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Positive association between a polymorphic locus near the *LBX1* gene and predisposition of idiopathic scoliosis in Southeastern European population

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Abstract
Idiopathic scoliosis (IS) is a common medical condition in children, characterized by three-dimensional spinal curve and strong evidence of genetic predisposition. The purpose of the present case-control study is to examine the association between the polymorphic variant rs11190870 (T/C), near the *LBX1* gene, and IS predisposition in distinct subgroups based on age at onset, family history and gender. A total of 127 IS patients and 254 unrelated controls of Southeastern European descent were recruited. The genotyping was carried out by TaqMan real-time amplification technology. The results were analyzed by the Pearson's Chi-squared Test and the Fisher's Exact Test with a value of p less than 0.05 as statistically significant. The T allele and homozygous TT genotype were associated with a greater incidence of IS. Our results suggest that there is a genetic association with IS in adolescents, familial and non-familial cases, and in females. Larger case-control studies are necessary to examine the genetic factors of IS/AIS etiology in infants, juveniles and males. In conclusion, the molecular genetic identification of diagnostic and prognostic molecular markers would make an early treatment including minimally invasive procedures possible.

Keywords: Association; Idiopathic scoliosis; *LBX1* gene; Predisposition

Highlights:
- There is a genetic association with IS in adolescents, familial and non-familial cases, and in females from Southeastern European population.
- The molecular genetic identification of diagnostic and prognostic molecular markers is a contemporary approach that could permit early treatment.
- The diagnostic and prognostic molecular markers would make minimally invasive procedures possible.

Introduction

Idiopathic scoliosis (IS) is a common medical condition in children, characterized by three-dimensional spinal curve and strong evidence of genetic predisposition (Nikolova et al., 2016, 2018; Raggio et al., 2009). There is a hypothesis that IS could be a consequence of moderate or large number of common genetic variants with modest to moderate individual effect probably reflecting multiple gene-gene interactions, differential regulatory mechanisms and significant environmental influences on the growing spine (Gorman et al., 2014). The main goal of a genome-wide association study (GWAS) is detection of statistically significant associations between common diseases and common genetic variants, mostly single nucleotide polymorphisms (SNPs), in population-based samples. During the period 2011–2017, two whole-genome studies have been reported (Chettier et al., 2015; Takahashi et al., 2011) followed by several replication studies confirming an association between an intergenic variant, rs11190870, near the ladybird homeobox 1 (*LBX1*) gene and the higher risk of development of adolescent idiopathic scoliosis (AIS) in Asian and Caucasian population groups. In 2011, Takahashi et al. 2011 reported a positive association in Japanese population (1050 cases/1474 controls; validation cohort: 326 cases/9823 controls) between AIS susceptibility and common genetic variants around the *LBX1* gene. The most significant association was that between SNP rs11190870 (p = 1.24 × 10^{-19}; OR =

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1.56) and AIS. During the period 2012–2017, the authors of four replication studies in Chinese subpopulations confirmed the association of rs11190870 with AIS predisposition (Fan et al., 2012; Gao et al., 2013; Jiang et al., 2013; Liu et al., 2017) and AIS progression (Jiang et al., 2013). In 2015, a new GWAS (Chettier et al., 2015) in a population sample of European descent (initial cohort: 906 cases/1,480 controls; final population: 620 cases/1287 controls) found the most significant association of AIS patients with rs11190870, downstream of the *LBX1* gene ($p = 5.43 \times 10^{-9}$) and rs11190878, upstream of the *LBX1* gene ($p = 4.18 \times 10^{-9}$). The results from a whole-exome sequencing study (Grauers et al., 2015) in a Scandinavian cohort (1,739 cases/1,812 controls) indicated that the genetic variant rs11190870, downstream of the *LBX1* gene, is significantly associated with AIS occurrence ($p = 7.0 \times 10^{-18}$). A meta-analysis of the *LBX1* locus (10q24.31) from the total number of case-control studies including 34,626 subjects (10,088 cases and 24,538 controls) provides significant evidence for association of this locus with AIS susceptibility in all Asian and Caucasian cohorts. The results show that the T allele of rs111090870 increases AIS susceptibility in Asians (T vs. C, OR = 1.22, 95% CI: 1.16–1.29, $p < 0.001$), Caucasians (T vs. C, OR = 1.17, 95% CI: 1.14–1.21, $p < 0.001$) and in females (T vs. C, OR = 1.21, 95% CI: 1.17–1.25, $p < 0.001$) (Cao et al., 2016).

The outcome measure of all the above studies was the most common form of scoliosis – AIS. At the same time, the available studies on specific forms of IS based on age at onset, family history of the patients and etc. are quite insufficient in the references. Different genetic factors may be involved in the etiopathogenesis of early and late onset scoliosis, progressive and non-progressive forms, familial and sporadic cases, and male and female scoliosis. Thus, additional research data are necessary to obtain in order to clarify their possible contribution. The purpose of the present case-control study is to examine: (i) the association between the polymorphic variant rs11190870 (T/C), near the *LBX1* gene, and IS predisposition in Southeastern European population and (ii) the genotype-phenotype correlations in distinct subgroups based on age at onset, family history and gender.

Materials and methods

Patients and controls

A total of 127 IS patients and 254 unrelated controls of Southeastern European descent were recruited. Informed consent was obtained from all individual participants included in the study. The clinical diagnosis included detailed anamnesis, physical examination and radiographic studies of the spine in patients. Any spinal curve greater than 10° is considered to be structural scoliosis (Nikolova et al., 2018). The range of Cobb angles for 127 cases was 12–125. The mean Cobb angle on standing radiographs was 53.8° (*SD* 21.2). Secondary scoliosis (congenital, neuromuscular, syndrome-related etc.) was excluded. Anamnesis, physical examination and previous available spinal roentgenographies excluded mild scoliosis among the control subjects. Mild scoliosis has been defined as a curve between 10° and 20° that could hardly be diagnosed without radiographic methods. For the aim of the study it was gathered important information concerning the onset of the disease. The patients were divided into three subgroups: infantile IS – up to 3 years of age ($n = 4$), juvenile IS – from 4 to 10 years of age ($n = 26$), and adolescent IS – from greater than 10 to 18 years of age ($n = 97$) according to the classification of Scoliosis Research Society. The mean age of IS onset was 11.2 years (*SD* 2.9). All the controls were skeletally matured individuals over 18 years of age. Each patient group was compared to the total number of controls. The patients were divided into two subgroups by gender – females ($n = 102$) and males ($n = 25$) and then were compared to twice as many gender-matched controls. Finally, familial ($n = 34$) and non-familial ($n = 93$) cases were compared to controls without family history of IS ($n = 254$).

DNA analysis

Automated magnetic bead-based extraction of genomic desoxy-ribonucleic acid (DNA) from peripheral venous blood samples by a chemagic DNA Blood 10k Kit (PerkinElmer, Baesweiler, Germany) using a chemagic Magnetic Separation Module I (PerkinElmer, Baesweiler, Germany) according to the manufacturer's instructions was performed. Real-time amplification by a TaqMan SNP genotyping assay available under a catalogue No C_1349874_20 (Thermo Fisher Scientific, USA) on an ABI Prism 7900HT sequence detection system (Thermo Fisher Scientific, USA) according to the manufacturer's recommendations was used for rs11190870 (T/C) genotyping. All samples were run in duplicate in a reaction mix of 5 µl containing 2 µl DNA (10 ng/µl), 2.5 µl 2× TaqMan Genotyping Master Mix (Thermo Fisher Scientific, USA), 0.125 µl 40× TaqMan Genotyping assay (Thermo Fisher Scientific, USA), and 0.375 µl dH_2O. TaqMan real-time polymerase chain reaction (PCR) was performed with an initial denaturation of 10 minutes at 95 °C. The following thermal cycle was repeated 40 times: denaturation at 95 °C for 15 seconds, annealing at 60 °C for 60 seconds, and extension at 72 °C for 15 seconds. The results were analyzed by the Pearson's Chi-squared Test for the general sample ($N = 381$) and the larger subgroups ($N > 300$) or the Fisher's Exact Test for the smaller subgroups ($N < 300$) with a value of p less than 0.05 as statistically significant. Odds ratios (OR) and risk ratios (RR) with 95% confidence interval (CI) were also calculated (IBM SPSS 19.0, NY, USA).

Results

Mutational analysis

The genotype and allele frequencies of rs11190870 (T/C) were analyzed in the general group and the distinct subgroups based on the age at onset, family history and gender of the patients. We examined the possible genetic predisposition using the following inheritance models: codominant (TT vs. CC), dominant (TT + TC vs. CC), recessive (TT vs. TC + CC) and allelic (T vs. C). The genotypes were in Hardy-Weinberg equilibrium. In the total sample, the frequencies of homozygous TT genotype and wild-type T allele of rs11190870 (T/C) were higher in the patients compared to the controls (TT vs. TC vs. CC, $p = 0.0038$, $\chi^2 = 11.15$ and T vs. C, $p = 0.0004$, $\chi^2 = 12.35$, respectively). The homozygous TT genotype was associated with a 1.36 times higher risk (TT vs. TC + CC, RR = 1.36, 95% CI: 1.13–1.63), and the T allele – with a 1.18 times higher risk (T vs. C, RR = 1.18, 95% CI: 1.08–1.29) for development of IS in Southeastern European patients. This suggests that the TT genotype should be considered as a risk factor with a modest individual impact (35% greater risk of IS) as a part of the genetic predisposition to disease in Southeastern European population.

Genetic models

In the subgroups of: (1) AIS; (2) familial IS; (3) sporadic IS, and (4) females with IS, the observed genotype and allele frequencies of rs11190870 (T/C) also differed considerably between

cases and controls ($p < 0.05$, χ^2-test). The highest RR value was recorded in the subgroup of familial IS (TT vs. TC + CC, RR = 1.48, 95% CI: 1.15–1.91). No statistically significant association was detected in the smaller subgroups of: (1) males and (2) juvenile IS ($p > 0.05$, Fisher's Exact Test). The four cases of infantile IS were excluded. By enrolling too few subjects, a study will not have enough statistical power to detect a difference (type II error). Larger sample surveys will be needed to detect a potential association with the disease in men and children under 10 years of age. Genotype and allele distributions are presented in Table 1.

Table 1. Genotype and allele distribution in different subgroups of IS

Group		Genotypes, *N*			Alleles, *N*	
		TT	TC	CC	T	C
General ($n_1 = 127$, $n_2 = 254$)	Cases	82	38	7	202	52
	Controls	121	100	33	342	166
AIS ($n_1 = 97$, $n_2 = 254$)	Cases	61	30	6	152	42
	Controls	121	100	33	342	166
JIS ($n_1 = 26$, $n_2 = 254$)	Cases	17	8	1	42	10
	Controls	121	100	33	342	166
Familial IS ($n_1 = 34$, $n_2 = 254$)	Cases	24	9	1	57	11
	Controls	121	100	33	342	166
Sporadic IS ($n_1 = 93$, $n_2 = 254$)	Cases	58	29	6	145	41
	Controls	121	100	33	342	166
Males ($n_1 = 25$, $n_2 = 50$)	Cases	16	8	1	40	10
	Controls	24	20	6	68	32
Females ($n_1 = 102$, $n_2 = 204$)	Cases	66	31	5	163	41
		97	80	27	274	134

IS, idiopathic scoliosis; AIS, adolescent idiopathic scoliosis; JIS, juvenile idiopathic scoliosis; *N*, number of genotypes or alleles; n_1, number of cases; n_2, number of controls.

P-values and ORs of the genotypes and alleles under the examined inheritance models are summarized in Table 2. These results show that the recessive model, as having the lowest *p*-value, best explains the inheritance pattern of the risk TT genotype. The genetic model in four subgroups is presented graphically at Fig. 1.

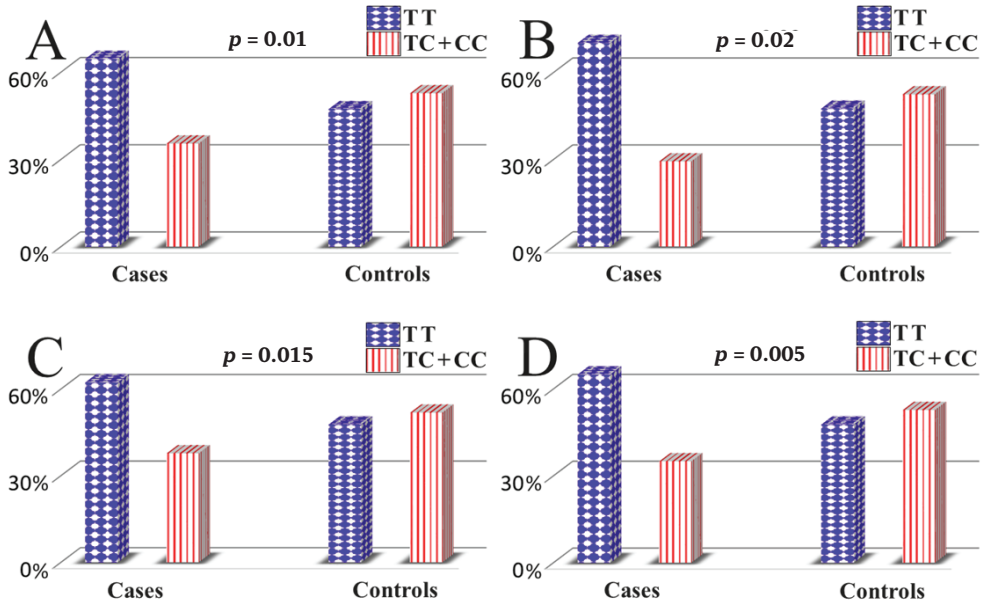

Fig. 1. Recessive genetic model TT / (TC + CC) in (**A**) AIS; (**B**) Familial IS; (**C**) Non-familial IS, and (**D**) Females with IS.

Table 2. Inheritance models in different subgroups of IS

Group	Genetic model	*p*-value	OR [95% CI]
General (n_1 = 127, n_2 = 254)	Codominant	0.0060	3.19 [1.35–7.57]
	Recessive	0.0018	2.00 [1.29–3.11]
	Dominant	0.0248	0.39 [0.17–0.91]
	Allelic	0.0004	1.89 [1.32–2.69]
AIS (n_1 = 97, n_2 = 254)	Codominant	0.0253	2.77 [1.10–6.98]
	Recessive	0.0105	1.86 [1.15–3.01]
	Dominant	0.0697	0.44 [0.18–1.09]
	Allelic	0.0042	1.76 [1.19–2.59]
JIS (n_1 = 26, n_2 = 254)	Codominant	0.1299	4.64 [0.59–36.1]
	Recessive	0.1008	2.08 [0.89–4.83]
	Dominant	0.2224	0.27 [0.04–2.04]
	Allelic	0.0588	2.04 [1.00–4.16]
Familial IS (n_1 = 34, n_2 = 254)	Codominant	0.0512	6.55 [0.85–50.2]
	Recessive	0.0167	2.64 [1.21–5.74]
	Dominant	0.0967	0.20 [0.03–1.53]
	Allelic	0.0074	2.52 [1.29–4.92]
Sporadic IS (n_1 = 93, n_2 = 254)	Codominant	0.0345	2.64 [1.05–6.65]
	Recessive	0.0151	1.82 [1.12–2.96]
	Dominant	0.0875	0.46 [0.19–1.14]
	Allelic	0.0067	1.72 [1.16–2.54]
Males (n_1 = 25, n_2 = 50)	Codominant	0.2419	4.00 [0.44–36.4]
	Recessive	0.2257	1.93 [0.72–5.17]
	Dominant	0.4129	0.31 [0.03–2.69]
	Allelic	0.1764	1.88 [0.84–4.23]
Females (n_1 = 102, n_2 = 204)	Codominant	0.0075	3.67 [1.35–10.0]
	Recessive	0.0046	2.02 [1.24–3.30]
	Dominant	0.0248	0.34 [0.13–0.91]
	Allelic	0.0010	1.94 [1.30–2.90]

IS, idiopathic scoliosis; AIS, adolescent idiopathic scoliosis; JIS, juvenile idiopathic scoliosis; *p*, probability value; OR, odds ratio; CI, confidence interval; n_1, number of cases; n_2, number of controls.

Discussion

The genotype and allele frequencies of rs11190870 (T/C), near the *LBX1* gene, associated with AIS from two previous GWAS in Asian and Caucasian population groups (Chettier et al., 2015; Takahashi et al., 2011), was compared between IS patients and controls of Southeastern European descent (case-control study). In the general group, the T allele and homozygous TT genotype were associated with a greater incidence of IS and can be considered as a low-impact risk allele and genotype, respectively, in Southeastern European population (RR < 1.5). The outcome measure of the most association studies (candidate-gene or GWA studies) is the commonest form of scoliosis – AIS (Chettier et al., 2015; Fan et al., 2012; Gao et al., 2013; Jiang et al., 2013; Liu et al., 2017; Takahashi et al., 2011). At the same time, available research on the early onset scoliosis (before 5 years of age) is insufficient to establish genotype-phenotype correlations (Grauers et al., 2015). Grauers et al. enrolled patients over 4 years of age (including 213 cases of juvenile IS) and found a statistically significant difference in the T allele distribution between cases and controls. However, the threshold of p-value less than 10^{-7}–10^{-8} is required in order to avoid false significant associations in the context of whole genome study design. We included patients between 1 and 15 years of age at the onset of primary scoliosis. In the AIS subgroup, the results were similar to those in the total sample but due to the low incidence of early onset scoliosis, we have not detected a statistically significant association in the other age subgroups (see Table 2). Thus, the previously reported genetic association between rs11190870 and AIS predisposition in a population sample of European descent (Grauers et al., 2015) was observed in Southeastern European patients. Larger replication studies will be needed to detect the potential association with the early onset IS, suggested by the overall results. Both in the familial and non-familial subgroup, the presence of T allele and TT genotype was associated with IS (see Table 2). No family history data from previous studies is available. The separation of cases according to this type of data is necessary because of the possible differences in genetic

predisposition to familial and non-familial forms of a specific complex disease (Donaldson et al., 2016).

IS occurs more frequently in females than in males (Cilli et al., 2009; Daruwalla et al., 1985; Kamtsiuris et al., 2007; Nery et al., 2010) and some studies include only female subjects (Chettier et al., 2015; Takahashi et al., 2011). A meta-analysis based on 34,626 subjects found significant increase of AIS susceptibility in females (Cao et al., 2016). In the current subgroup analysis stratified by gender, significant increasing IS susceptibility was found for females in the recessive genetic model and allele contrast model, but no significant association between rs111090870 and IS risk was found in the smaller male subgroup (see Table 2). On the basis of the current results, rs11190870 can be regarded as an independent predisposing factor to IS and AIS with a modest effect on IS/AIS susceptibility in Southeastern European patients. Our results suggest that there is genetic predisposition to IS in familial and non-familial cases, and in females but larger case-control studies will be needed to examine the genetic factors of IS etiology in infantiles, juveniles and males.

Variant alleles which occur with a higher frequency in patients than controls, possibly mark genomic regions containing genetic correlates with a certain complex disease (Guo et al., 2006). The *LBX1* candidate-gene is expressed in the central nervous system (dorsal spinal cord and hindbrain) and skeletal muscles (Jiang et al., 2013). It is important for the development of inhibitory interneurons in the posterior horns, and for the migration and further differentiation of muscle cell precursors (Cheng et al., 2005; Gross et al., 2002; Huang et al., 2008; Jagla et al., 1995; Müller et al., 2002; Sieber et al., 2007). In knock-out mice with an inactivated gene (*LbX1* – / –), the morphology of neurons and neural circuits in the dorsal horns are aberrant, suggesting that the *LBX1* protein is a critical factor for the development of sensory pathways in the spinal cord (Müller et al., 2002). Studies on dorsal rhizotomized animals show a higher incidence of scoliosis and assume the role of somatosensory dysfunction in the etiopathogenesis of scoliosis (Barrios et al., 1987; Suk et al., 1989). In clinical studies, it was found that growing children with functional or organic disorders of the somatosensory pathways are more prone to scoliosis compared with their peers (Kouwenhoven and Castelein, 2008). In AIS patients, the frequency of somatosensory disorders is greater than that in general population. It is possible that differential *LBX1* gene expression contributes to IS development as a part of more complex genetic predisposition.

Conclusions

As a conclusion, the osteogenetics is a perspective field that involves the study of genetic changes in locomotory diseases. The current study revealed a genetic association with IS/AIS predisposition in adolescents, familial and non-familial cases, and in females from Southeastern European population. Larger case-control studies are necessary to examine the genetic factors of IS/AIS etiology in infants, juveniles and males. Molecular genetic identification of diagnostic and prognostic molecular markers is a contemporary approach that would make an early treatment including minimally invasive procedures possible.

Conflict of interests

The authors have no conflict of interests to disclose. All patients and parents provided informed consent.

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