Eur Spine J (2013) 22:282–286
DOI 10.1007/s00586-012-2532-4

CHINESE SECTION

Association of rs11190870 near *LBX1* with adolescent idiopathic scoliosis susceptibility in a Han Chinese population

Hua Jiang · Xusheng Qiu · Jin Dai · Huang Yan · Zezhang Zhu · Bangping Qian · Yong Qiu

Received: 27 June 2012 / Accepted: 2 October 2012 / Published online: 21 October 2012

Abstract
Purpose To investigate whether rs11190870 near *LBX1* correlates with the susceptibility or curve progression of adolescent idiopathic scoliosis (AIS) in a Han Chinese population.
Methods A total of 949 AIS patients and 976 age-matched healthy controls were recruited. All the subjects were genotyped using the PCR-based invader assay. Case–control study and case-only study were performed to define the contribution of rs11190870 to predisposition and curve severity of AIS. Additionally, we further conducted a meta-analysis of the study findings together with those of previously reported studies.
Results A significant association of rs11190870 with AIS was observed in the Han Chinese population ($P = 1.8 \times 10^{-9}$; odd ratio = 1.51; 95 % confidence interval = 1.33–1.71), and AIS patients with TT genotype had a larger Cobb angle than those with TC or CC genotype ($P = 0.005$). The meta-analysis confirmed that the positive association of this SNP with AIS in the East Asian population.
Conclusions The SNP rs11190870 near *LBX1* is associated with both susceptibility and curve progression of AIS.

Keywords Adolescent idiopathic scoliosis · Han Chinese · rs11190870 · Genetic · Etiology

Introduction

Adolescent idiopathic scoliosis (AIS) is the most common structural spine deformity, with a prevalence of 2–4 % in adolescent, leading to functional disabilities and significant cosmetic problems [1–3]. Despite decades of researches, the specific cause of AIS has not been determined [4]. Many hypotheses have been proposed, including genetic factors, nervous system, skeletal growth, hormones and metabolic dysfunction [5]. It is widely accepted that genetic factors play an important role in the development of AIS [6, 7]. Previous studies have indicated that AIS may be a complex genetic disorder resulting from one or more genetic loci with complex genetic-environment interactions [8–13].

Genome-wide association study (GWAS) is the most robust approach to identify predisposition genes for common diseases and complex traits [14]. Hence, the development of GWAS provides a more comprehensive picture on the possible genes involved in AIS. Recently, Takahashi et al. [15] used a GWAS to compare 1,376 Japanese female AIS patients with 11,297 female controls, and detected a strong association between AIS and a single nucleotide polymorphism (SNP) rs11190870 located at the 3′-flanking region of the *LBX1* (ladybird homeobox 1) gene on chromosome 10q24.31. Their association reached genome-wide significance ($P = 1.24 \times 10^{-19}$). The replication study in Hong Kong showed the association between AIS and rs11190870 ($P = 9.1 \times 10^{-10}$) [16]. However, this evidence is limited by its small sample size and little information on the association of rs11190870 with curve

H. Jiang, X. Qiu and J. Dai contributed equally to this work.

H. Jiang · X. Qiu · H. Yan · Z. Zhu · B. Qian · Y. Qiu (✉)
Department of Spine Surgery, The Affiliated Drum Tower Hospital of Nanjing University Medical School, Zhongshan Road No 321, 210008 Nanjing, China
e-mail: scoliosis2002@sina.com

J. Dai
Department of Orthopedic Surgery, The Affiliated Drum Tower Hospital of Nanjing University Medical School, Zhongshan Road No 321, 210008 Nanjing, China

severity. Furthermore, Hong Kong Chinese may not be considered equal to Han Chinese of mainland China. Given the history of British colonization and European immigration, Hong Kong Chinese may have more complex genetic background than Han Chinese of mainland China.

To confirm the association between this SNP and AIS, we performed a validation study for the association of rs11190870 with AIS in Han Chinese population residing in Yangtze River region of mainland China. Case–control study and case-only study were conducted to define the contribution of rs11190870 to predisposition and disease severity of AIS.

Materials and methods

Subjects

A total of 1925 subjects were studied. 949 AIS patients (820 girls and 129 boys) with Cobb angle above 20° were recruited from the Spine Surgery, The Affiliated Drum Tower Hospital of Nanjing University Medical School between May 2005 and August 2011. Curve severity of scoliosis was measured by the Cobb method on the posteroanterior radiograph images of the whole spine. Diagnosis of scoliosis was made for initial Cobb angle $\geq$10 °. Secondary scoliosis with known etiology was ruled out from the study, including congenital scoliosis, neuromuscular scoliosis, or scoliosis with connective tissue abnormalities. 976 normal control subjects (662 females and 314 males) were age-matched adolescent enrolled from local secondary schools. The control subjects were clinically examined by experienced orthopedic surgeons to rule out any potential spinal deformity. All participants were Han Chinese who lived in and around the Yangtze River region.

The effect of rs11190870 on the curve severity of AIS was determined by a case-only analysis. Since skeletal maturity and bracing can affect natural history of AIS, the patients included in the case-only analysis should be met the following criteria to ensure the intact natural history of AIS: (1) no history of bracing or any other conservative treatment; (2) growth maturation (age >16 years or Risser 5) [17]. Informed consent to DNA analysis was obtained from all subjects and/or their parents. Ethical approval was obtained from the Nanjing University and Drum Tower Hospital Research Ethics Committee.

Genotyping

Genomic DNA was extracted from peripheral blood leukocytes using genomic DNA isolation kits (Promega, Madison, WI) according to the manufacturer's instructions. The primers, probes and reaction conditions were available upon request. SNPs were genotyped by the PCR-based invader assay (Third Wave Technologies) using ABI 7900 (Applied Biosystems, Foster City, CA, WI) [18]. Genotyping was done by laboratory personnel blinded to subject status. Of the samples, 10 % were tested twice to validate the genotyping results with 100 % reproducibility. Two authors independently reviewed the genotyping results, data entry, and statistical analysis.

Statistical analysis

Standard χ^2 analysis of contingency tables were used to examine differences of allelic frequencies and genotype distributions between AIS patients and controls. Hardy–Weinberg equilibrium was tested by a goodness-of-fit χ^2 test. Odds ratio (OR) and 95 % confidence interval (CI) were calculated using the reported risk allele (T allele) as a reference. One-way ANOVA test was used in the comparison of Cobb angles with different genotypes in the case-only analysis. Meta-analysis was performed with STATA version 11.0 (Stata Corporation, College Station, TX). Statistically significance was considered at $P < 0.05$.

Results

A total of 1925 subjects (949 cases and 976 controls) were successfully genotyped and subjected to statistical analysis. The distributions of the alleles and genotypes for rs11190870 were presented in Table 1. Genotype frequencies of AIS group and the control group were conformed to the Hardy–Weinberg equilibrium ($P = 0.60$ and 0.61, respectively).

Case–control association study

In accordance with the GWAS and replication study reported previously [15, 16], the risk allele (T allele) lead to a higher risk for AIS in the Han Chinese population (Table 1). T allele frequency of rs11190870 was significantly different between total cases and total controls ($P = 1.8 \times 10^{-9}$) as well as female cases and female controls ($P = 2.1 \times 10^{-13}$).

Case-only association study

A subgroup of 314 skeletally mature AIS patients receiving no bracing or any other conservative treatment previously were analyzed to define the contribution of rs11190870 on curve severity. In general, AIS patients with TT genotype showed significantly higher Cobb angle than the patients with TC and CC genotype ($P = 0.005$, Table 2). Female AIS patients ($n = 258$) were isolated alone for one-way

Table 1 Allele and genotype frequencies of rs11190870 in adolescent idiopathic scoliosis in a Han Chinese population

	Genotype (%)			*P*	Allele (%)		*P**	OR (95 % CI)
	TT	TC	CC		T	C		
Total cases	335 (35.3)	464 (48.9)	150 (15.8)		1134 (59.7)	764 (40.3)		
Total controls	236 (24.2)	496 (50.8)	244 (25.0)	<0.001	968 (49.6)	984 (50.4)	1.8×10^{-9}	1.51 (1.33–1.71)
Female cases	307 (37.4)	399 (48.7)	114 (13.9)		1013 (61.8)	627 (38.2)		
Female controls	156 (23.6)	321 (48.5)	185 (27.9)	<0.001	633 (47.8)	691 (52.2)	2.1×10^{-13}	1.76 (1.52–2.04)

OR odds ratio, *CI* confidence interval

* The Cochran–Armitage test

ANOVA test, validating a significantly higher Cobb angle in TT genotype compared with TC and CC genotype ($P = 0.012$, Table 2).

Meta-analysis

We performed meta-analysis between the previous studies [15, 16] and our study. The results showed a significant association between rs11190870 and AIS in East Asian populations. (all $P < 0.001$, Table 3).

Discussion

GWAS is a powerful method for the detecting genetic contributions to polygenic diseases, and has been increasingly used to study genetic predisposition in AIS that is regarded as one of the most common complex genetic disorders of the musculoskeletal system [19]. However, this method may produce spurious association [20, 21]. Therefore, replications of the associations in different ethnic groups and studies with large sample sizes are important to confirm the results of GWAS [22].

Recently, a GWAS has identified a positive association of a SNP rs11190870 near *LBX1* with AIS susceptibility in Japanese [15]. This association has been replicated in the Hong Kong Chinese population [23]. However, Hong Kong Chinese is regarded as a special ethnic group in China. Hong Kong is located to the extreme south of China. Historically, the inhabitants of Guangdong, ancestor of Hong Kong Chinese, have remained rather separate from other parts of China, being spared significant traffic from the north and west by mountain ranges and rivers [24, 25]. Because of the British Colony since the middle of the 19th century, Hong Kong has a long history of European immigration and a high degree of ethnic admixture. As a result, Hong Kong Chinese would be more genetically heterogeneous than Han Chinese of mainland China. Furthermore, a relatively small sample was recruited in the Hong Kong's study, which may be limited to distinguish false-positives from real associations and conduct a subgroup analysis. Hence, validation of the associations in a large sample size of Han Chinese AIS patients was necessary.

Table 2 Association between curve severity and rs11190870 in adolescent idiopathic scoliosis

Population	Genotype	Number	Maximum Cobb angle [mean ± SD]	*P*
Total cases	TT	110	34.1 ± 11.6	0.175[a]
	TC	151	32.0 ± 13.8	0.018[b]
	CC	53	27.2 ± 9.4	0.001[c]
		314		0.005[d]
Female cases	TT	87	34.7 ± 11.9	0.137[a]
	TC	129	32.2 ± 14.4	0.035[b]
	CC	42	27.3 ± 9.9	0.003[c]
		258		0.012[d]

[a] The difference among TT and TC compared using LSD test

[b] The difference among TC and CC compared using LSD test

[c] The difference among TT and CC compared using LSD test

[d] The difference among TT, TC, and CC compared using one-way ANOVA test

Table 3 Meta-analysis of the association between rs11190870 and AIS in various populations

Population	Test for allele frequency of the risk allele (T allele)[a]		
	P value	OR	95 % CI
Chinese female[b]	<0.001	1.80	1.59–2.03
East Asian female[c]	<0.001	1.64	1.53–1.76
Chinese[d]	<0.001	1.60	1.44–1.79
East Asian[e]	<0.001	1.58	1.48–1.69

OR odds ratio, *CI* confidence interval

[a] Mantel–Haenszel meta-analysis

[b] Hongkongese female and Han Chinese female

[c] Hongkongese female, Han Chinese female and Japanese female

[d] Hongkongese and Han Chinese

[e] Hongkongese, Han Chinese and Japanese

In this study, we identified that rs11190870 near *LBX1* was associated with both susceptibility and disease severity of AIS. The total number of AIS patients of the current study was more than three times of that of previous replication study [16], which provided more powerful evidence to support that rs11190870 may account for disease predisposition of AIS. Our study confirmed the earlier finding of positive association in Japanese and Hong Kong Chinese [15, 16]. Furthermore, the results of meta-analysis implicated that the effect of rs11190870 polymorphism near *LBX1* would be present across East Asians.

The effect of rs11190870 on AIS severity was also analyzed in a subgroup of skeletally mature AIS. The possible role of rs11190870 in disease progression was assessed quantitatively with the Cobb angle. In the case-only study, rs11190870 was associated with curve severity. AIS patients with TT genotype had a larger Cobb angle than those with TC or CC genotype, indicating that the T allele had an effect on curve development. This was consistent with the findings that T allele was considered as the same risk allele in previous GWAS and replication study [15, 16]. It suggested that rs11190870 might be involved in initiation and progression of AIS.

LBX1 is a homeobox gene and plays an important role in developmental processes. This gene encodes ladybird homeobox 1 and expressed in the central nervous system and skeletal muscle [26–28]. Among the hypothesis of AIS etiology, abnormalities in the central nervous system have long been thought a key AIS etiology [29, 30]. Disturbance of central nervous system may impair somatosensory function and motor adaptation which lead to asymmetry of neuromuscular condition causing AIS [30, 31]. Correspondingly, functional studies in AIS subjects have shown abnormalities in the postural balance and somatosensory function [32–35]. Thus, it remains plausible that *LBX1* may be involved in the etiology of scoliosis through somatosensory dysfunction [15]. The SNP rs11190870 is located in the 3′-flanking region of *LBX1* on chromosome 10q24.31. As its proximity to gene loci, this SNP is supposed to have an impact on gene regulation; however, the precise role of rs11190870 is still unknown. Functional analysis of the *LBX1* and other genes in the 10q24.31 locus might help to understand the real genetic effect on the etiopathogenesis of AIS.

Conclusions

This study suggested that rs11190870 polymorphism was associated with the susceptibility and curve progression of AIS in a Han Chinese population. The results indicated that rs11190870 polymorphism near *LBX1* might be both risk factor and disease modifier in the pathogenesis of AIS.

Acknowledgments This work was supported by the Natural Science Foundation of China (30901570) (81171767). The Manuscript submitted does not contain information about medical device(s)/drug(s). No benefits in any form have been or will be received from a commercial party related directly or indirectly to the subject of this manuscript.

Conflicts of interest None.

References

1. Kane WJ (1977) Scoliosis prevalence: a call for a statement of terms. Clin Orthop Relat Res 126:43–46
2. Rogala EJ, Drummond DS, Gurr J (1978) Scoliosis: incidence and natural history. A prospective epidemiological study. J Bone Joint Surg Am 60:173–176
3. Weinstein SL, Dolan LA, Cheng JC et al (2008) Adolescent idiopathic scoliosis. Lancet 371:1527–1537
4. Lowe TG, Edgar M, Margulies JY et al (2000) Etiology of idiopathic scoliosis: current trends in research. J Bone Joint Surg Am 82-A:1157–1168
5. Wang WJ, Yeung HY, Chu WC et al (2011) Top theories for the etiopathogenesis of adolescent idiopathic scoliosis. J Pediatr Orthop 31:S14–S27
6. Ogilvie JW, Braun J, Argyle V et al (2006) The search for idiopathic scoliosis genes. Spine (Phila Pa 1976) 31:679–681
7. Miller NH (2011) Idiopathic scoliosis: cracking the genetic code and what does it mean? J Pediatr Orthop 31:S49–S52
8. Salehi LB, Mangino M, De Serio S et al (2002) Assignment of a locus for autosomal dominant idiopathic scoliosis (IS) to human chromosome 17p11. Hum Genet 111:401–404
9. Qiu XS, Tang NL, Yeung HY et al (2007) Melatonin receptor 1B (MTNR1B) gene polymorphism is associated with the occurrence of adolescent idiopathic scoliosis. Spine (Phila Pa 1976) 32:1748–1753
10. Ocaka L, Zhao C, Reed JA et al (2008) Assignment of two loci for autosomal dominant adolescent idiopathic scoliosis to chromosomes 9q31.2-q34.2 and 17q25.3-qtel. J Med Genet 45:87–92
11. Chen Z, Tang NL, Cao X et al (2009) Promoter polymorphism of matrilin-1 gene predisposes to adolescent idiopathic scoliosis in a Chinese population. Eur J Hum Genet 17:525–532
12. Chan V, Fong GC, Luk KD et al (2002) A genetic locus for adolescent idiopathic scoliosis linked to chromosome 19p13.3. Am J Hum Genet 71:401–406
13. Gao X, Gordon D, Zhang D et al (2007) CHD7 gene polymorphisms are associated with susceptibility to idiopathic scoliosis. Am J Hum Genet 80:957–965
14. Hirschhorn JN, Daly MJ (2005) Genome-wide association studies for common diseases and complex traits. Nat Rev Genet 6:95–108
15. Takahashi Y, Kou I, Takahashi A et al (2011) A genome-wide association study identifies common variants near LBX1 associated with adolescent idiopathic scoliosis. Nat Genet 43:1237–1240
16. Fan YH, Song YQ, Chan D et al (2012) SNP rs11190870 near LBX1 is associated with adolescent idiopathic scoliosis in southern Chinese. J Hum Genet 57:244–246
17. Risser JC (1958) The Iliac apophysis; an invaluable sign in the management of scoliosis. Clin Orthop 11:111–119
18. Ohnishi Y, Tanaka T, Ozaki K et al (2001) A high-throughput SNP typing system for genome-wide association studies. J Hum Genet 46:471–477
19. Sharma S, Gao X, Londono D et al (2011) Genome-wide association studies of adolescent idiopathic scoliosis suggest candidate susceptibility genes. Hum Mol Genet 20:1456–1466

20. Kingsmore SF, Lindquist IE, Mudge J et al (2007) Genome-wide association studies: progress in identifying genetic biomarkers in common, complex diseases. Biomark Insights 2:283–292
21. McCarthy MI, Abecasis GR, Cardon LR et al (2008) Genome-wide association studies for complex traits: consensus, uncertainty and challenges. Nat Rev Genet 9:356–369
22. Siontis KC, Patsopoulos NA, Ioannidis JP (2010) Replication of past candidate loci for common diseases and phenotypes in 100 genome-wide association studies. Eur J Hum Genet 18:832–837
23. Fan YH, Song YQ, Chan D et al (2012) SNP rs11190870 near LBX1 is associated with adolescent idiopathic scoliosis in southern Chinese. J Hum Genet 57(4):244–246
24. Chu JY, Huang W, Kuang SQ et al (1998) Genetic relationship of populations in China. Proc Natl Acad Sci U S A 95:11763–11768
25. Deng W, Shi B, He X et al (2004) Evolution and migration history of the Chinese population inferred from Chinese Y-chromosome evidence. J Hum Genet 49:339–348
26. Jagla K, Dolle P, Mattei MG et al (1995) Mouse Lbx1 and human LBX1 define a novel mammalian homeobox gene family related to the Drosophila lady bird genes. Mech Dev 53:345–356
27. Chao Z, Wu J, Zheng R et al (2011) Molecular characterization and expression patterns of Lbx1 in porcine skeletal muscle. Mol Biol Rep 38:3983–3991
28. Cheng L, Samad OA, Xu Y et al (2005) Lbx1 and Tlx3 are opposing switches in determining GABAergic versus glutamatergic transmitter phenotypes. Nat Neurosci 8:1510–1515
29. Potoupnis M, Kapetanos G, Kimiskidis VK et al (2002) Is the central nervous system a causative factor in idiopathic scoliosis? Stud Health Technol Inform 91:10–11
30. Herman R, Mixon J, Fisher A (1985) Idiopathic scoliosis and the central nervous system: a motor control problem. The Harrington lecture, 1983. Scoliosis research society. Spine (Phila Pa 1976) 10:1–14
31. Veldhuizen AG, Wever DJ, Webb PJ (2000) The aetiology of idiopathic scoliosis: biomechanical and neuromuscular factors. Eur Spine J 9:178–184
32. Guo X, Chau WW, Hui-Chan CW et al (2006) Balance control in adolescents with idiopathic scoliosis and disturbed somatosensory function. Spine (Phila Pa 1976) 31:E437–E440
33. Simoneau M, Richer N, Mercier P et al (2006) Sensory deprivation and balance control in idiopathic scoliosis adolescent. Exp Brain Res 170:576–582
34. Lao ML, Chow DH, Guo X et al (2008) Impaired dynamic balance control in adolescents with idiopathic scoliosis and abnormal somatosensory evoked potentials. J Pediatr Orthop 28: 846–849
35. Beaulieu M, Toulotte C, Gatto L et al (2009) Postural imbalance in non-treated adolescent idiopathic scoliosis at different periods of progression. Eur Spine J 18:38–44