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Candidate gene analysis and exome sequencing confirm *LBX1* as a susceptibility gene for idiopathic scoliosis

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Abstract

Background: Idiopathic scoliosis is a spinal deformity affecting approximately 3% of otherwise healthy children or adolescents. The etiology is still largely unknown but has an important genetic component. Genome-wide association studies have identified a number of common genetic variants that are significantly associated with idiopathic scoliosis in Asian and Caucasian populations, rs11190870 close to the LBX1 gene being the most replicated finding.

Purpose: The aim of the present study was to investigate the genetics of idiopathic scoliosis in a Scandinavian cohort by performing a candidate gene study of four variants previously shown to be associated with idiopathic scoliosis and exome sequencing of idiopathic scoliosis patients with a severe phenotype to identify possible novel scoliosis risk variants.

Study design: This was a case control study.

Patient sample: A total of 1,739 patients with idiopathic scoliosis and 1,812 controls were included.

Outcome measure: The outcome measure was idiopathic scoliosis.

Methods: The variants rs10510181, rs11190870, rs12946942, and rs6570507 were genotyped in 1,739 patients with idiopathic scoliosis and 1,812 controls. Exome sequencing was performed on pooled samples from 100 surgically treated idiopathic scoliosis patients. Novel or rare missense, nonsense, or splice site variants were selected for individual genotyping in the 1,739 cases and 1,812 controls. In addition, the 5'UTR, noncoding exon and promoter regions of LBX1, not covered by exome sequencing, were Sanger sequenced in the 100 pooled samples.

Results: Of the four candidate genes, an intergenic variant, rs11190870, downstream of the LBX1 gene, showed a highly significant association to idiopathic scoliosis in 1,739 cases and 1,812 controls ($p=7.0 \times 10^{-18}$). We identified 20 novel variants by exome sequencing after filtration and an initial genotyping validation. However, we could not verify any association to idiopathic scoliosis in the large cohort of 1,739 cases and 1,812 controls. We did not find any variants in the 5'UTR, noncoding exon and promoter regions of LBX1.

Conclusions: Here, we confirm LBX1 as a susceptibility gene for idiopathic scoliosis in a Scandinavian population and report that we are unable to find evidence of other genes of similar or stronger effect.

Keywords: Idiopathic scoliosis; Genetics; Etiology; LBX1; Association study; Exome sequencing; Adolescent; Juvenile

Introduction

Idiopathic scoliosis is a spinal deformity affecting approximately 3% of otherwise healthy children or adolescents. In approximately 10% of the affected individuals, the deformity is progressive, requiring treatment with brace or spinal fusion surgery [1].

The etiology of idiopathic scoliosis is still largely unknown; heredity, however, is an important contributing factor [2,3]. Recent genome-wide association studies have identified common genetic variants close to, or within, the ladybird homeobox 1 (LBX1), cell adhesion molecule L1-like (CHL1), and G protein-coupled receptor 126 (GPR126) genes, as well as an intergenic variant on 17q, in Asian and Caucasian populations [4–7]. One exome sequencing study has identified novel, rare variants associated with idiopathic scoliosis in the Fibrillin 1 and 2 genes in a European population [8]. However, these variants explain only a small part of the heritability of this disorder. The aim of the present study was to investigate the genetics of idiopathic scoliosis in a Scandinavian cohort by performing a candidate gene study of variants previously shown to be associated with idiopathic scoliosis and exome sequencing of idiopathic scoliosis patients with a severe phenotype.

Methods

This is a Swedish and Danish multicenter case-control study. Five Swedish and one Danish orthopedic department participated: the Karolinska University Hospital, the Skåne University Hospital, the Sahlgren University Hospital, the Sundsvall and Härnösand County Hospital, and the University Hospital of Umeå, all in Sweden, and Middelfart Hospital, Denmark.

Subjects and samples collection

Cases

Between 2004 and 2013, individuals with idiopathic scoliosis were invited to participate in the study. Details of the recruitment of patients have been published elsewhere [9–11]. Inclusion criteria were a juvenile or adolescent idiopathic scoliosis with a Cobb angle greater than or equal to 10° [12]. Only patients with a normal neurologic examination and without history or clinical sign of a non-idiopathic scoliosis were eligible for the study. A magnetic resonance imaging (MRI) was not mandatory for inclusion in the study, but all cases with an MRI of the spine showing neural abnormalities were excluded. In total, 4,404 patients were invited; 1,244 did not answer, 955 declined to participate, and 320 were excluded because of signs of nonidiopathic scoliosis, incomplete medical records, or age at onset less than 4 years of age, leaving 1,885 individuals for this study. Sampling and DNA extraction was successful in 1,739 patients, Table 1.

Among the 1,739 patients, 495 were untreated, 641 were brace treated, and 603 were surgically treated. To allow for grading of the severity of the scoliosis, curve size was measured on routine radiographs from the occasion when the patient was considered to have the largest curve size: The last available radiograph before the age of 27 years in the untreated patients; on the last radiograph before bracing in the brace-treated patients; and on the preoperative radiograph in the surgically treated patients. Measurements were performed by the method by Cobb [12] and showed a mean (standard deviation) of 25 (12) for untreated, 33 (8) for braced, and 57 (14) ° for operated patients. Curve location was defined according to the apex of the major curve; thoracic curves had an apex between the first and eleventh thoracic vertebrae, thoracolumbar curves between the twelfth thoracic and first lumbar vertebra, and lumbar curves from the second lumbar to fifth lumbar vertebrae. If an individual had two curves with a difference between the two measurements of less than 5°, the patient was defined as having a double primary curve. Individuals who were aged 4 to 9 years old at onset of scoliosis were defined as having juvenile idiopathic scoliosis. Of the patients, 1,485 (85%) had parents who both were born in Scandinavia, 109 (6%) had one parent who was born in Scandinavia and one parent who was born outside Scandinavia, 113 (6%) had parents who were both born outside Scandinavia, and in 35 (2%), data on place of birth of parents were not available.

DNA was prepared from blood in 98% and saliva in 2% of the samples. In 1,216 patients, a salt precipitation method on the Autopure LS system (Qiagen, Hilden, Germany) was used, and in 523 samples, the QIAamp 96 DNA Blood Kit (Qiagen, Hilden, Germany) was used for DNA extraction according to instructions from the manufacturer.

From this cohort, 100 patients with a severe phenotype and Swedish ancestry were chosen for exome sequencing, Table 1. Additional DNA was extracted with the Gentra Puregene Blood Kit (Qiagen, Hilden, Germany) according to instructions from the manufacturer.

Concentration of the DNA was measured at least twice with the Qubit dsDNA BR Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA). An equal amount of DNA from each subject was added to make 10 pools, with DNA from 10 patients in each pool.

Controls

Osteoporosis Prospective Risk Assessment (OPRA) and PEAK-25 cohorts

Individuals from the population-based cohorts OPRA and PEAK-25 were used as controls [13,14]. The OPRA cohort consists of elderly women, and the PEAK-25 cohort consists of young women living in Malmö, Sweden. Deoxyribonucleic acid was available in 2,011 individuals. In both these cohorts, dual-energy X-ray absorptiometry (DXA) for studying bone density had been part of the research protocol. Dual-energy X-ray absorptiometry has been reported to be a reliable method to evaluate scoliosis, comparable to conventional radiographs [15]. However, DXA is performed with the patient in a supine position, and the Cobb angle may be underestimated compared with standing radiographs. We therefore chose to exclude all individuals showing any sign of a curved spine on a whole body or lumbar spine DXA (n=199). We included those individuals missing DXA information

(n=69), because the prevalence of idiopathic scoliosis in the normal population is low [1], leaving 1,812 individuals for this study, Table 1.

In the 909 controls from the OPRA cohort, data on place of birth of parents were not available. All except three were born in Sweden. Place of birth is not specified for two individuals. In the 903 controls from the PEAK-25 cohort, 833 (92%) had parents who both were born in Scandinavia, 49 (5%) had one parent who was born in Scandinavia, and 21 (2%) had parents who both were born outside Scandinavia. Deoxyribonucleic acid was extracted using the QIAamp 96 DNA Blood Kit (Qiagen, Hilden, Germany) according to the instructions from the manufacturer.

Control data for exome sequencing

We used already existing anonymized exome sequencing data from 100 preeclampsia patients [16,17] and 100 patients with morbid obesity [18] as control data for the exome sequencing, Table 1. There is no phenotype information on scoliosis for these controls. The same pooling strategy, target enrichment, and sequencing platform as in the present study were used in both these cohorts.

Candidate gene study

Genotyping

We genotyped the previously reported variants rs10510181, rs11190870, rs12946942, and rs6570507 in 1,739 patients with idiopathic scoliosis and in 1,812 controls from the OPRA and PEAK-25 cohorts [4–7]. Genotyping was performed at the Mutation Analysis Facility at Karolinska University Hospital (Huddinge, Sweden) using iPLEX Gold chemistry and MassARRAY mass spectrometry system (Agena Bioscience, San Diego, CA, USA) [19]. Multiplexed assays were designed using the MassARRAY Assay Design v4.0 Software (Sequenom, Agena Bioscience, San Diego, CA, USA). Genotype calls were manually checked by two persons individually using the MassARRAY Typer v4.0 Software (Agena BioScience, San Diego, CA, USA).

Sequencing of LBX1 locus

We found a strong association to rs11190870, approximately 6 kb downstream of the LBX1 gene. To further study the role of LBX1 in idiopathic scoliosis, we chose to sequence the LBX1 promoter and exon regions. There are two known transcripts of LBX1, one confirmed (NM_006562) and one predicted (XM_005269443), described in the Genome Reference Consortium, human reference 38, Figure S1. The coding regions of the confirmed transcript were covered by exome sequencing, in the following section. The predicted transcript (XM_005269443) of LBX1 contains an extra noncoding exon that was not covered by the exome sequencing target enrichment platform that we used. According to the FANTOM5 human promoter centric expression atlas, there are three transcription start sites (TSSs) of LBX1 and one TSS of the new LBX1 isoform, detected in both adult and fetal skeletal muscle, Figure S2 [20]. To cover the new noncoding exon and the TSSs, we designed three primer pairs and performed polymerase chain reaction (PCR) amplification with

HotStarTaq Plus DNA Polymerase kit (Qiagen, Hilden, Germany) on genomic DNA from the 100 pooled samples used in exome sequencing, Table 1. PCR products were sequenced using cycle sequencing technology on ABI 3730XL sequencing machine at Eurofins Genomics (Ebersberg, Germany).

Exome sequencing

Exome sequencing was performed at the Science for Life laboratory, Stockholm, Sweden. Each DNA library was prepared from 3 µg of pooled genomic DNA sheared to 300 bp using a Covaris S2 instrument (Covaris, MA, USA) and enriched using the SureSelectXT Human All Exon 50 Mb (Agilent technologies, CA, USA) and an Agilent NGS workstation according to the manufacturer's instructions (SureSelectXT Automated Target Enrichment for Illumina Paired-End Multiplexed Sequencing, version A; Agilent technologies, CA, USA).

Sequencing data analysis and variant selection

Exome sequencing reads were aligned to the human reference genome hg19, Genome Reference Consortium Human Build 37, with the Burrows-Wheeler Aligner, BWA software package, version 0.6.1 (<http://sourceforge.net/projects/bio-bwa/files/bwa-0.6.1.tar.bz2>) [21]. We used SAMtools, version 0.1.18 (<http://sourceforge.net/projects/samtools/files/samtools/0.1.18/>), to remove PCR duplicates on each lane, filter out reads with low mapping quality (Phred quality score <20), and call variants [22]. Both single and multiple pool calls were used. Annotation was completed using the ANNOVARsoftware (Biobase, Wolfenbuettel, Germany) [23]. We applied BEDTools, version 2.16.2 (<http://bedtools.readthedocs.org/>) for read depth and coverage evaluation [24].

We estimated the minor allele frequency of each variant by pooling all the reads at each position together and calculating the percentage of reads supporting the minor allele. We filtered out singleton variants and selected missense, nonsense, and splice site rare variants that had a minor allele frequency less than or equal to 0.05 in the 1000G project European population [25]. We compared our data with the exome sequencing data from the 100 preeclampsia and the 100 obesity patients. We filtered out the variants that were found in more than one of the preeclampsia and obesity pools as likely innocent variants or sequencing errors. Among the remaining variants (n=353), we selected those that were related to skeleton, muscle or nervous system development, or cell adhesion (n=29) in the Gene Ontology Consortium database [26]. In addition, we checked the 353 variants by manual visualization and kept those not shown in obesity or preeclampsia patients at all (n=21).

Variant validation and association analysis

In an initial validation association analysis, 45 of the variants selected from the exome sequencing were successfully genotyped in 180 cases, including the previously exome sequenced subjects, and in 245 controls from the PEAK-25 cohort. In a follow-up, 20 of the variants with an odds ratio (OR) greater than or equal to 1.5 or less than or equal to 0.67 in the initial association analyses were genotyped in the remaining 1,567 cases and 1,567 controls

from the OPRA and PEAK-25 cohorts. The same genotyping platform was used as described previously for the candidate gene study, Table S1. Finally, an association analysis combining the two data sets was performed.

Statistical analysis

Association was evaluated by the chi-square test using PLINK v1.07 (<http://pngu.mgh.harvard.edu/purcell/plink/>) [27]. For subgroup analyses, curve severity was defined as mild if the patient had a Cobb angle of 10° to 30°, moderate if the patient had a Cobb angle of 31° to 44°, and severe if the patient had a Cobb angle greater than or equal to 45°. Patients with a thoracic major curve with a right convexity were defined as “Right Thoracic” and patients with a thoracic major curve with a left convexity as “Left Thoracic.” The group “All Except Right Thoracic” includes all patients except those defined as “Right Thoracic.” Patients were subgrouped according to the age at onset in 4 to 8, 9 to 12, and 13 years and older, as well as in juvenile (4–9 years) and adolescent (10–20 years).

Results

Association analysis of candidate genes

Of the four candidate genes, only an intergenic variant, rs11190870, downstream of the LBX1 gene, showed a strong association to idiopathic scoliosis in individual genotyping of cases and controls, Table 2. The other three variants showed weaker or no association. In subgroup analyses of the association of rs11190870, there seemed to be a stronger association with females than males, and with right thoracic curve types compared with all other curve types together, yielding higher point estimates for the ORs, Table 3. The curve severity and juvenile or adolescent age of onset did not appear to have a significant effect on the association, Table 3. However, comparisons between patient subgroups did not yield significant differences (data not shown). In addition, when comparing untreated, brace-treated and surgically treated individuals, we found no significant differences in association with the risk allele.

We analyzed age at onset as a continuous outcome variable in a logistic regression of cases only and found no significant association with the risk allele (data not shown). In a subgroup analysis comparing age at onset 4 to 8 years, 9 to 12 years, and 13 years and older, we found no significant differences in association with the risk allele either (data not shown).

Sequencing of LBX1 locus

Because we observed a highly significant association of the previously identified LBX1 variant to scoliosis, we performed Sanger sequencing to discover further genetic variation at this locus. We did not find any variants in the 5'UTR, noncoding exon or promoter regions of LBX1, data not shown.

Exome sequencing

We obtained 307 to 418 million sequence reads in each sample pool, 93.3% to 98.5% of which could be mapped to the human reference genome hg19. Eighty-one to eighty-nine percent of the SureSelect target regions were covered by a depth of 30x. We identified a total of 1.7 million single nucleotide variants. After filtration and an initial genotyping validation, we selected 20 novel variants for further follow-up. We were, however, unable to find statistically significant association of any of these to idiopathic scoliosis in the large cohort of 1,739 cases and 1,812 controls, Table S1.

In the LBX1 exon region, we identified one synonymous variant, rs941909, in 6 of the 10 pools in the idiopathic scoliosis patients and in 8 of the 10 pools in the preeclampsia patients, suggesting that it is a common innocent variant (data not shown).

Discussion

In a candidate gene study of four common single nucleotide variants previously shown to be associated with idiopathic scoliosis, we found a highly significant association of rs11190870, downstream of the LBX1 gene ($p=7.0\times 10^{-18}$, OR 1.53) to idiopathic scoliosis, in a Scandinavian cohort of 1,739 idiopathic scoliosis cases and 1,812 controls. The other three variants showed weaker or no association. To identify novel high-risk variants contributing to scoliosis, we then performed exome sequencing on 100 pooled samples with a severe idiopathic scoliosis phenotype. Multiple variants were identified by exome sequencing, but we could not verify any association to idiopathic scoliosis by direct genotyping in the cohort of 1,739 cases and 1,812 controls. No new functional variant in the LBX1 gene was found. In addition, we Sanger sequenced the promoter region of LBX1 without identifying any variants.

In a genome wide association study, Takahashi et al. [5] identified the common variant rs11190870 in the 10q24.31 region to be strongly associated with idiopathic scoliosis in a Japanese population of 1,033 cases and 1,473 controls with a p value and OR (1.2×10^{-19} and 1.56, respectively) in parity with the present study. This association has subsequently been replicated in several North American and East Asian cohorts, suggesting the region on 10q24.31 harboring the LBX1 gene, to be a true susceptibility locus for idiopathic scoliosis [28–30]. In this study, we found a highly significant association of rs11190870 to idiopathic scoliosis also in a Scandinavian population, and as such, confirm the association of the region 10q24.31 to idiopathic scoliosis across populations.

Of the remaining three variants genotyped in our study, two showed a weak association to idiopathic scoliosis (rs6570507, $p=1.6\times 10^{-4}$, OR 1.22 and rs12946942, $p=.005$, OR 1.27). The variant rs6570507 has previously been shown to be associated with idiopathic scoliosis in Japanese, Chinese Han, and European populations ($p=1.27\times 10^{-14}$, OR 1.27) and rs12946942 in a Japanese and Han Chinese population ($p=6.43\times 10^{-12}$, OR=2.21) [4,6]. Our results lend support to the previously observed associations at a moderate level of genetic effect, but these variants do not appear to have a major effect on scoliosis risk in the Scandinavian population. Because of an obvious lack of power, we did not follow up subgroup associations to these three variants in the present study.

LBX1 is expressed in dorsal spinal neurons and hindbrain, muscle precursor cells, and certain cardiac crest cells and is a plausible candidate in the pathogenesis of idiopathic scoliosis [31–35]. In an attempt to further elucidate the potential role of LBX1 in idiopathic scoliosis, we used the FANTOM5 database to identify four putative TSSs of LBX1, detected in both adult and fetal skeleton muscles. We Sanger sequenced these regions in pooled DNA from 100 surgically treated idiopathic scoliosis patients. We were, however, unable to identify any variants in these regions.

The variant rs11190870 seems to be associated to idiopathic scoliosis irrespective of scoliosis curve severity [28–30]. This is supported by our findings, where we found no substantial differences in OR between mild, moderate, or severe scoliosis compared with controls. In a previous meta-analysis of several cohorts, Londono et al. [30] showed evidence for genetic susceptibility to scoliosis of rs11190870 in both males and females. In the current cohort, including the largest number of males reported, the point estimate of risk is higher in females, but not significantly different compared with that in males. In addition, the association of rs11190870 seems to be as strong in both adolescent and juvenile idiopathic scoliosis patients as well.

Patients with idiopathic scoliosis have normal neurology and no signs of any disorders associated with scoliosis. However, MRI may reveal subclinical neural axis abnormalities in these patients. The most common curve type is a right convex thoracic scoliosis and it is most often associated with a normal neural axis MRI. On the other hand, neural axis malformations have been reported to be more common in left thoracic curves, and it may be speculated that these may represent another clinical entity [36,37]. Based on this, we hypothesized that the genetic basis could be different in patients with different curve types. In a subgroup analysis, we found that the association of rs11190870 seems to be stronger to right thoracic curves than all other curve types taken together (1.4×10^{-19} , OR 1.68 and 5.4×10^{-5} , OR 1.32, respectively) and to patients with left thoracic curves. The number of patients in the latter group is however very small, limiting the conclusions that can be drawn. To our knowledge, data on association with curve types have not been presented previously.

The variants previously shown to be associated with idiopathic scoliosis, in other studies and ours, explain only a small part of the heritability of idiopathic scoliosis. To identify novel rare high-risk variants, we exome sequenced 100 pooled samples from individuals with a severe idiopathic scoliosis. The variants we identified were validated but were not strongly associated by individual genotyping in our large cohort of 1,739 and 1,812 controls. In addition, we could not find any putative functional variants in the LBX1 gene. In a recent exome sequencing study of a European population, rare variants in Fibrillin 1 and 2 were found to contribute to scoliosis [8]. We could not replicate these findings (data not shown). This might be due to different population background or different selection of subgroups of scoliosis individuals.

A limitation of the pooled exome sequencing strategy is the need for filtering. Variants found in only one pool and variants with a very low frequency, despite being found in several pools, were removed to avoid false positives. One might question the use of exome sequencing data

from morbidly obese and preeclampsia patients without any scoliosis phenotype information as control data. However, the prevalence of scoliosis is low in the general population, and an above-normal BMI is shown to be protective against spinal deformities [38]. Another limitation is the difficulty of finding association by genotyping of the variants identified by exome sequencing. This might be due to a lack of power in the follow-up, looking for rare variants demands large sample sizes to reach significance.

In conclusion, we confirm LBX1 as a susceptibility gene for idiopathic scoliosis in a Scandinavian population and do not find evidence of other scoliosis risk genes of similar or stronger effect. In the future, possible strategies to succeed in elucidating the role of the LBX1 region and discovering new risk factors for adolescent idiopathic scoliosis, include metastudies, detailed studying of subphenotypes, family studies, and epigenetic approaches.

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References

- [1] S. Willner and A. Uden, A prospective prevalence study of scoliosis in Southern Sweden, *Acta Orthop Scand* 53, 1982, 233–237.
- [2] R. Wynne-Davies, Familial (idiopathic) scoliosis. A family survey, *J Bone Joint Surg Br* 50, 1968, 24–30.
- [3] A. Grauers, I. Rahman and P. Gerdhem, Heritability of scoliosis, *Eur Spine J* 21, 2012, 1069–1074.
- [4] I. Kou, Y. Takahashi, T.A. Johnson, A. Takahashi, L. Guo, J. Dai, et al., Genetic variants in GPR126 are associated with adolescent idiopathic scoliosis, *Nat Genet* 45, 2013, 676–679.
- [5] Y. Takahashi, I. Kou, A. Takahashi, T.A. Johnson, K. Kono, N. Kawakami, et al., A genome-wide association study identifies common variants near LBX1 associated with adolescent idiopathic scoliosis, *Nat Genet* 43, 2011, 1237–1240.
- [6] A. Miyake, I. Kou, Y. Takahashi, T.A. Johnson, Y. Ogura, J. Dai, et al., Identification of a susceptibility locus for severe adolescent idiopathic scoliosis on chromosome 17q24.3, *PLoS One* 8, 2013, e72802.
- [7] S. Sharma, X. Gao, D. Londono, S.E. Devroy, K.N. Mauldin, J.T. Frankel, et al., Genome-wide association studies of adolescent idiopathic scoliosis suggest candidate susceptibility genes, *Hum Mol Genet* 20, 2011, 1456–1466.
- [8] J.G. Buchan, D.M. Alvarado, G.E. Haller, C. Cruchaga, M.B. Harms, T. Zhang, et al., Rare variants in FBN1 and FBN2 are associated with severe adolescent idiopathic scoliosis, *Hum Mol Genet* 23, 2014, 5271–5282.
- [9] A. Grauers, A. Danielsson, M. Karlsson, A. Ohlin, P. Gerdhem, Family history and its association to curve size and treatment in 1,463 patients with idiopathic scoliosis, *Eur Spine J* 22, 2013, 2421–2426.
- [10] M.O. Andersen, S.B. Christensen and K. Thomsen, Outcome at 10 years after treatment for adolescent idiopathic scoliosis, *Spine* 31, 2006, 350–354.
- [11] A. Grauers, C. Topalis, H. Moller, H. Normelli, M. Karlsson, A. Danielsson, et al., Prevalence of back problems in 1069 adults with idiopathic scoliosis and 158 adults without scoliosis, *Spine* 39, 2014, 886–892.
- [12] J. Cobb, Technique for study of scoliosis, In: W.P. Blount and AAOS, (Eds.), *AAOS Instructional Course Lectures*, 1948, J. Edwards Inc; Ann Arbor, 261–275.
- [13] F.E. McGuigan, E. Larzenius, M. Callreus, P. Gerdhem, H. Luthman, K. Akesson, Variation in the BMP2 gene: bone mineral density and ultrasound in young adult and elderly women, *Calcif Tissue Int* 81, 2007, 254–262.

- [14] P. Gerdhem and K. Akesson, Rates of fracture in participants and non-participants in the Osteoporosis Prospective Risk Assessment study, *J Bone Joint Surg Br* 89, 2007, 1627–1631.
- [15] I.P. Pappou, F.P. Girardi, H.S. Sandhu, H.K. Parvataneni, F.P. Cammisa, R. Schneider, et al., Discordantly high spinal bone mineral density values in patients with adult lumbar scoliosis, *Spine* 31, 2006, 1614–1620.
- [16] H. Laivuori, P. Lahermo, V. Ollikainen, E. Widen, L. Haiva-Mallinen, H. Sundstrom, et al., Susceptibility loci for preeclampsia on chromosomes 2p25 and 9p13 in Finnish families, *Am J Hum Genet* 72, 2003, 168–177.
- [17] T. Kaartokallio, M.M. Klemetti, A. Timonen, J. Uotila, S. Heinonen, E. Kajantie, et al., Microsatellite polymorphism in the heme oxygenase-1 promoter is associated with nonsevere and late-onset preeclampsia, *Hypertension* 64, 2014, 172–177.
- [18] Jiao H, Arner P, Gerdhem P, R.J. Strawbridge, E. Naslund, A. Thorell, et al. Exome sequencing followed by genotyping suggests SYPL2 as a susceptibility gene for morbid obesity. *Eur J Hum Genet* 2014 Nov 19. [Epub ahead of print].
- [19] C. Jurinke, D. van den Boom, C.R. Cantor, Köster H, Automated genotyping using the DNA MassArray technology, *Methods Mol Biol* 187, 2002, 179–192.
- [20] FANTOM Consortium and the RIKEN PMI and CLST (DGT), A.R. Forrest, H. Kawaji, M. Rehli, J.K. Baillie, M.J.L. de Hoon, T. Lassmann, et al., A promoter-level mammalian expression atlas, *Nature* 507, 2014, 462–470.
- [21] H. Li and R. Durbin, Fast and accurate short read alignment with Burrows-Wheeler transform, *Bioinformatics* 25, 2009, 1754–1760.
- [22] H. Li, B. Handsaker, A. Wysoker, T. Fennell, J. Ruan, N. Homer; 1000 Genome Project Data Processing Subgroup, et al., The sequence alignment/map format and SAMtools, *Bioinformatics* 25, 2009, 2078–2079.
- [23] K. Wang, M. Li and H. Hakonarson, ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data, *Nucleic Acids Res* 38, 2010, e164.
- [24] A.R. Quinlan and I.M. Hall, BEDTools: a flexible suite of utilities for comparing genomic features, *Bioinformatics* 26, 2010, 841–842.
- [25] 1000 Genomes Project Consortium, G.R. Abecasis, A. Auton, L.D. Brooks, M.A. DePristo, R.M. Durbin, R.E. Handsaker, et al., An integrated map of genetic variation from 1,092 human genomes, *Nature* 491, 2012, 56–65.
- [26] Gene Ontology Consortium, database online, 2014, Available at: <http://geneontology.org>. Accessed September 1, 2013.
- [27] S. Purcell, B. Neale, K. Todd-Brown, L. Thomas, M.A.R. Ferreira, D. Bender, et al., PLINK: a tool set for whole-genome association and population-based linkage analyses, *Am J Hum Genet* 81, 2007, 559–575.

- [28] Y.H. Fan, Y.Q. Song, D. Chan, Y. Takahashi, S. Ikegawa, M. Matsumoto, et al., SNP rs11190870 near LBX1 is associated with adolescent idiopathic scoliosis in southern Chinese, *J Hum Genet* 57, 2012, 244–246.
- [29] W. Gao, Y. Peng, G. Liang, A. Liang, W. Ye, L. Zhang, et al., Association between common variants near LBX1 and adolescent idiopathic scoliosis replicated in the Chinese Han population, *PLoS One* 8, 2013, e53234.
- [30] D. Londono, I. Kou, T.A. Johnson, S. Sharma, Y. Ogura, T. Tsunoda, Japanese Scoliosis Clinical Research Group, TSRHC IS Clinical Group, International Consortium for Scoliosis Genetics, et al., A meta-analysis identifies adolescent idiopathic scoliosis association with LBX1 locus in multiple ethnic groups, *J Med Genet* 51, 2014, 401–406.
- [31] L. Cheng, O.A. Samad, Y. Xu, R. Mizuguchi, P. Luo, S. Shirasawa, et al., Lbx1 and Tlx3 are opposing switches in determining GABAergic versus glutamatergic transmitter phenotypes, *Nat Neurosci* 8, 2005, 1510–1515.
- [32] M.K. Gross, M. Dottori and M. Goulding, Lbx1 specifies somatosensory association interneurons in the dorsal spinal cord, *Neuron* 34, 2002, 535–549.
- [33] K. Jagla, P. Dolle, M.G. Mattei, T. Jagla, B. Schuhbaur, G. Dretzen, et al., Mouse Lbx1 and human LBX1 define a novel mammalian homeobox gene family related to the Drosophila lady bird genes, *Mech Dev* 53, 1995, 345–356.
- [34] K. Schafer and T. Braun, Early specification of limb muscle precursor cells by the homeobox gene Lbx1h, *Nat Genet* 23, 1999, 213–216.
- [35] K. Schafer, P. Neuhaus, J. Kruse, et al., T. Braun, The homeobox gene Lbx1 specifies a subpopulation of cardiac neural crest necessary for normal heart development, *Circ Res* 92, 2003, 73–80.
- [36] M. Inoue, S. Minami, Y. Nakata, Y. Otsuka, M. Takaso, H. Kitahara, et al., Preoperative MRI analysis of patients with idiopathic scoliosis: a prospective study, *Spine* 30, 2005, 108–114.
- [37] L. Wu, Y. Qiu, B. Wang, Z.Z. Zhu, W.W. Ma, The left thoracic curve pattern: a strong predictor for neural axis abnormalities in patients with “idiopathic” scoliosis, *Spine* 35, 2010, 182–185.
- [38] O. HersHKovich, A. Friedlander, B. Gordon, H. Arzi, E. Derazne, D. Tzur, et al., Association between body mass index, body height, and the prevalence of spinal deformities, *Spine J* 14, 2014, 1581–1587.

Tables

Table 1. Summary of samples used in association analysis and exome sequencing

	Association analysis		Exome sequencing		
	Cases	Controls	Cases	Control data set (preeclampsia)	Control data set (obesity)
Sample size	1,739	1,812	100	100	100
Gender(male/female)	241/1,498	0/1,812	24/76	0/100	31/69
Cobb Angle (Mean±SD)	38.8±17.5*		56.3±13.2		

SD, standard deviation.

*Missing data on Cobb angles in 11 individuals.

Table 2. Association of candidate genes to idiopathic scoliosis in a Scandinavian cohort of 1,739 cases and 1,812 controls.

Variant name	Gene	A1	A2	RAF cases	RAF controls	p Value	OR (95% CI)
rs6570507	<i>GPR126</i> intron	A	G	0.32	0.28	1.6×10^{-4}	1.22(1.10–1.35)
rs11190870	<i>LBX1</i> downstream	T	C	0.69	0.59	7.0×10^{-18}	1.53(1.39–1.69)
rs12946942	Intergenic	T	G	0.09	0.08	.005	1.27(1.08–1.51)
rs10510181	<i>CHL1</i> upstream	A	G	0.36	0.36	.97	1.0(0.91–1.1)

A1, allele 1 (risk allele); A2, allele 2; RAF, risk allele frequency; OR, odds ratio; CI, confidence interval; GPR126, G protein-coupled receptor; LBX1, ladybird homeobox 1; CHL1, cell adhesion molecule L1-like.

Table 3. Associations of rs11190870, downstream the LBX1 gene, to subgroups of idiopathic scoliosis cases. The same number of controls is used in all analyses (n=1812).

Subgroup	n	Risk allele	RAF cases	RAF controls	p Value	OR	95% CI
All	1,739	T	0.69	0.59	7.0×10^{-18}	1.53	1.39 to 1.69
Females	1,498	T	0.69	0.59	8.2×10^{-18}	1.56	1.41 to 1.73
Males	241	T	0.66	0.59	.001	1.38	1.13 to 1.68
Right Thoracic curve	1,107	T	0.71	0.59	1.4×10^{-19}	1.68	1.50 to 1.88
Left Thoracic	74	T	0.64	0.59	.17	1.27	0.90 to 1.79
All except Right Thoracic*	603	T	0.66	0.59	5.4×10^{-5}	1.32	1.15 to 1.50
Juvenile onset	213	T	0.69	0.59	3.7×10^{-5}	1.57	1.27 to 1.95
Adolescent onset	1,526	T	0.69	0.59	1.6×10^{-16}	1.53	1.38 to 1.69
Mild (10–30)†	616	T	0.67	0.59	1.9×10^{-7}	1.43	1.25 to 1.64
Moderate (31–44)†	527	T	0.70	0.59	6.6×10^{-11}	1.63	1.41 to 1.89
Severe (45–)†	585	T	0.69	0.59	4.5×10^{-10}	1.56	1.36 to 1.80

RAF, risk allele frequency; OR, odds ratio; CI, confidence interval.

*The group “All except Right Thoracic” includes the “Left Thoracic” group. Missing data on curve type in 29 individuals.

†Curve severity and Cobb angle of the largest curve. Missing data in 11 individuals.

Supplementary material

Table S1. Oligonucleotide primers used for PCR amplification of LBX1 promoter regions

#primer	Sequence	Orientation	Amplicon length (bp)	GC contents
1	5' - GCCTTGCCGTCCTCCTTG - 3'	sense	444	75.68 %
	5' - AATTGCCCCGTCTCTCCG - 3'	antisense		
2	5' - TAAAGGTGTCAGGTGGCTCG - 3'	sense	512	57.42 %
	5' - AGGTTCGGGTGCAATAAAGG - 3'	antisense		
3	5' - GAGAGCTCAGCCTTCTCCAC - 3'	sense	395	72.92 %
	5' - CGAGTTCCTGAGAGCAAAGC - 3'	antisense		

Table S2. Scoliosis pooled DNA exome sequencing mapping statistic

Pooled sample	Uniquely mapped reads (PCR duplicate removed)
Pool1	111,199,355
Pool2	138,036,076
Pool3	115,158,113
Pool4	127,845,939
Pool5	119,736,656
Pool6	103,399,069
Pool7	83,341,128
Pool8	68,241,716
Pool9	102,034,437
Pool10	112,423,514

Table S3. Association analysis of 20 variants identified by exome sequencing

SNP	A1	A2	F_A	F_U	P	OR
rs3795424	G	T	0.03385	0.02956	0.3017	1.15
rs41265897	A	G	0.04102	0.03333	0.087	1.241
rs74857529	G	C	0.006613	0.004171	0.1601	1.589
rs141826063	A	G	0.002009	0.0008338	0.1879	2.413
rs34372645	A	G	0.02583	0.02309	0.4557	1.122
rs41272699	T	C	0.02183	0.0172	0.1591	1.275
rs61734638	A	G	0.03096	0.02998	0.8103	1.034
rs106287	A	G	0.01696	0.02139	0.1752	0.7895
rs61748674	A	G	0.0218	0.02627	0.2192	0.826
rs41279597	C	T	0.03129	0.02361	0.04785	1.336
rs2437100	A	G	0.02411	0.02585	0.6396	0.9311
rs11575580	T	C	0.01263	0.01051	0.4032	1.204
rs150621117	G	A	0.01406	0.01444	0.8927	0.9733
rs76708142	C	T	0.009466	0.009439	0.9905	1.003
rs118105704	G	A	0.02875	0.02738	0.7261	1.052
rs141855950	C	T	0.006024	0.00221	0.0117	2.736
rs63750072	G	A	0.04744	0.04712	0.9488	1.007
rs55780333	A	G	0.1117	0.09712	0.04432	1.169
rs144151494	C	T	0.004303	0.003057	0.3861	1.409
rs1800273	A	G	0.04426	0.03929	0.3044	1.132

A1: major allele

A2: minor allele

F_A: frequency of minor allele in cases

F_U: frequency of minor allele in controls

OR: odds ratio

Table S4. Primers for genotyping

SNP_ID	2nd-PCR (PCR primer reverse)	1st-PCR (PCR primer forward)	UEP_SEQ (extension primer)
rs74857529	ACGTTGGATGAGGACCCCCATTTACTTC	ACGTTGGATGTCTGCTGTTTGAGAAGATGG	TCTTCCCGCCTTACCTT
rs2437100	ACGTTGGATGAGTCACCTGTCGGTCTGGTC	ACGTTGGATGATAGACTTGCTGAGCAGCAC	GCCGTGACGCATGACCC
rs41265897	ACGTTGGATGTGCAGAGTTCACACTCTGGG	ACGTTGGATGTCCTACCGCACATGTCTCTC	TTGAGGAGCTGGGCTGCT
rs76708142	ACGTTGGATGGGTTGACCAGTTCACAAGAC	ACGTTGGATGTCTATAGCTGACTTGGCTCG	AACACATCTGCCCTTGATA
rs141826063	ACGTTGGATGCATCCCCTAATCAACATACC	ACGTTGGATGAGGAATCCCACTGTAAGATG	ACCTGTGCAAACCTTCATAG
rs12946942	ACGTTGGATGGAATGTTTGGGCATGGAGAG	ACGTTGGATGCCAAACAATGCCTTCATCCC	GTTTCATTGAGTTAGACTGG
rs144151494	ACGTTGGATGTGACTTCAGAGGGAGACAAG	ACGTTGGATGGAACCCCTAGATTCTCTAAG	ACAAGCCCCCAAGACTGTCTG
rs150621117	ACGTTGGATGACCAGCAGCATAAGGCCTAT	ACGTTGGATGAGCCAGAAGTACTCAATGGG	GCATAAGGCCTATTAAATCCA
rs41279597	ACGTTGGATGTGCTGGCCTAGATGATAACC	ACGTTGGATGAAATGAGGCTCAGGACAATG	AGGCAGAGAAAATGCTTACCTG
rs61734638	ACGTTGGATGCACTGGAAGTCATTCACGC	ACGTTGGATGAGCGCTAATCGTCATTGAGG	CTGGAAGTCATTCACGCTGACCA
rs34372645	ACGTTGGATGACTCGAGTATGCAGTCGGTG	ACGTTGGATGCAAAGTGACGGACCTGGATG	CCTGCACACGGCGTCTCCTCCACA
rs106287	ACGTTGGATGGGTGATGGCAGCCATATCTC	ACGTTGGATGCTGCTCTCCCCTTTTCACAG	ATATCTCCTGGCTGGAGCTTGACCA
rs41272699	ACGTTGGATGTGCAGGAGGAGATTCAGTTG	ACGTTGGATGCATGCAAGGGGAAAAGAAGG	GAGAACAATTTGGCTGCCTTCCGAG
rs11575580	ACGTTGGATGACTGAGGCCAAGAACCCAG	ACGTTGGATGGAGACAGCTGCCTTTCTATG	TCCATCCTTCCCACCCC
rs11190870	ACGTTGGATGATATGGAGCTGTTTGCCTGC	ACGTTGGATGCCAACACCAGAGGAATTATC	GTTTGCCTGCGATTTGC
rs10510181	ACGTTGGATGCAAATGTGTAGGAATAGCAG	ACGTTGGATGGAGACCAACGTCTACTTAAGA	AGGAATAGCAGAGGGTC
rs55780333	ACGTTGGATGACGGACTCCCAAATCCTCT	ACGTTGGATGCACAAGGAAGCCTTCTTCAG	CCAAATCCTCTCATGAAC
rs1800273	ACGTTGGATGCCCAGTTGCATTCAATGTTT	ACGTTGGATGTACAGGAAGTCCAGGATGGC	GTTCTGACAACAGTTTGCC
rs141855950	ACGTTGGATGATGCTCCCTCTGAACCACTG	ACGTTGGATGGGAAGACAGACTAGAAGCAC	TGAACCACTGAAAGCTGTG
rs61748674	ACGTTGGATGTGATGGTAGATGGGTCGATG	ACGTTGGATGTAAGACTGATCCGAGCACAC	AGATGGGTGCGATGTCGAGA
rs63750072	ACGTTGGATGGCTTCTCTGGCGGCTGTCT	ACGTTGGATGAAGACCGCGACGTCGATGAG	CTTGGAGGGAGGGGAGTCT
rs3795424	ACGTTGGATGAGTATGAGCCTCAGTTCCTG	ACGTTGGATGGTTCTCTGCAGAGCTTTGG	CTCAGTTCCTGCGGCTCCTAG
rs6570507	ACGTTGGATGAGCAGTTAGGCTCACTTCAG	ACGTTGGATGAACTTAGCAGTCACAGTGGC	GAATTCACTCCTTGTAATAA
rs118105704	ACGTTGGATGTCCTAGCCCTCCTTATGGTC	ACGTTGGATGCGATTCTCCCAAACCTCTTG	TCATATGGAAACACATCTCCTTC

Figure S1. LBX1 promoter region