive model for response to atypical antipsychotic monotherapy treatment in south Indian schizophrenia patients. *Genomics*, **2013**, *102*(2), 131-135. [http://dx.doi.org/10.1016/j.ygeno.2013.02.002] [PMID: 23402766]
- [25] 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), 1757-1764. [http://dx.doi.org/10.1007/s00586-011-1874-7] [PMID: 21691901]