Clin Orthop Relat Res (2014) 472:3216–3225
DOI 10.1007/s11999-014-3766-8

Clinical Orthopaedics and Related Research®
A Publication of The Association of Bone and Joint Surgeons®

BASIC RESEARCH

Are Copy Number Variants Associated With Adolescent Idiopathic Scoliosis?

Jillian G. Buchan MS, David M. Alvarado PhD, Gabe Haller PhD, Hyuliya Aferol BS, Nancy H. Miller MD, Matthew B. Dobbs MD, Christina A. Gurnett MD, PhD

Received: 7 February 2014 / Accepted: 13 June 2014 / Published online: 9 July 2014

Abstract

Background Adolescent idiopathic scoliosis (AIS) is a complex genetic disorder that causes spinal deformity in approximately 3% of the population. Candidate gene, linkage, and genome-wide association studies have sought to identify genetic variation that predisposes individuals to AIS, but the genetic basis remains unclear. Copy number variants are associated with several isolated skeletal phenotypes, but their role in AIS, to our knowledge, has not been assessed.

Questions/Purposes We determined the frequency of recurrent copy number rearrangements, chromosome aneuploidy, and rare copy number variants in patients with AIS.

Methods Between January 2010 and August 2014, we evaluated 150 patients with isolated AIS and spinal curvatures measuring 10° or greater, and 148 agreed to participate. Genomic copy number analysis was performed on patients and 1079 control subjects using the Affymetrix® Genome-wide Human SNP Array 6.0. After removing poor quality samples, 143 (97%) patients with AIS were evaluated for copy number variation.

Results We identified a duplication of chromosome 1q21.1 in 2.1% (N = 3/143) of patients with AIS, which was enriched compared with 0.09% (N = 1/1079) of control subjects (p = 0.0057) and 0.07% (N = 6/8329) of a large published control cohort (p = 0.0004). Other notable findings include trisomy X, which was identified in 1.8% (N = 2/114) of female patients with AIS, and rearrangements of chromosome 15q11.2 and 16p11.2 that previously have been associated with spinal phenotypes. Finally, we report rare copy number variants that will be useful in future studies investigating candidate genes for AIS.

Funding for this project was provided by National Institutes of Health (NIH, K12 HD001459-08), Shriners Hospital for Children, and the Children's Discovery Institute of Washington University, and St. Louis Children's Hospital.
Dr. Dobbs is a Senior Editor for Clinical Orthopaedics and Related Research.
The authors report no other conflicts of interest.
All ICMJE Conflict of Interest Forms for authors and *Clinical Orthopaedics and Related Research* editors and board members are on file with the publication and can be viewed on request.
Each author certifies that his or her institution approved the human protocol for this investigation, that all investigations were conducted in conformity with ethical principles of research, and that informed consent for participation in the study was obtained.
This work was performed at Washington University School of Medicine.

Electronic supplementary material The online version of this article (doi:10.1007/s11999-014-3766-8) contains supplementary material, which is available to authorized users.

J. G. Buchan, C. A. Gurnett
Department of Genetics, Washington University School of Medicine, 660 S Euclid Ave, Box 8111, St. Louis, MO 63110, USA

D. M. Alvarado, G. Haller, H. Aferol, M. B. Dobbs, C. A. Gurnett
Department of Orthopaedic Surgery, Washington University School of Medicine, St. Louis, MO, USA

M. B. Dobbs
e-mail: dobbsm@wudosis.wustl.edu

C. A. Gurnett (✉)
Department of Neurology, Washington University School of Medicine, St. Louis, MO, USA
e-mail: gurnettc@neuro.wustl.edu

Conclusions Copy number variation and chromosomal aneuploidy may contribute to the pathogenesis of adolescent idiopathic scoliosis.
Clinical Relevance Chromosomal microarray may reveal clinically useful abnormalities in some patients with AIS.

Introduction

Scoliosis is a common spine deformity that is defined as a 10° or greater lateral curvature of the spine measured by the Cobb method on a standing radiograph [12]. Although scoliosis may result from congenital abnormalities, neuromuscular disorders or other conditions, the majority (approximately 80%) are idiopathic. Adolescent idiopathic scoliosis (AIS) develops during late childhood in otherwise healthy individuals and affects up to 3% of the population. In patients with mild scoliosis (spinal curves measuring 10°–25°), AIS affects males and females equally, but severe spinal curves (> 40°) affect adolescent females at a ratio of 10:1 [29]. While patients with mild scoliosis typically are not treated, approximately 10% of patients with AIS have a progressive deformity develop [33], and bracing or surgery may be indicated to mitigate negative physical and psychologic morbidities. Risk factors for progressive AIS include skeletal immaturity, female gender, and larger spinal curvature at initial diagnosis [43].

Monozygotic twins have higher concordance for AIS (73%) compared with dizygotic twins (36%) [23], suggesting that there are genetic factors contributing to AIS. Additionally, the incidence of AIS is greater among family members of affected patients, with 6% to 11% of first-degree relatives also affected [44, 61]. Most affected families have complex, non-Mendelian inheritance, and emerging views of AIS heritably favor a complex genetic model with large genetic heterogeneity [26, 27, 34, 35, 60]. Numerous candidate genes and linkage associations have been reported in AIS [17, 26, 58], but the importance of these loci remain unclear. Genome-wide association studies have uncovered common polymorphisms associated with AIS [25, 48, 55]. However, common polymorphisms account for only a small amount of AIS heritability, suggesting that other forms of genetic variation also play a role in AIS etiology.

Copy number variant analysis has been used successfully for patients with intellectual disability, neuropsychiatric disorders, and multiple congenital abnormalities, revealing multiple genes and genomic regions contributing to disease susceptibility [13, 38, 54]. In comparison, relatively few copy number variant studies have assessed patients with isolated skeletal phenotypes, although copy number variants have been associated with idiopathic short stature [57, 62], idiopathic clubfoot [4–6], adult-onset degenerative lumbar scoliosis [52], and bone mineral density [11]. Moreover, a recent study showed that recurrent rearrangements of chromosome 16p11.2 are risk factors for nonidiopathic scoliosis and vertebral abnormalities in children with additional developmental, neurologic, and congenital abnormalities [2]. These results show the potential for recurrent and other rare copy number variants to affected skeletal phenotypes, like scoliosis, and warrant evaluation in patients with AIS.

In this study, we determine the frequency of copy number rearrangements, chromosome aneuploidy, and rare copy number variants from a genome-wide copy number variant screen of 143 patients with AIS and report a role for copy number variants in AIS pathogenesis.

Materials and Methods

Patient Samples

Between January 2010 and August 2014, we evaluated 150 patients with isolated AIS and spinal curvatures measuring 10° or greater using the Cobb method [12], and 148 agreed to participate. The AIS cohort included primarily familial AIS with moderate to severe scoliosis (> 25°) resulting in treatment and 92% were of European-American ancestry. The average spinal curve (Cobb angle) for patients in this cohort was 49° and 80% were female (Table 1). Patients with known or suspected scoliosis etiologies (eg, Marfan syndrome, congenital abnormalities) were excluded. Growth parameters were calculated based on data from the National Center for Health Statistics [10]. Blood or saliva samples were collected for probands and available relatives after obtaining informed consent. DNA isolations were performed using the DNA Isolation Kit for Mammalian Blood (Roche, Indianapolis, IN, USA) or the Oragene® Purifier (DNA Genotek, Kanata, ON, Canada) according to the manufacturer's instructions. Replication cohorts for chromosome 1q21.1 (N = 120) and trisomy X (N = 172) included patients with AIS recruited from St. Louis Children's Hospital, St. Louis Shriners Hospital for Children, and the University of Colorado using identical inclusion criteria. Human Subjects Committees at all three institutions approved this study.

Control subjects of European-American ancestry (N = 1079) were analyzed concurrently. Control subjects were described previously and included 666 healthy subjects from a bipolar disorder study [15] and 413 patients

N. H. Miller
Department of Orthopaedic Surgery, University of Colorado, Anschutz Medical Campus, Aurora, CO, USA

M. B. Dobbs
St. Louis Shriners Hospital for Children, St. Louis, MO, USA

Table 1. Demographics of 143 AIS probands analyzed for copy number variation

Variable	Familial	Nonfamilial	Total
Patients with AIS	134 (94%)	9 (6%)	143
Males	29 (100%)	0 (0%)	29 (20%)
Females	105 (92%)	9 (8%)	114 (80%)
Cobb angle			
Mean	49° (N = 122)	50° (N = 9)	49° (N = 131)
Range	10°–97° (N = 122)	26°–74° (N = 9)	10°–97° (N = 131)

AIS = adolescent idiopathic scoliosis.

with idiopathic clubfoot [5]. Copy number variation data from 8329 published control subjects previously described in a study of developmental delay [14] were included as an additional control cohort in a subset of analyses.

Copy Number Variant Analysis

Copy number analysis was performed for 148 AIS probands and 1079 control subjects using the Genome-wide Human SNP Array 6.0 (Affymetrix®, Santa Clara, CA, USA). Copy number calls were generated with the Genotyping Console software (Affymetrix®) using a reference set of 270 HapMap controls [56]. AIS samples with contrast quality control less than 0.04 and median absolute pairwise difference greater than 0.35 or total copy number variants greater than two standard deviations more than the average were excluded (N = 5). Analysis of the remaining 143 AIS samples and 1079 control subjects was limited to copy number variants of 125 kb or greater with 50 or more markers and 10 kb or less average distance between markers to enrich our dataset with high confidence copy number variants. Copy number variants with more than 50% overlap with assembly gaps were removed. We identified rare and novel copy number variants by limiting our analysis to those that had less than 50% overlap with copy number variants at greater than 1% frequency or with all copy number variants, respectively, in the Database of Genomic Variants [32] when 100 or more individuals were evaluated. To evaluate known, clinically significant copy number disorders in patients with AIS, we identified copy number variants that overlapped genomic regions associated with recurrent copy number disorders, including 45 recurrent genomic disorder regions (< 10 Mb) examined in 8329 published controls [14]. Copy number variants in published controls were identified as previously described [14]. Quantitative PCR (qPCR) using three or more PCR primer pairs validated selected copy number variants. All sequence coordinates are reported using National Center for Biotechnology Information (NCBI) assembly build 36 (hg18) [39].

Results

Recurrent Copy Number Rearrangements

Recurrent rearrangements of chromosome 16p11.2 are associated with scoliosis and other abnormalities [2], but this locus, to our knowledge, has not been evaluated for copy number variants in a patient cohort with isolated scoliosis, and the role of 16p11.2 and other recurrent copy number variants in AIS remains unknown. Therefore, we identified patients with AIS with copy number variants overlapping 45 loci associated with recurrent copy number disorders that contribute to a broad range of phenotypes [14]. Seven patients (4.9%; N = 7/143) in our AIS cohort had copy number variants overlapping one of the recurrent genomic disorder regions. These copy number variants caused rearrangements of chromosome 1q21.1 (N = 3), chromosome 2q13 (N = 1), chromosome 15q11.2 (N = 2), and chromosome 16p11.2 (N = 1) (Table 2).

A duplication of chromosome 1q21.1 was the most frequent finding and was present in 2.1% (N = 3/143) of patients with AIS compared with 0.09% (N = 1/1079) of control subjects (p = 0.0057, one-tailed Fisher's exact test) and 0.07% (N = 6/8329) of published control subjects (p = 0.0004, one-tailed Fisher's exact test) [14]. After Bonferroni correction for 45 tests, the enrichment of chromosome 1q21.1 duplications in AIS remained significant compared with that of published control subjects (p < 0.001). Large segmental duplication blocks mediate four recurrent breakpoints in chromosome 1q21.1 copy number variants and define distinct proximal and distal regions, but only the proximal region was duplicated in all three patients (Fig. 1A). To test segregation of the proximal 1q21.1 duplication with AIS, available family members were evaluated for the presence of the copy number variant using qPCR. The proximal 1q21.1 duplication segregated with reduced penetrance in all three pedigrees (Fig. 1B). AIS ranged in severity from mild to severe in the five affected individuals with the duplication (Table 3). Two carriers of the duplication were unaffected, although this was not confirmed radiographically. Clinical

Table 2. Frequency of 45 genomic copy number disorder regions in patients with AIS

Abnormality	Genomic disorder region (Mb)	Size (kb)	Patients with AIS (this study)					Control subjects (this study)		Control subjects (published) [16]	
			Patient(s)	Phenotype(s)	CNV (Mb)	Size (kb)	Frequency	Frequency	p value	Frequency	p value
1q21.1 duplication (proximal)	chr1: 144–144.34	340	6041-001	AIS	chr1:142.72–144.97	2241	2.10% (N = 3/143)	0.09% (N = 1/1079)	0.0057	0.07% (N = 6/8329)	0.0004
			6267-001	AIS	chr1:144.08–144.50	420					
			6035-004	AIS	chr1:144.08–144.46	379					
2q13 duplication	chr2: 110.18–110.34	160	6126-001	AIS	chr2:110.13–110.52	394	0.70% (N = 1/143)	0.65% (N = 7/1079)	0.6316	0.38% (N = 32/8329)	0.4304
15q11.2 deletion	chr15: 20.35–20.64	290	6053-001	AIS	chr15:19.09–21.04	1949	0.70% (N = 1/143)	0.37% (N = 4/1079)	0.4639	0.23% (N = 19/8329)	0.2888
15q11.2 duplication	chr15: 20.35–20.64	290	6036-001	AIS	chr15:20.22–20.97	744	0.70% (N = 1/143)	0.46% (N = 5/1079)	0.5269	0.43% (N = 36/8329)	0.4681
16p11.2 duplication	chr16: 29.56–30.11	550	6032-001	AIS, spina bifida occulta	chr16:29.50–30.09	587	0.70% (N = 1/143)	0.19% (N = 2/1079)	0.3118	0.02% (N = 2/8329)	0.0498

Genomic coordinates are reported using NCBI assembly build 36 (hg18); AIS = adolescent idiopathic scoliosis; CNV = copy number variant; chr = chromosome.

Fig. 1A–B Duplications of the proximal region of chromosome 1q21.1 segregate with AIS. (**A**) Log2 ratios show duplications (shaded) of chromosome 1q21.1 in three AIS probands. The four recurrent breakpoints (BP1-BP4) and approximate locations of the proximal and distal regions are shown, but only duplications of the proximal region were common to all three patients with AIS. (Image modified from Genome Browser [http://genome.ucsc.edu]). All genomic coordinates are shown for the NCBI assembly build 36 (hg18). (**B**) The proximal chromosome 1q21.1 duplication identified in AIS probands (arrow) segregated with affected family members with reduced penetrance. Some individuals, including 001 in Family 6035 did not have DNA available to test. Dup = duplication; − = wild type.

information and family histories did not reveal evidence for intellectual or developmental disability or other significant comorbidities in the individuals with the duplication.

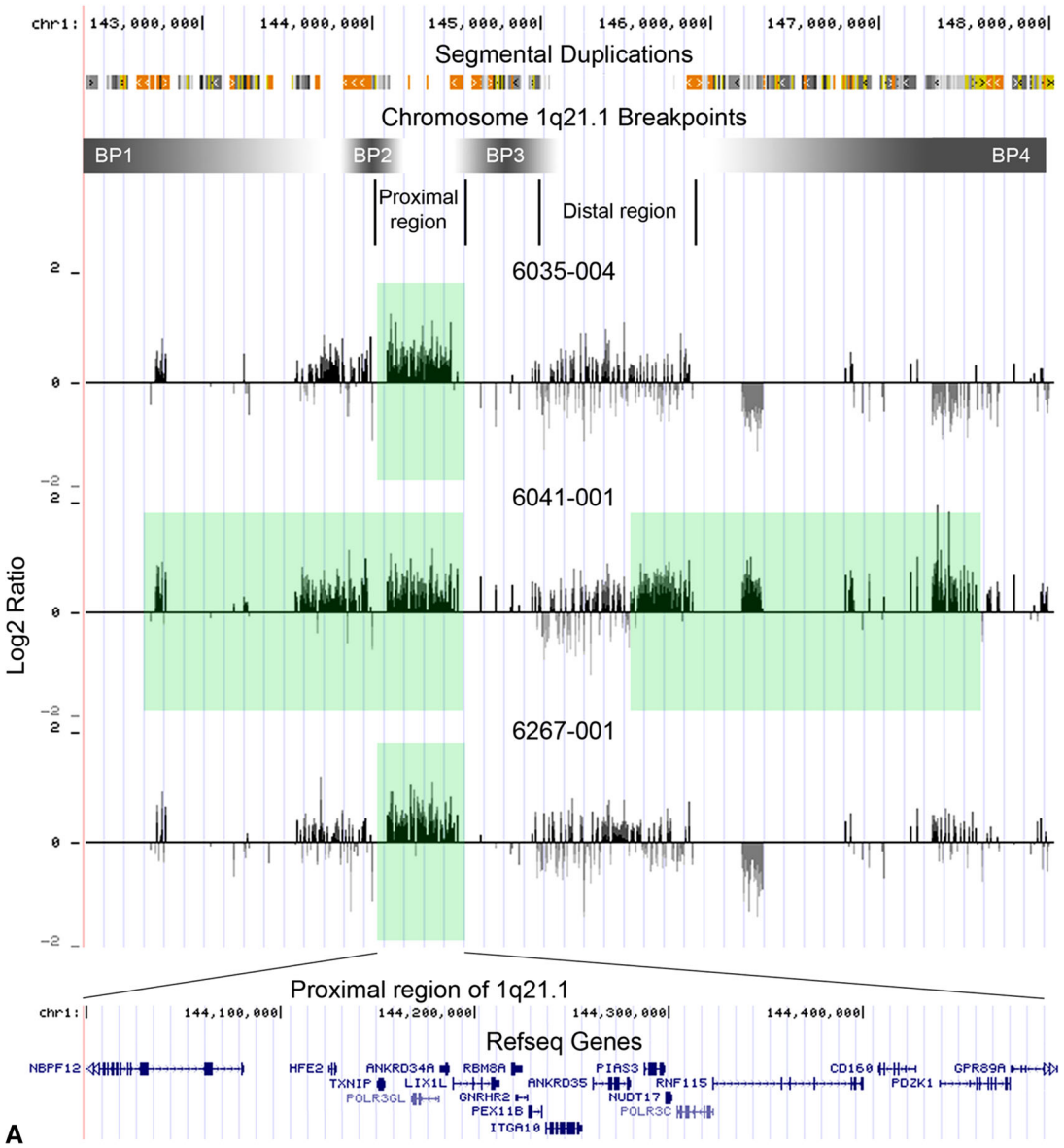
To screen for additional patients with AIS with copy number variants in chromosome 1q21.1, we used qPCR to identify proximal 1q21.copy number variants in an independent replication cohort (N = 120), but no additional chromosome 1q21.1 duplications were identified. However, even combined with the Affymetrix® Genome-wide Human SNP Array 6.0 results, the frequency of proximal 1q21.1 duplications in patients with AIS was enriched compared with that of control subjects from this study (N = 3/263 versus N = 1/1079; p = 0.0255, one-tailed Fisher's exact test) and published control subjects (N = 3/263 versus N = 6/8329; p = 0.0021, one-tailed Fisher's exact test) [14].

Trisomy X

We identified chromosomal aneuploidy in 1.4% (N = 2/143) of patients with AIS. Both patients were female with trisomy of the X chromosome (47, XXX). Trisomy X therefore was present in 1.75% (N = 2/114) of female patients with AIS and was confirmed by qPCR. Both patients had tall stature (> 99th percentile), but no additional physical or developmental comorbidities, and neither previously had been diagnosed with this condition. Only one of 529 females (0.19%) in our control cohort was identified with trisomy X, similar to the 0.11% frequency observed by Nielsen and Wohlert [40] in a large study of 17,038 newborn females. We screened an additional 172 female patients with AIS for trisomy X by qPCR and no additional patients were identified. Thus, the overall frequency of trisomy X in females with AIS was 0.7% (N = 2/286).

Rare Copy Number Variants in Patients with AIS

To determine if the overall burden of rare copy number variants differs between patients with AIS and control


chr1: 143,000,000 144,000,000 145,000,000 146,000,000 147,000,000 148,000,000
Segmental Duplications
Chromosome 1q21.1 Breakpoints
BP1
BP2
BP3
BP4
Proximal region
Distal region
6035-004
6041-001
6267-001
Log2 Ratio
Proximal region of 1q21.1
chr1: 144,100,000 144,200,000 144,300,000 144,400,000
Refseq Genes
NBPF12
HFE2
ANKRD34A
RBM8A
PIAS3
CD160
GPR89A
TXNIP
LIX1L
ANKRD35
RNF115
PDZK1
POLR3GL
GNRHR2
NUDT17
PEX11B
POLR3C
ITGA10
A

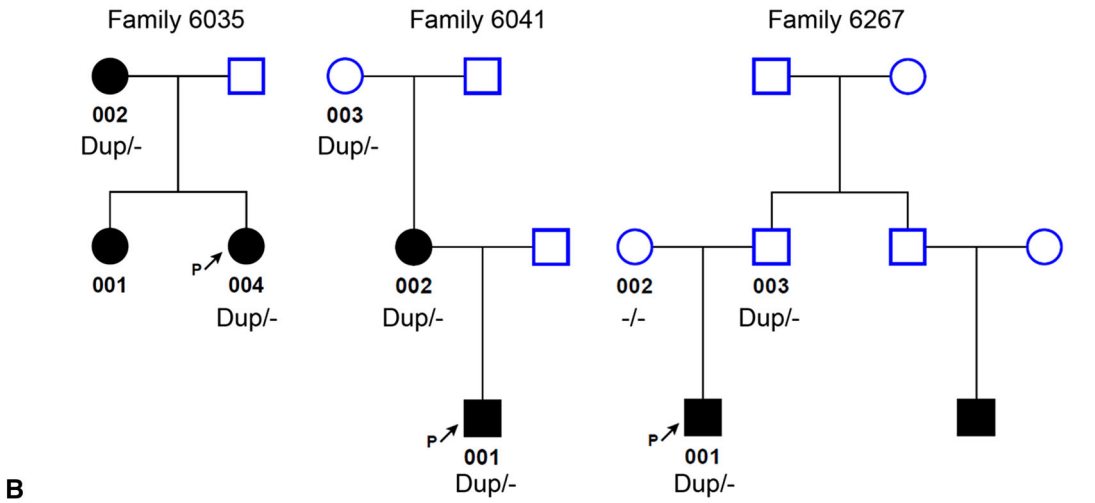

Family 6035
Family 6041
Family 6267
002
Dup/-
001
P
004
Dup/-
003
Dup/-
002
Dup/-
P
001
Dup/-
002
-/-
003
Dup/-
P
001
Dup/-
B

Table 3. Clinical information for individuals identified with chromosome 1q21.1 duplications

Family	Individual	Sex	1q21.1 status	Affected (Cobb angle)	Curve type	Treatment
6035	004*	F	Dup/−	Yes (15°)	NA	None
	002	F	Dup/−	Yes, mild	NA	None
	001	F	NA	Yes (56°)	Right thoracic	Surgery
6041	001*	M	Dup/−	Yes (31°)	Right thoracic	Brace
	002	F	Dup/−	Yes	NA	Surgery
	003	F	Dup/−	No	Not affected	Not affected
6267	001*	M	Dup/−	Yes (44°)	Right thoracic	Surgery
	002	F	−/−	No	Not affected	Not affected
	003	M	Dup/−	No	Not affected	Not affected

NA = not available; Dup = duplication; − = wild type; *proband.

subjects, we identified variants that occurred at less than 1% in the Database of Genomic Variants. We identified 177 rare copy number variants in 92 patients with AIS (Table 4). Of these variants, 118 were duplications, 59 were deletions, and the average variant size was 328 kb (Appendix 1. Supplemental material is available with the online version of CORR [21]). The frequency of large (> 750 kb), autosomal copy number variants was similar between patients with AIS (4.90%; N = 7/143) and control subjects (5.65%; N = 61/1079).

Table 4. Summary of rare copy number variants (< 1% frequency in DGV) identified in patients with AIS

Variable	Number
Patients with AIS	143
Patients with AIS with rare copy number variants	92
Total rare copy number variants	177
Rare copy number variants containing Refseq genes	130
Average copy number variant size	328 kb
Median copy number variant size	217 kb
Duplications	118
Deletions	59
Large autosomal copy number variants (> 750 kb)	7
Patients with AIS with large copy number variants	4.90%
In-house control subjects with large copy number variants	5.65%

AIS = adolescent idiopathic scoliosis; DGV = Database of Genomic Variants.

Discussion

AIS affects up to 3% of children but its genetic basis remains poorly understood. Copy number variation has been associated with isolated skeletal phenotypes such as idiopathic clubfoot [4–6], suggesting that they may be associated with additional skeletal phenotypes, such as AIS. Here, we report rare and recurrent copy number variation in a cohort of patients with AIS. In this study, 6.3% (N = 9/143) of patients with AIS were identified with a clinically relevant copy number rearrangement that previously was associated with a known condition, including trisomy X (N = 2/143) or a copy number variant affecting a region previously associated with a recurrent genomic disorder (N = 7/143). Our findings illustrate how frequently clinically relevant copy number variants will be encountered during microarray testing of patients with isolated scoliosis. Although several of the clinically important copy number variants identified in this study are unlikely to be related to AIS, others may have a role in AIS pathogenesis or contribute more generally to multiple spinal phenotypes.

There are several limitations to our study, including the relatively small sample size. For the rare copy number variants discussed here, a larger sample size would have improved frequency estimates and provided more statistical power. Moreover, in many patients, additional family members were unavailable for testing and limited our ability to provide additional support for causality. Second, control subjects used in this study included a large number of patients with idiopathic clubfoot and copy number variants already have been associated with this phenotype [4–6]. However, we also reference the frequency of recurrent copy number variants in an even larger published independent control dataset [14] and these frequencies are similar to our control dataset. Third, although the majority of patients with AIS were of European-American ancestry, including patients of other ancestral backgrounds could lead to population stratification. Finally, because our study cohort was ascertained in an orthopaedic clinic population and because patients with obvious developmental and intellectual impairment were excluded from the study, the frequency of copy number variation likely would have been greater if patients with additional comorbidities were included.

Proximal chromosome 1q21.1 duplications were the most frequently observed chromosomal abnormality identified in

patients with AIS. Duplications of 1q21.1 were present in 2.1% of AIS probands but were found in only 0.07% to 0.08% of healthy control subjects [14, 46]. The distal region of chromosome 1q21.1 is associated with many phenotypes, whereas there are far fewer disease associations with the proximal region. The strongest association is with thrombocytopenia with absent radii (TAR) syndrome [24], which frequently is caused by compound inheritance of proximal 1q21.1 deletion and a rare single nucleotide variant in *RBM8A* [3], although 1q21.1 duplications and deletions of varying sizes have been noted to include congenital heart defects [9, 20, 59], Mayer-Rokitansky-Küster-Hauser syndrome [28], autism [50], fetal urogenital abnormalities [30], and additional diverse phenotypes [22, 46]. Although the three patients with AIS in our cohort had different chromosome 1q21.1 duplication breakpoints, only the proximal region was common to all three individuals and no individuals had additional phenotypes previously associated with chromosome 1q21.1 rearrangements. Scoliosis has not been described in patients with proximal chromosome 1q21.1 duplications, although the overall number of patients with this genomic abnormality is relatively small. Scoliosis was not reported in 17 patients with proximal duplications, although two had other spine phenotypes (lumbar lordosis and lumbosacral hyperlordosis) [46]. Of 34 patients with proximal deletions, three had a spinal phenotype (scoliosis, kyphosis, and C6-C7 vertebral fusion) [46]. Although some studies [30, 46] suggest that scoliosis is not a commonly associated phenotype, scoliosis often is not detected until adolescence and therefore is likely to be underreported in younger patient cohorts. Furthermore, mild scoliosis may be underreported in children with multiple congenital anomalies whose other major medical problems are of greater concern. Duplications of 1q21.1 segregated with AIS in small families with incomplete penetrance, but incomplete penetrance has also been noted in TAR syndrome [24] and other phenotypes [45, 46]. Because additional chromosome 1q21.1 duplications were not detected after screening an additional 120 patient samples, proximal duplications are likely rarer in AIS than estimated from our original cohort of 143 patients. Nevertheless, the combined dataset of 263 patients still showed a significant enrichment of proximal 1q21.1 duplications in AIS. Additional larger studies are needed to determine the importance of proximal chromosome 1q21.1 duplications in the etiology of AIS.

Several recurrent copy number variants identified in our patient cohort with AIS primarily are associated with cognitive impairment or other related phenotypes, but also may contribute to scoliosis and other spinal phenotypes. Hemivertebrae were present in 20% (N = 2/10) of patients with chromosome 15q11.2 duplications, although none of 15 patients with the reciprocal deletion had vertebral abnormalities [1]. Two patients in our AIS cohort had chromosome 15q11.2 copy number variants. Chromosome 15q11.2 deletions are strongly associated with developmental delay [14], but the reciprocal duplication is not and the significance and associated phenotypes of 15q11.2 duplications, if any, are not well established. Neurocognitive or developmental phenotypes were not present in either of our patients with AIS with the 15q11.2 copy number variant.

Likewise, various spinal abnormalities, including hemivertebrae, syringomyelia, and scoliosis have been described in patients with rearrangements of chromosome 16p11.2 [2, 8, 16, 19, 47, 49, 51] and this locus was hypothesized to be a risk factor for idiopathic scoliosis [2]. We identified a chromosome 16p11.2 duplication in one patient with AIS with spina bifida occulta. This patient did not have intellectual or developmental disability, which is consistent with the large phenotypic variability and reduced penetrance observed in individuals with chromosome 16p11.2 duplications [53]. Interestingly, chromosome 16 previously was associated with idiopathic scoliosis by linkage analysis in 202 families [36], and fine-mapping linkage analysis of 544 individuals from an additional 95 families narrowed the association to two regions on chromosome 16, which included 16p11.2 [37], providing further support for an association of the 16p11.2 locus with AIS.

We identified trisomy of the X chromosome (47, XXX), also called triple X syndrome, in 0.7% (N = 2/286) female patients with AIS. Triple X syndrome is estimated to occur in one of 1000 female births and generally causes mild phenotypes, such as premature ovarian failure and learning disabilities, which results in many patients being undiagnosed [18]. Scoliosis is known to occur more frequently in patients with triple X syndrome than in the general population [7, 41, 42]. Specifically, Olanders described scoliosis in 15.2% (N = 5/33) of patients with triple X syndrome [41]. Patients with triple X syndrome also typically are taller than average, with the average height being greater than the 80th percentile by the age of 14 years [31]. Both patients with AIS with trisomy X in our series were tall (> 99th percentile for height) but reported no additional phenotypes. Testing for triple X syndrome should be considered in tall females with AIS so that other associated phenotypes, such as premature ovarian failure and developmental delay, can be monitored.

Finally, we report a similar burden of rare copy number variants in patients with AIS compared with control subjects, which is in contrast to many other neurodevelopmental disorders. Unfortunately, most of the AIS families included in our study were small and relatively few family members were available for testing, so the role of individual rare copy number variants in the etiology of AIS remains unexplored. Although we did not have large families for segregation analysis, rare copy number variants identified in patients with AIS are ideal resources for identifying new candidate

genes for AIS. None of the genes located in the rare or novel copy number variants has, to our knowledge, been associated with AIS previously, but this list of candidate genes will be a rich source of information for future investigations of AIS.

We analyzed copy number variation in a large cohort of patients with AIS. Our data show that more than 6% of patients with AIS harbor a clinically important copy number abnormality and many of these are possible risk factors for scoliosis and other spinal phenotypes. Our results suggest that microarray analysis for copy number variants may be a useful clinical test in this patient population. The rare number variants we provided will be useful for future studies evaluating candidate genes for AIS.

Acknowledgments We thank Drs. Keith Bridwell, Lawrence Lenke, and Scott Luhmann for allowing us to recruit patients from their clinics. We thank the patients and their families for their role in this work. The Genome Technology Access Center in the Department of Genetics at Washington University School of Medicine processed the microarrays. The Center is partially supported by NCI Cancer Center Support Grant #P30 CA91842 to the Siteman Cancer Center and by ICTS/CTSA Grant #UL1RR024992 from the National Center for Research Resources (NCRR), a component of the NIH, and NIH Roadmap for Medical Research. The control dataset used for the analysis described in this manuscript was obtained from the database of Genotype and Phenotype (dbGaP) (http://www.ncbi.nlm.nih.gov/gap) through dbGaP accession number phs000017.v3.p1. Data for control samples were provided by John R Kelsoe and John Nurnberger as part of the NIMH Bipolar Genetics Collaborative. Control samples were genotyped through the Genetic Association Information Network (GAIN). This publication is solely the responsibility of the authors and does not necessarily represent the official view of NCRR or NIH.

References

1. Abdelmoity AT, LePichon JB, Nyp SS, Soden SE, Daniel CA, Yu S. 15q11.2 proximal imbalances associated with a diverse array of neuropsychiatric disorders and mild dysmorphic features. *J Dev Behav Pediatr.* 2012;33:570–576.
2. Al-Kateb H, Khanna G, Filges I, Hauser N, Grange DK, Shen J, Smyser CD, Kulkarni S, Shinawi M. Scoliosis and vertebral anomalies: additional abnormal phenotypes associated with chromosome 16p11.2 rearrangement. *Am J Med Genet A.* 2014;164A:1118–1126.
3. Albers CA, Paul DS, Schulze H, Freson K, Stephens JC, Smethurst PA, Jolley JD, Cvejic A, Kostadima M, Bertone P, Breuning MH, Debili N, Deloukas P, Favier R, Fiedler J, Hobbs CM, Huang N, Hurles ME, Kiddle G, Krapels I, Nurden P, Ruivenkamp CA, Sambrook JG, Smith K, Stemple DL, Strauss G, Thys C, van Geet C, Newbury-Ecob R, Ouwehand WH, Ghevaert C. Compound inheritance of a low-frequency regulatory SNP and a rare null mutation in exon-junction complex subunit RBM8A causes TAR syndrome. *Nat Genet.* 2012;44:435–439.
4. Alvarado DM, Aferol H, McCall K, Huang JB, Techy M, Buchan J, Cady J, Gonzales PR, Dobbs MB, Gurnett CA. Familial isolated clubfoot is associated with recurrent chromosome 17q23.1q23.2 microduplications containing TBX4. *Am J Hum Genet.* 2010;87:154–160.
5. Alvarado DM, Buchan JG, Frick SL, Herzenberg JE, Dobbs MB, Gurnett CA. Copy number analysis of 413 isolated talipes equinovarus patients suggests role for transcriptional regulators of early limb development. *Eur J Hum Genet.* 2013;21:373–380.
6. Alvarado DM, McCall K, Aferol H, Silva MJ, Garbow JR, Spees WM, Patel T, Siegel M, Dobbs MB, Gurnett CA. Pitx1 haploinsufficiency causes clubfoot in humans and a clubfoot-like phenotype in mice. *Hum Mol Genet.* 2011;20:3943–3952.
7. Barr ML, Sergovich FR, Carr DH, Saver EL. The triplo-X female: an appraisal based on a study of 12 cases and a review of the literature. *Can Med Assoc J.* 1969;101:247–258.
8. Bijlsma EK, Gijsbers AC, Schuurs-Hoeijmakers JH, van Haeringen A, Fransen van de Putte DE, Anderlid BM, Lundin J, Lapunzina P, Perez Jurado LA, Delle Chiaie B, Loeys B, Menten B, Oostra A, Verhelst H, Amor DJ, Bruno DL, van Essen AJ, Hordijk R, Sikkema-Raddatz B, Verbruggen KT, Jongmans MC, Pfundt R, Reeser HM, Breuning MH, Ruivenkamp CA. Extending the phenotype of recurrent rearrangements of 16p11.2: deletions in mentally retarded patients without autism and in normal individuals. *Eur J Med Genet.* 2009;52:77–87.
9. Brunet A, Armengol L, Heine D, Rosell J, Garcia-Aragones M, Gabau E, Estivill X, Guitart M. BAC array CGH in patients with Velocardiofacial syndrome-like features reveals genomic aberrations on chromosome region 1q21.1. *BMC Med Genet.* 2009;10:144.
10. CDC Centers for Disease Control and Prevention. National Center for Health Statistics. Health, United States, 2013. Available at: http://www.cdc.gov/nchs/. Accessed January 3, 2014.
11. Chew S, Dastani Z, Brown SJ, Lewis JR, Dudbridge F, Soranzo N, Surdulescu GL, Richards JB, Spector TD, Wilson SG. Copy number variation of the APC gene is associated with regulation of bone mineral density. *Bone.* 2012;51:939–943.
12. Cobb JR. Outline for the study of scoliosis. *Instr Course Lect.* 1948;5:261–265.
13. Cook EH Jr, Scherer SW. Copy-number variations associated with neuropsychiatric conditions. *Nature.* 2008;455:919–923.
14. Cooper GM, Coe BP, Girirajan S, Rosenfeld JA, Vu TH, Baker C, Williams C, Stalker H, Hamid R, Hannig V, Abdel-Hamid H, Bader P, McCracken E, Niyazov D, Leppig K, Thiese H, Hummel M, Alexander N, Gorski J, Kussmann J, Shashi V, Johnson K, Rehder C, Ballif BC, Shaffer LG, Eichler EE. A copy number variation morbidity map of developmental delay. *Nat Genet.* 2011;43:838–846.
15. Dick DM, Foroud T, Flury L, Bowman ES, Miller MJ, Rau NL, Moe PR, Samavedy N, El-Mallakh R, Manji H, Glitz DA, Meyer ET, Smiley C, Hahn R, Widmark C, McKinney R, Sutton L, Ballas C, Grice D, Berrettini W, Byerley W, Coryell W, DePaulo R, MacKinnon DF, Gershon ES, Kelsoe JR, McMahon FJ, McInnis M, Murphy DL, Reich T, Scheftner W, Nurnberger JI Jr. Genomewide linkage analyses of bipolar disorder: a new sample of 250 pedigrees from the National Institute of Mental Health Genetics Initiative. *Am J Hum Genet.* 2003;73:107–114.
16. Fernandez BA, Roberts W, Chung B, Weksberg R, Meyn S, Szatmari P, Joseph-George AM, Mackay S, Whitten K, Noble B, Vardy C, Crosbie V, Luscombe S, Tucker E, Turner L, Marshall CR, Scherer SW. Phenotypic spectrum associated with de novo and inherited deletions and duplications at 16p11.2 in individuals ascertained for diagnosis of autism spectrum disorder. *J Med Genet.* 2010;47:195–203.
17. Gorman KF, Julien C, Moreau A. The genetic epidemiology of idiopathic scoliosis. *Eur Spine J.* 2012;21:1905–1919.
18. Gustavson KH. [Triple X syndrome deviation with mild symptoms: the majority goes undiagnosed][in Swedish]. *Lakartidningen.* 1999;96:5646–5647.
19. Hernando C, Plaja A, Rigola MA, Perez MM, Vendrell T, Egocue J, Fuster C. Comparative genomic hybridisation shows a partial

de novo deletion 16p11.2 in a neonate with multiple congenital malformations. *J Med Genet.* 2002;39:E24.
20. Hitz MP, Lemieux-Perreault LP, Marshall C, Feroz-Zada Y, Davies R, Yang SW, Lionel AC, D'Amours G, Lemyre E, Cullum R, Bigras JL, Thibeault M, Chetaille P, Montpetit A, Khairy P, Overduin B, Klaassen S, Hoodless P, Awadalla P, Hussin J, Idaghdour Y, Nemer M, Stewart AF, Boerkoel C, Scherer SW, Richter A, Dube MP, Andelfinger G. Rare copy number variants contribute to congenital left-sided heart disease. *PLoS Genet.* 2012;8:e1002903.
21. Huang N, Lee I, Marcotte EM, Hurles ME. Characterising and predicting haploinsufficiency in the human genome. *PLoS Genet.* 2010;6:e1001154.
22. Kaminsky EB, Kaul V, Paschall J, Church DM, Bunke B, Kunig D, Moreno-De-Luca D, Moreno-De-Luca A, Mulle JG, Warren ST, Richard G, Compton JG, Fuller AE, Gliem TJ, Huang S, Collinson MN, Beal SJ, Ackley T, Pickering DL, Golden DM, Aston E, Whitby H, Shetty S, Rossi MR, Rudd MK, South ST, Brothman AR, Sanger WG, Iyer RK, Crolla JA, Thorland EC, Aradhya S, Ledbetter DH, Martin CL. An evidence-based approach to establish the functional and clinical significance of copy number variants in intellectual and developmental disabilities. *Genet Med.* 2011;13:777–784.
23. Kesling KL, Reinker KA. Scoliosis in twins: a meta-analysis of the literature and report of six cases. *Spine (Phila Pa 1976).* 1997;22:2009–2014; discussion 2015.
24. Klopocki E, Schulze H, Strauss G, Ott CE, Hall J, Trotier F, Fleischhauer S, Greenhalgh L, Newbury-Ecob RA, Neumann LM, Habenicht R, Konig R, Seemanova E, Megarbane A, Ropers HH, Ullmann R, Horn D, Mundlos S. Complex inheritance pattern resembling autosomal recessive inheritance involving a microdeletion in thrombocytopenia-absent radius syndrome. *Am J Hum Genet.* 2007;80:232–240.
25. Kou I, Takahashi Y, Johnson TA, Takahashi A, Guo L, Dai J, Qiu X, Sharma S, Takimoto A, Ogura Y, Jiang H, Yan H, Kono K, Kawakami N, Uno K, Ito M, Minami S, Yanagida H, Taneichi H, Hosono N, Tsuji T, Suzuki T, Sudo H, Kotani T, Yonezawa I, Londono D, Gordon D, Herring JA, Watanabe K, Chiba K, Kamatani N, Jiang Q, Hiraki Y, Kubo M, Toyama Y, Tsunoda T, Wise CA, Qiu Y, Shukunami C, Matsumoto M, Ikegawa S. Genetic variants in GPR126 are associated with adolescent idiopathic scoliosis. *Nat Genet.* 2013;45:676–679.
26. Kouwenhoven JW, Castelein RM. The pathogenesis of adolescent idiopathic scoliosis: review of the literature. *Spine (Phila Pa 1976).* 2008;33:2898–2908.
27. Kruse LM, Buchan JG, Gurnett CA, Dobbs MB. Polygenic threshold model with sex dimorphism in adolescent idiopathic scoliosis: the Carter effect. *J Bone Joint Surg Am.* 2012;94:1485–1491.
28. Ledig S, Schippert C, Strick R, Beckmann MW, Oppelt PG, Wieacker P. Recurrent aberrations identified by array-CGH in patients with Mayer-Rokitansky-Kuster-Hauser syndrome. *Fertil Steril.* 2011;95:1589–1594.
29. Lenke L, Dobbs MB. Idiopathic scoliosis. In: Frymoyer JW, Wiesel SW, eds. *The Adult and Pediatric Spine*. Philadelphia, PA: Lippincott, Williams & Wilkins; 2004;337–360.
30. Liao C, Fu F, Yi CX, Li R, Yang X, Xu Q, Li DZ. Prenatal diagnosis of an atypical 1q21.1 microdeletion and duplication associated with foetal urogenital abnormalities. *Gene*. 2012;507:92–94.
31. Linden MG, Bender BG, Harmon RJ, Mrazek DA, Robinson A. 47,XXX: what is the prognosis? *Pediatrics.* 1988;82:619–630.
32. MacDonald JR, Ziman R, Yuen RK, Feuk L, Scherer SW. The Database of Genomic Variants: a curated collection of structural variation in the human genome. *Nucleic Acids Res.* 2014;42(Database issue):D986–992.
33. Miller NH. Cause and natural history of adolescent idiopathic scoliosis. *Orthop Clin North Am.* 1999;30:343–352.
34. Miller NH. Genetics of familial idiopathic scoliosis. *Clin Orthop Relat Res.* 2007;462:6–10.
35. Miller NH. Idiopathic scoliosis: cracking the genetic code and what does it mean? *J Pediatr Orthop.* 2011;31(1 suppl):S49–52.
36. Miller NH, Justice CM, Marosy B, Doheny KF, Pugh E, Zhang J, Dietz HC 3rd, Wilson AF. Identification of candidate regions for familial idiopathic scoliosis. *Spine (Phila Pa 1976).* 2005;30:1181–1187.
37. Miller NH, Justice CM, Marosy B, Swindle K, Kim Y, Roy-Gagnon MH, Sung H, Behneman D, Doheny KF, Pugh E, Wilson AF. Intra-familial tests of association between familial idiopathic scoliosis and linked regions on 9q31.3–q34.3 and 16p12.3–q22.2. *Hum Hered.* 2012;74:36–44.
38. Morrow EM. Genomic copy number variation in disorders of cognitive development. *J Am Acad Child Adolesc Psychiatry*. 2010;49:1091–1104.
39. NCBI. Build 36 (hg18). Available at: http://www.ncbi.nlm.nih.gov/mapview/stats/BuildStats.cgi?taxid=9606&build=36&ver=1. Accessed 04 Jan 2014.
40. Nielsen J, Wohlert M. Chromosome abnormalities found among 34,910 newborn children: results from a 13-year incidence study in Arhus, Denmark. *Hum Genet.* 1991;87:81–83.
41. Olanders S. *Females with Supernumerary X Chromosomes; A Study of 39 Psychiatric Cases*, Stockholm, Sweden: Esselte Studium; 1977;12–16.
42. Otter M, Schrander-Stumpel CT, Curfs LM. Triple X syndrome: a review of the literature. *Eur J Hum Genet.* 2010;18:265–271.
43. Reamy BV, Slakey JB. Adolescent idiopathic scoliosis: review and current concepts. *Am Fam Physician.* 2001;64:111–116.
44. Riseborough EJ, Wynne-Davies R. A genetic survey of idiopathic scoliosis in Boston, Massachusetts. *J Bone Joint Surg Am.* 1973;55:974–982.
45. Rosenfeld JA, Coe BP, Eichler EE, Cuckle H, Shaffer LG. Estimates of penetrance for recurrent pathogenic copy-number variations. *Genet Med.* 2013;15:478–481.
46. Rosenfeld JA, Traylor RN, Schaefer GB, McPherson EW, Ballif BC, Klopocki E, Mundlos S, Shaffer LG, Aylsworth AS; 1q21.1 Study Group. Proximal microdeletions and microduplications of 1q21.1 contribute to variable abnormal phenotypes. *Eur J Hum Genet.* 2012;20:754–761.
47. Schaaf CP, Goin-Kochel RP, Nowell KP, Hunter JV, Aleck KA, Cox S, Patel A, Bacino CA, Shinawi M. Expanding the clinical spectrum of the 16p11.2 chromosomal rearrangements: three patients with syringomyelia. *Eur J Hum Genet.* 2011;19:152–156.
48. Sharma S, Gao X, Londono D, Devroy SE, Mauldin KN, Frankel JT, Brandon JM, Zhang D, Li QZ, Dobbs MB, Gurnett CA, Grant SF, Hakonarson H, Dormans JP, Herring JA, Gordon D, Wise CA. Genome-wide association studies of adolescent idiopathic scoliosis suggest candidate susceptibility genes. *Hum Mol Genet.* 2011;20:1456–1466.
49. Shen Y, Chen X, Wang L, Guo J, Shen J, An Y, Zhu H, Zhu Y, Xin R, Bao Y, Gusella JF, Zhang T, Wu BL. Intra-family phenotypic heterogeneity of 16p11.2 deletion carriers in a three-generation Chinese family. *Am J Med Genet B Neuropsychiatr Genet.* 2011;156:225–232.
50. Shen Y, Dies KA, Holm IA, Bridgemohan C, Sobeih MM, Caronna EB, Miller KJ, Frazier JA, Silverstein I, Picker J, Weissman L, Raffalli P, Jeste S, Demmer LA, Peters HK, Brewster SJ, Kowalczyk SJ, Rosen-Sheidley B, McGowan C, Duda AW 3rd, Lincoln SA, Lowe KR, Schonwald A, Robbins M, Hisama F, Wolff R, Becker R, Nasir R, Urion DK, Milunsky JM, Rappaport L, Gusella JF, Walsh CA, Wu BL, Miller DT, Autism Consortium Clinical Genetics/DNA Diagnostics Collaboration. Clinical

genetic testing for patients with autism spectrum disorders. *Pediatrics.* 2010;125:e727–735.
51. Shimojima K, Inoue T, Fujii Y, Ohno K, Yamamoto T. A familial 593-kb microdeletion of 16p11.2 associated with mental retardation and hemivertebrae. *Eur J Med Genet.* 2009;52:433–435.
52. Shin JH, Ha KY, Jung SH, Chung YJ. Genetic predisposition in degenerative lumbar scoliosis due to the copy number variation. *Spine (Phila Pa 1976).* 2011;36:1782–1793.
53. Shinawi M, Liu P, Kang SH, Shen J, Belmont JW, Scott DA, Probst FJ, Craigen WJ, Graham BH, Pursley A, Clark G, Lee J, Proud M, Stocco A, Rodriguez DL, Kozel BA, Sparagana S, Roeder ER, McGrew SG, Kurczynski TW, Allison LJ, Amato S, Savage S, Patel A, Stankiewicz P, Beaudet AL, Cheung SW, Lupski JR. Recurrent reciprocal 16p11.2 rearrangements associated with global developmental delay, behavioural problems, dysmorphism, epilepsy, and abnormal head size. *J Med Genet.* 2010;47:332–341.
54. Stankiewicz P, Lupski JR. Structural variation in the human genome and its role in disease. *Annu Rev Med.* 2010;61:437–455.
55. Takahashi Y, Kou I, Takahashi A, Johnson TA, Kono K, Kawakami N, Uno K, Ito M, Minami S, Yanagida H, Taneichi H, Tsuji T, Suzuki T, Sudo H, Kotani T, Watanabe K, Chiba K, Hosono N, Kamatani N, Tsunoda T, Toyama Y, Kubo M, Matsumoto M, Ikegawa S. A genome-wide association study identifies common variants near LBX1 associated with adolescent idiopathic scoliosis. *Nat Genet.* 2011;43:1237–1240.
56. The International HapMap Consortium. The International HapMap Project. *Nature*. 2003;426:789–796.
57. van Duyvenvoorde HA, Lui JC, Kant SG, Oostdijk W, Gijsbers AC, Hoffer MJ, Karperien M, Walenkamp MJ, Noordam C, Voorhoeve PG, Mericq V, Pereira AM, Claahsen-van de Grinten HL, van Gool SA, Breuning MH, Losekoot M, Baron J, Ruivenkamp CA, Wit JM. Copy number variants in patients with short stature. *Eur J Hum Genet.* 2014;22:602–609.
58. Wang WJ, Yeung HY, Chu WC, Tang NL, Lee KM, Qiu Y, Burwell RG, Cheng JC. Top theories for the etiopathogenesis of adolescent idiopathic scoliosis. *J Pediatr Orthop.* 2011;31(1 suppl):S14–27.
59. Warburton D, Ronemus M, Kline J, Jobanputra V, Williams I, Anyane-Yeboa K, Chung W, Yu L, Wong N, Awad D, Yu CY, Leotta A, Kendall J, Yamrom B, Lee YH, Wigler M, Levy D. The contribution of de novo and rare inherited copy number changes to congenital heart disease in an unselected sample of children with conotruncal defects or hypoplastic left heart disease. *Hum Genet.* 2014; 133:11–27.
60. Ward K, Ogilvie J, Argyle V, Nelson L, Meade M, Braun J, Chettier R. Polygenic inheritance of adolescent idiopathic scoliosis: a study of extended families in Utah. *Am J Med Genet A.* 2010;152A:1178–1188.
61. Wynne-Davies R. Familial (idiopathic) scoliosis: a family survey. *J Bone Joint Surg Br.* 1968;50:24–30.
62. Zahnleiter D, Uebe S, Ekici AB, Hoyer J, Wiesener A, Wieczorek D, Kunstmann E, Reis A, Doerr HG, Rauch A, Thiel CT. Rare copy number variants are a common cause of short stature. *PLoS Genet.* 2013;9:e1003365.