Original Article

Polymorphisms in paired box 1 gene were associated with susceptibility of adolescent idiopathic scoliosis: A case–control study

ABSTRACT

Background: Association of genetic polymorphisms in paired box 1 (*PAX-1*) gene can influence the development of adolescent idiopathic scoliosis (AIS). PAX-1 is mainly expressed in the region of the vertebral bodies and intervertebral discs, being important for the proper formation of spinal structures.

Objectives: The objective of this study was to evaluate the association of polymorphisms in *PAX-1* gene with the susceptibility of AIS.

Settings and Design: This was an analytical observational case–control study.

Materials and Methods: Samples of 59 AIS indicated for surgical treatment, and 119 controls, without spinal disease were genotyped for *PAX-1* rs6137473 and rs169311 polymorphisms.

Statistical Analysis: The association of the polymorphisms with AIS was evaluated by a multivariable logistic regression model, using odds ratios (OR) and 95% confidence intervals (CI).

Results: According to Lenke's classification, 89.8% had Type I and 10.2% II curves. The mean value of the Cobb angle of the proximal thoracic curve was 30.8°, 58.7° thoracic, and 30.4° for the lumbar and on the bending films 14.6°, 40.7°, and 11°, respectively. Among the AIS group, there was a predominance of females (8.8:1). The *PAX-1* rs169311 and rs6137473 polymorphisms were positively associated with developing the AIS (OR = 1.98; 95% CI = 1.2–3.3 and OR = 3.16; 95% CI = 1.4–7.3, respectively). The rs6137473 polymorphism was associated with the lumbar modifier B and C compared to A (OR = 2.52; 95% CI = 1.1–5.8).

Conclusions: *PAX-1* polymorphisms were associated with an increased risk of developing the AIS and with curve severity and can be used as a biomarker to map the risk of developing surgical-grade AIS, guiding the treatment of patients.

Keywords: Adolescent idiopathic scoliosis, genetic polymorphisms, Lenke's classification, paired box 1

Antônio Eulálio Pedrosa[1,2], Gustavo Borges Laurindo de Azevedo[1,2], Jessica Vilarinho Cardoso[3], João Antonio Matheus Guimarães[4], Helton Luiz Aparecido Defino[2], Jamila Alessandra Perini[3,4]

[1]Spine Surgery Center, National Institute of Traumatology and Orthopaedics (INTO), Rio de Janeiro, RJ, [2]Departments of Orthopaedic and Anesthesiology, Ribeirão Preto Medical School, University of São Paulo, de São Paulo-Brazil, [3]Research Laboratory of Pharmaceutical Sciences (LAPESF), State University of Rio de Janeiro (UERJ), Rio de Janeiro, RJ, [4]Research Division of National Institute of Traumatology and Orthopaedics (INTO), Rio de Janeiro, RJ, Brazil

Address for correspondence: Prof. Jamila Alessandra Perini, Pharmaceutical Sciences Research Laboratory (LAPESF), State University of Rio de Janeiro (UERJ) (https://lapesfuezo.wixsite.com/website), Av. Manuel Caldeira de Alvarenga, 1.203. Zip-code: 23070-200, Rio de Janeiro, Brazil.
E-mail: jamilaperini@yahoo.com.br

Submitted: 14-Apr-22 **Accepted:** 09-May-22
Published: 14-Sep-22

INTRODUCTION

The curve progression in adolescent idiopathic scoliosis (AIS) depends on several factors such as skeletal age, sex, magnitude, and type of curves.[1-4] Only 0.1% of patients have surgical treatment indication (Cobb angle >40°), and according to Lenke's classification, Types I and II are the most common phenotypes.[5,6]

Access this article online	
Website: www.jcvjs.com	Quick Response Code
DOI: 10.4103/jcvjs.jcvjs_54_22	

How to cite this article: Pedrosa AE, Azevedo GB, Cardoso JV, Guimarães JA, Defino HL, Perini JA. Polymorphisms in paired box 1 gene were associated with susceptibility of adolescent idiopathic scoliosis: A case–control study. J Craniovert Jun Spine 2022;13:318-24.

Although the etiology of AIS is still unknown, there are some theories that try to explain the onset of the disease, such as metabolic errors, nervous system dysfunction, biomechanical, bone growth, and genetic alterations.[7] Several candidate genes, associated with the AIS development, participate in the formation of the intervertebral disc, among them paired box 1 (*PAX-1*) stands out,[8-11] due to its predominant expression in the region of the vertebral bodies and intervertebral discs, being crucial for the proper formation of spinal structures.[12,13]

The *PAX-1* gene is a transcription factor, characterized by the presence of a conserved DNA-binding domain, located on chromosome 20p11.[14] Considering the single-nucleotide polymorphisms (SNPs) described in the *PAX-1* gene, which have already been associated with the AIS development, the rs6137473 A>G and the rs169311 C>A SNPs stand out, mainly because of their ability to affect the *PAX-1* expression and because of the availability of previous studies describing this association.[9,10,15,16] The aim of this study was to describe the clinical data of Lenke I and II AIS patients and to evaluate the association of *PAX-1* rs6137473 and rs169311 SNPs with the development and severity of the disease.

MATERIALS AND METHODS

Study population

An analytical observational case–control study was carried out in a sample of patients diagnosed with AIS, treated by the Spine Surgery Service of a public orthopedic referral hospital in Brazil, in the period from July 2018 to January 2019. The study was conducted in accordance with the Helsinki Declaration and with the ethical standards.

All included patients (N = 59) were over 10 years old and were clinically diagnosed with AIS, classified as Lenke type I and II, with Cobb angle >40° and coronal decompensation, with the intention of homogenizing the scoliosis phenotype, since both forms have a lumbar curve flexible. Patients with incomplete radiographic data, absence of biological material for genetic analysis, or those with any underlying disease that could justify nonidiopathic scoliosis, such as neurological disorders, neuromuscular disorders, and syndromic diseases, were excluded. In the control group (N = 119), healthy volunteers recruited at INTO's blood bank without spinal disease were included.

Radiographic parameters

Panoramic radiographs of the spine in AP, lateral, and supine bending tests [Figure 1] were taken to define the Cobb angle and to identify the patients classified as Lenke 1 and 2, the lumbar modifiers (A, B, and C), and the sagittal modifiers, according to the thoracic kyphosis angle measured from T5–T12 (being hyper for kyphosis >40°; hypo, <10°; and normal, when kyphosis is between 11° at 40°).[17,18] The evaluation of radiographs was performed using the Surgimap Spine software (Nemaris Inc., New York, USA), version 2.2.15.5.

Polymorphisms' genotyping

Genomic DNA was extracted from an oral mucosal sample using the Invisorb® Spin DNA Extraction Kit (Qiagen, Hilden,

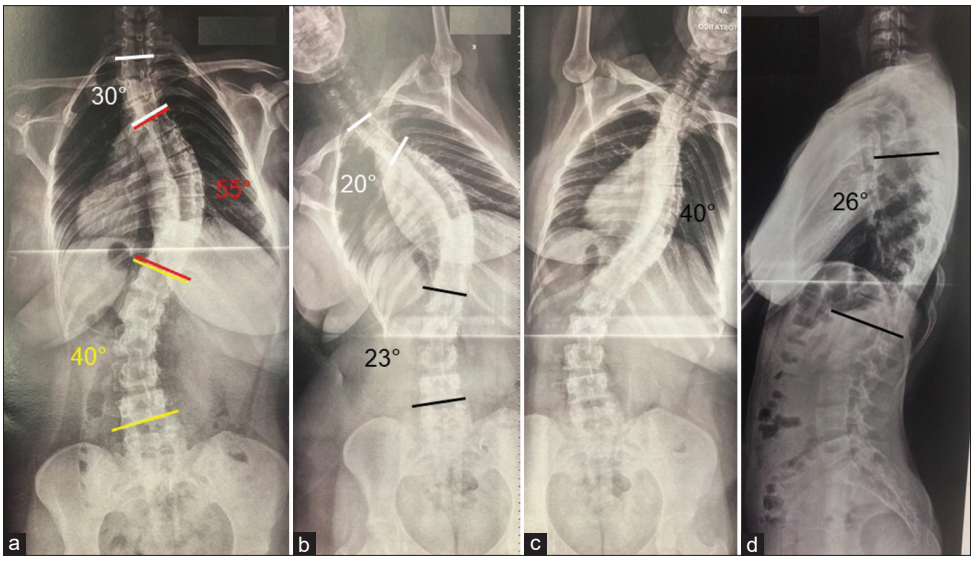

Figure 1: Spine radiographs of scoliosis patients. (a-d) Cobb angle measurements. (a) Posteroanterior radiograph with proximal thoracic (white lines), main thoracic (red lines), and thoracolumbar curves (yellow lines) measured. (b) Left-side bending radiograph showing proximal thoracic (white lines) and thoracolumbar flexibility (black lines). (c) Right-side bending radiograph showing main thoracic curve flexibility. (d) Lateral spine radiograph showing thoracic kyphosis measured from T5 to T12

Germany), according to the procedures recommended by the manufacturer. Analysis of the rs6137473 and rs169311 SNPs of the *PAX-1* gene was performed by the real-time polymerase chain reaction (PCR) technique using the QuantStudio™ 3 Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA USA). PCR amplification was performed in 8-μL reactions with ~30 ng of template DNA, ×1 TaqMan Universal Master Mix (Applied Biosystems, Foster City, CA, USA), ×1 each primer and probe assay. Thermal cycling was initiated with a first denaturation step of 10 min at 95°C, followed by 40 cycles of denaturation at 92°C for 15 s and annealing at 60°C for 1 min. To assure genotyping quality, in each reaction, two standardized negative and positive controls of each polymorphism genotype were used, as previously described.[19]

Statistical analysis

A descriptive analysis about demographic and clinical data of population studied was performed, using relative frequencies for each categorical variable. Continuous variables were presented as mean ± standard deviation and differences between means were evaluated using Student's *t*-test. Categorical data were expressed as percentages and evaluated by Person's Chi-square test (x^2) or Fisher's exact test, when necessary.

The allelic and genotypic frequency of the studied polymorphisms was determined by direct gene counting and the Hardy–Weinberg equilibrium (HWE) was calculated by the Chi-square test for goodness-of-fit. To assess the association of polymorphisms with the development and severity of AIS, the odds ratios with their respective 95% confidence intervals were estimated and $P < 0.05$ was considered statistically significant. A binary logistic regression model was performed to control for possible confounding factors, considering the biological and statistical significance of the univariate analysis of each sociodemographic and clinical variable. A value of $P \leq 0.25$ was considered the input significance level and $P \leq 0.05$ as the output significance for the final regression model. The Statistical Package for Social Sciences program (SPSS Inc., Chicago, IL, USA, version 20.0) was used for all statistical analyses.

RESULTS

Radiographic data of adolescent idiopathic scoliosis

Of the 59 patients, 89.8% ($N = 53$) were classified as Lenke type I and 10.2% ($N = 6$) as Lenke II. Lumbar modifier A was observed in 67.8% ($N = 40$) of patients, followed by modifiers B ($N = 16$, 27.1%) and C ($n = 3$ or 5.1%). There was a predominance of the normal type (74.6%, $N = 44$), 23.7% hyperkyphotic ($N = 14$), and 1.7% hypokyphotic ($N = 1$) for the sagittal modifier. Considering the lumbar and sagittal modifier, the most frequent form of AIS was the normal Lenke IA ($N = 25$, 42.4%), followed by normal Lenke IB ($N = 13$, 22.0%) and hyperkyphotic Lenke IA ($N = 8$, 13.6%). Only one patient (1.7%) had a hypokyphotic Lenke IA and another one had a hyperkyphotic Lenke IC (1.7%). Three patients (5.1%) were classified as Lenke IB hyperkyphotic and another two (3.4%) as IC normal. Of the Lenke II, four patients (6.8%) were classified as A (normal) and two (3.4%) as A hyperkyphotic [Figure 2]. The Cobb angles of proximal thoracic, main thoracic, thoracolumbar/lumbar of the 59 AIS patients are shown in Table 1.

Case–control study and polymorphisms association

Approximately 89.8% ($N = 53$) of AIS cases were female (8.8:1), with a mean age of 22.8 ± 5.7 years at recruitment, 42.4% ($N = 25$) aged 20 years old or less, and 79.7% ($N = 47$) with low weight or normal BMI (21.5 ± 5.2 Kg/m^2). The control group ($N = 119$) consisted of 108 women (90.8%), with a mean age of 28.6 ± 6.6 years and a mean BMI of 25.7 ± 4.4 Kg/m^2. All variables with $P \leq 0.25$ were inserted in the logistic regression model to identify possible

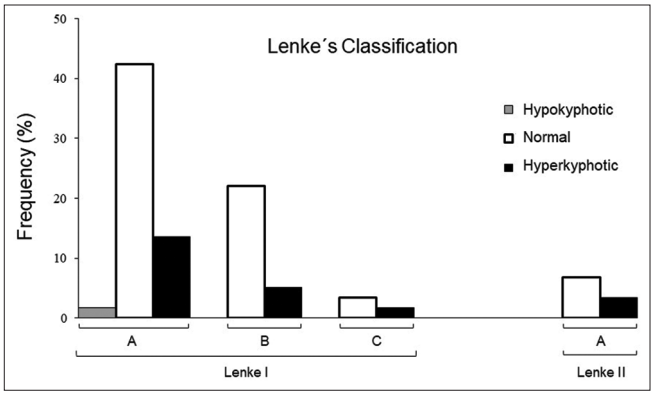

Figure 2: Adolescent idiopathic scoliosis patients (n = 59) distribution categorized by Lenke's classification system. A, B, and C are the lumbar spine modifier. Type Lenke I main thoracic and Lenke II double thoracic curves

Table 1: Curve angle information (proximal thoracic, main thoracic, thoracolumbar/lumbar) of adolescent idiopathic scoliosis patients

Angles	AIS cases (n=59)	
	Mean±SD	Range
Proximal thoracic		
Cobb	30.8±9.2	13.4-52
Inclination	14.6±10.2	0-41.7
Main thoracic		
Cobb	58.7±10.3	40-92.9
Inclination	40.7±13.5	16.9-75
Lumbar		
Cobb	30.4±12.6	2.3-54.2
Inclination	11.0±8.0	0-24.8

SD - Standard deviation; Range - Minimum and maximum value; AIS - Adolescent idiopathic scoliosis

confounding factors involved with the association between SNPs and AIS. After multivariate analysis, the variables age, sex, and BMI remained in the model.

The frequency of the *PAX-1* rs6137473 and rs169311 SNPs was in HWE. There was a significant difference in the frequency distribution of both polymorphisms comparing AIS cases and controls [Figure 3]. After adjustment for confounding factors, the variant homozygous *PAX-1* genotypes (rs6137473 *GG* and rs169311 *AA*) were associated with higher risk of developing AIS than patients with the wild-type genotype, since in the control group, the frequency of the variant genotypes was lower and null, respectively, compared with case group [Table 2].

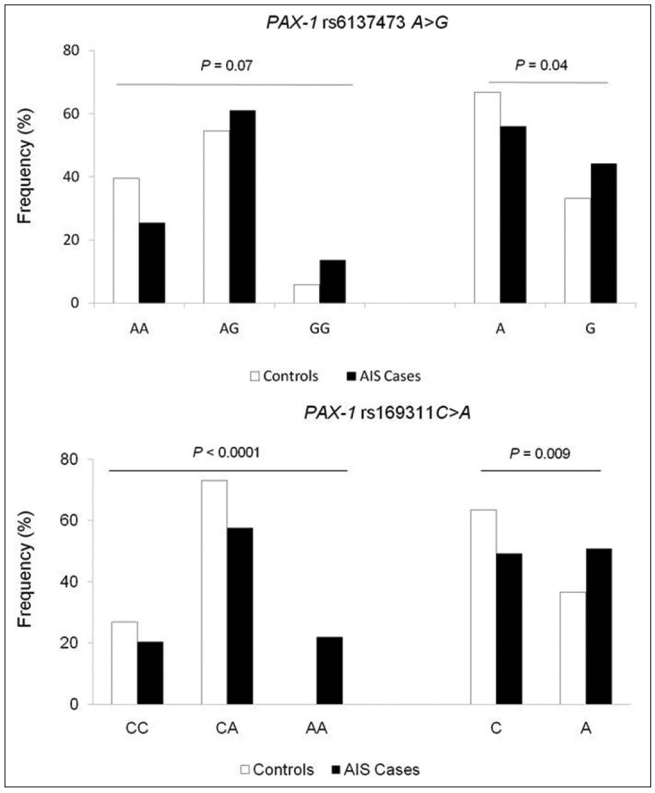

Figure 3: Allelic and genotypic distribution of the *PAX-1* polymorphisms in adolescent idiopathic scoliosis cases and controls (*n* = 178). *P* value calculated by Chi-square test or Fisher's exact test, when necessary. *PAX-1* - Paired box 1

The presence of *PAX-1* rs6137473 and rs169311 polymorphisms was also evaluated regarding the severity of the AIS attributed by the lumbar and sagittal modifier and the value of the curve angle. An approximately 5-fold increased risk was observed for the more severe scoliosis phenotype for patients who had the variant genotype rs6137473 *GG* compared to individuals with the wild-type genotype [Table 3]. Regarding the thoracic kyphosis measured from T5-T12, no significant difference was observed in the presence of both polymorphisms (rs6137473 and rs169311) in *PAX-1* gene between the AIS cases that presented sagittal modifier normal compared to those with hyperkyphosis (data not shown). Only one case of scoliosis had thoracic hypokyphosis, which did not allow for comparison between subgroups.

DISCUSSION

The *PAX-1* SNPs (rs6137473 and rs169311) were associated with the susceptibility of AIS, and *PAX-1* rs6137473 can significantly add the risk of AIS severity in patients classified as Lenke type I and II.

As far as we know, the present work is the first study to describe the frequency of *PAX-1* rs6137473 and rs169311 SNPs in the Brazilian population, a notoriously admixed and heterogeneous population.[20] The discrepancies between different studies involving the influence of genetic variations on the susceptibility to complex disease may be impacted

Table 2: Association analysis between the *PAX-1* polymorphisms and adolescent idiopathic scoliosis development (*n*=178)

PAX-1 SNP	Controls (*n*=119), *n* (%)	AIS cases (*n*=59), *n* (%)	*P**	OR_{crude} (95% CI)	ORa‡ (95% CI)
rs6137473					
AA	47 (39.5)	15 (25.4)		1†	1†
AG	65 (54.6)	36 (61.0)	0.126	1.74 (0.85-3.53)	1.52 (0.65-3.54)
GG	7 (5.9)	8 (13.6)	0.027	3.58 (1.11-11.5)	3.16 (1.37-7.29)
A	159 (66.8)	66 (55.9)		1†	1†
G	79 (33.2)	52 (44.1)	0.045	1.59 (1.01-2.49)	1.62 (0.99-2.66)
rs169311					
CC	32 (26.9)	12 (20.4)		1†	1†
CA	87 (73.1)	34 (57.6)	0.917	1.04 (0.48-2.26)	1.00 (0.39-2.53)
AA	0	13 (22.0)	<0.001	70.2 (3.87-1273)	-
C	151 (63.4)	58 (49.2)		1†	1†
A	87 (36.6)	60 (50.8)	0.009	1.80 (1.15-2.81)	1.98 (1.21-3.25)

*Pearson's Chi-square test or Fisher's exact test, when necessary; †Reference group; ‡ORa - Odds ratio adjusted by age, gender, and BMI. (-) As no individual in the control group had *PAX-1 rs169311 AA* genotype, it was not possible to perform a binary regression analysis. SNP - Single-nucleotide polymorphism; OR - odds ratio; CI - 95% confidence interval; AIS - Adolescent idiopathic scoliosis; BMI - Body mass index

Table 3: Association analysis between the *PAX-1* polymorphisms and scoliosis severity, considering the lumbar spine modifier of Lenke's classification (*n*=59)

PAX-1 SNP	A (*n*=40),	B and C (*n*=19)	*P**	ORa‡ (95% CI)
rs6137473	*n* (%)	*n* (%)		
AA	14 (35.0)	1 (5.3)		1†
AG	22 (55.0)	14 (73.7)	0.041	8.50 (0.99-73.0)
GG	4 (10.0)	4 (21.0)		4.83 (1.15-20.4)
A	50 (62.5)	16 (42.1)		1†
G	30 (37.5)	22 (57.9)	0.047	2.52 (1.10-5.77)
rs169311				
CC	11 (27.5)	1 (5.3)		1†
CA	21 (52.5)	13 (68.4)	0.141	7.00 (0.77-64.0)
AA	8 (20.0)	5 (26.3)		2.48 (0.69-8.95)
C	43 (53.8)	15 (39.5)	0.171	1†
A	37 (46.2)	23 (60.5)		1.95 (0.86-4.43)

*Pearson's Chi-square test or Fisher's exact test, when necessary; †Reference group; ‡ORa - Odds ratio adjusted by age, gender, and BMI. SNP - Single-nucleotide polymorphism; OR - Odds ratio; CI - 95% confidence interval; BMI - Body mass index

by distinct allele frequencies and heterogeneity in the studied populations, besides variation on environmental backgrounds.[15] Furthermore, to successfully replicate the association between *PAX1* SNPs and the susceptibility of AIS in the mixed population, our study has distinct strengths. First, despite the small number of included patients, we selected only patients with surgical indication for the treatment of scoliosis, classified as Lenke I and II, with the view to homogenize the group of cases to assess the influence of genetic variation in *PAX-1* gene with the development and severity of the disease. AIS patients were recruited from an orthopedic referral hospital that reflects real-life community for diagnosing and treating AIS in a developing country. Second, all control individuals were evaluated by experienced spine surgeons, excluding spine deformities in the control group. However, the small number of patients in the Lenke's classification groups and no family history data to evaluate the differences between familial and nonfamilial AIS cases were the main limitations of this study. Relatives of individuals with idiopathic scoliosis have a higher incidence of the disease than the general population.[21,22]

As observed in the present study, AIS Lenke type I is the most frequent type in literature. A study involving 606 patients with AIS observed that 51% of cases corresponded to Lenke type I and 20% Type II.[6] Furthermore, most AIS cases were female and normal or low BMI according to previous studies.[4,23-25] Women have 5 and 10-fold higher risk than male to develop progressive deformity and most severe forms of the AIS, respectively.[2,4,26,27] In addition, patients with severe curves had significantly lower BMI and it is hypothesized that this association may be related to the pathogenesis of the disease.[24,25,28]

Although the etiology of scoliosis is still unknown, extrinsic and intrinsic factors can be associated with the disease.[7] Genetic factors significantly influence the determination of clinical, anatomical, and biomechanical characteristics of scoliosis.[22,29] The association of *PAX-1* SNPs with the development of AIS has been described recently in a meta-analysis involving GWAS studies that evaluated 7956 cases of scoliosis and 88,459 controls.[15] Other previous GWAS work had already identified AIS susceptibility within putative enhancers of the PAX1.[9] PAX1 is required for adequate formation of vertebral bodies and can regulate the transformation of the notochord in the intervertebral disc.[30]

The rs6137473 (*G* > *A*) SNP, located downstream of the *PAX-1* gene, was also associated with an increased risk of developing AIS in the Japanese, American,[9] Chinese population[10,16] and now in the Brazilian population. A higher frequency of the *PAX-1* rs6137473 *GG* genotype was observed in cases of AIS and in the group of patients with lumbar modifier B and C, conferring an increased risk of developing the disease and its severity. The risk of severe scoliosis was previously observed in a northern Chinese Han population,[16] using the Peking Union Medical College (PUMC) classification, which stratifies into three phenotypic groups.[31] This study was the first to associate SNPs in *PAX-1* gene with the curve-type phenotype,[16] however the PUMC classification is little used in clinical practice.[31,32] It has been observed that the presence of *PAX-1* rs6137473 SNP did not influence the difference in *PAX-1* expression between the genotypic groups (GG, GA, and AA), after analysis of mRNA extracted from bilateral paravertebral muscles of 84 patients with AIS.[10]

The *PAX-1* rs169311 (*A* > *C*) SNP is within an active enhancer site and may affect the binding of transcription factors and the expression of the *PAX-1* gene.[9,10,21] Individuals with the *PAX-1* rs169311 *AA* genotype showed a significant reduction in *PAX-1* expression compared to the rs169311 *CC* genotype.[10] The *PAX-1* rs169311 SNP was already associated with an increased risk for the development of idiopathic scoliosis in Chinese women, after analyzing 2914 patients with scoliosis with a curve magnitude variation of 21.2°–62.7° of the Cobb angle.[10] In the present study, the presence of the *PAX-1* rs169311 *A* allele conferred a risk for the development of AIS, in accordance with what has already been observed in the Chinese population.[10]

Despite the ancestral diversity, these results replicated the association observed in homogeneous populations, which supports the hypothesis that SNPs in *PAX-1* gene may serve as a biomarker to track the risk of development and the severity of AIS. It is becoming increasingly important to build a database to better understand and identify risk factors

associated with AIS development and progression. Thus, future studies with a larger number of patients, including less severe curves, are needed to validate the influence of SNPs in *PAX-1* gene on disease progression.

CONCLUSIONS

PAX-1 SNPs (rs169311 and rs6137473) were associated with increased risk of developing AIS and curve severity. This study may contribute to the evaluation of the influence of the genetic component involved in the development and severity of the disease, to elucidate possible etiological mechanisms, and to suggest policies for the prevention, diagnosis, and treatment of AIS.

Acknowledgment

The authors thank for the technical support of the INTO scientific initiation students from the Research Laboratory of Pharmaceutical Sciences (https://lapesfuezo.wixsite.com/website) and staff of the Research Division from the National Institute of Traumatology and Orthopaedics (INTO). This work was supported by the Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro – FAPERJ, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES and Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq.

Financial support and sponsorship

This study was financially supported by the Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro – FAPERJ, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES and Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Negrini S, Donzelli S, Aulisa AG, Czaprowski D, Schreiber S, de Mauroy JC, *et al.* 2016 SOSORT guidelines: Orthopaedic and rehabilitation treatment of idiopathic scoliosis during growth. Scoliosis Spinal Disord 2018;13:3.
2. Hresko MT. Clinical practice. Idiopathic scoliosis in adolescents. N Engl J Med 2013;368:834-41.
3. El-Hawary R, Chukwunyerenwa C. Update on evaluation and treatment of scoliosis. Pediatr Clin North Am 2014;61:1223-41.
4. Konieczny MR, Senyurt H, Krauspe R. Epidemiology of adolescent idiopathic scoliosis. J Child Orthop 2013;7:3-9.
5. Slattery C, Verma K. Classifications in brief: The Lenke classification for adolescent idiopathic scoliosis. Clin Orthop Relat Res 2018;476:2271-6.
6. Lenke LG, Betz RR, Clements D, Merola A, Haher T, Lowe T, *et al.* Curve prevalence of a new classification of operative adolescent idiopathic scoliosis: Does classification correlate with treatment? Spine (Phila Pa 1976) 2002;27:604-11.
7. Burwell RG, Clark EM, Dangerfield PH, Moulton A. Adolescent idiopathic scoliosis (AIS): A multifactorial cascade concept for pathogenesis and embryonic origin. Scoliosis Spinal Disord 2016;11:8.
8. Karner CM, Long F, Solnica-Krezel L, Monk KR, Gray RS. Gpr126/Adgrg6 deletion in cartilage models idiopathic scoliosis and pectus excavatum in mice. Hum Mol Genet 2015;24:4365-73.
9. Sharma S, Londono D, Eckalbar WL, Gao X, Zhang D, Mauldin K, *et al.* A PAX1 enhancer locus is associated with susceptibility to idiopathic scoliosis in females. Nat Commun 2015;6:6452.
10. Xu L, Sheng F, Xia C, Qin X, Tang NL, Qiu Y, *et al.* Genetic variant of PAX1 gene is functionally associated with adolescent idiopathic scoliosis in the Chinese population. Spine (Phila Pa 1976) 2018;43:492-6.
11. Xu L, Wu Z, Xia C, Tang N, Cheng JC, Qiu Y, *et al.* A genetic predictive model estimating the risk of developing adolescent idiopathic scoliosis. Curr Genomics 2019;20:246-51.
12. Wilm B, Dahl E, Peters H, Balling R, Imai K. Targeted disruption of Pax1 defines its null phenotype and proves haploinsufficiency. Proc Natl Acad Sci U S A 1998;95:8692-7.
13. Giampietro PF, Raggio CL, Reynolds CE, Shukla SK, McPherson E, Ghebranious N, *et al.* An analysis of PAX1 in the development of vertebral malformations. Clin Genet 2005;68:448-53.
14. Underhill DA. Genetic and biochemical diversity in the Pax gene family. Biochem Cell Biol 2000;78:629-38.
15. Khanshour AM, Kou I, Fan Y, Einarsdottir E, Makki N, Kidane YH, *et al.* Genome-wide meta-analysis and replication studies in multiple ethnicities identify novel adolescent idiopathic scoliosis susceptibility loci. Hum Mol Genet 2018;27:3986-98.
16. Liu G, Liu S, Li X, Chen J, Chen W, Zuo Y, *et al.* Genetic polymorphisms of PAX1 are functionally associated with different PUMC types of adolescent idiopathic scoliosis in a northern Chinese Han population. Gene 2019;688:215-20.
17. Lenke LG, Betz RR, Bridwell KH, Clements DH, Harms J, Lowe TG, *et al.* Intraobserver and interobserver reliability of the classification of thoracic adolescent idiopathic scoliosis. J Bone Joint Surg Am 1998;80:1097-106.
18. Lenke LG, Betz RR, Harms J, Bridwell KH, Clements DH, Lowe TG, *et al.* Adolescent idiopathic scoliosis: A new classification to determine extent of spinal arthrodesis. J Bone Joint Surg Am 2001;83:1169-81.
19. Perini JA, Lopes LR, Guimarães JA, Goes RA, Pereira LF, Pereira CG, *et al*. Influence of type I collagen polymorphisms and risk of anterior cruciate ligament rupture in athletes: A case-control study. BMC Musculoskelet Disord 2022;23:154.
20. Pena SD, Di Pietro G, Fuchshuber-Moraes M, Genro JP, Hutz MH, Kehdy Fde S, *et al.* The genomic ancestry of individuals from different geographical regions of Brazil is more uniform than expected. PLoS One 2011;6:e17063.
21. Ward K, Ogilvie J, Argyle V, Nelson L, Meade M, Braun J, *et al.* Polygenic inheritance of adolescent idiopathic scoliosis: A study of extended families in Utah. Am J Med Genet A 2010;152A: 1178-88.
22. Grauers A, Einarsdottir E, Gerdhem P. Genetics and pathogenesis of idiopathic scoliosis. Scoliosis Spinal Disord 2016;11:45.
23. Raggio CL. Sexual dimorphism in adolescent idiopathic scoliosis. Orthop Clin North Am 2006;37:555-8.
24. Tarrant RC, Queally JM, Moore DP, Kiely PJ. Prevalence and impact of low body mass index on outcomes in patients with adolescent idiopathic scoliosis: A systematic review. Eur J Clin Nutr 2018;72:1463-84.
25. Goodbody CM, Asztalos IB, Sankar WN, Flynn JM. It's not just the big kids: Both high and low BMI impact bracing success for adolescent idiopathic scoliosis. J Child Orthop 2016;10:395-404.
26. Karol LA, Johnston CE 2nd, Browne RH, Madison M. Progression of the curve in boys who have idiopathic scoliosis. J Bone Joint Surg Am 1993;75:1804-10.
27. Lenke LG, Dobbs MB. "Idiopathic scoliosis." In: The Adult and Pediatric Spine. 3rd ed., Vol. 1. Philadelphia: Lippincott, Williams & Wilkins;

2004. p. 337-60.
28. Miyagi M, Saito W, Imura T, Nakazawa T, Shirasawa E, Kawakubo A, *et al.* Body composition in Japanese girls with adolescent idiopathic scoliosis. Spine Surg Relat Res 2021;5:68-74.
29. Kikanloo SR, Tarpada SP, Cho W. Etiology of adolescent idiopathic scoliosis: A literature review. Asian Spine J 2019;13:519-26.
30. Wallin J, Wilting J, Koseki H, Fritsch R, Christ B, Balling R. The role of Pax-1 in axial skeleton development. Development 1994;120:1109-21.
31. Qiu G, Li Q, Wang Y, Yu B, Qian J, Yu K, *et al.* Comparison of reliability between the PUMC and Lenke classification systems for classifying adolescent idiopathic scoliosis. Spine (Phila Pa 1976) 2008;33:E836-42.
32. Lenke LG. Lenke classification system of adolescent idiopathic scoliosis: Treatment recommendations. Instr Course Lect 2005;54:537-42.