Exome sequencing identifies a rare HSPG2 variant associated with familial idiopathic scoliosis

Erin E. Baschal  
*University of Colorado, Denver*

Cambria I. Wethey  
*University of Colorado, Denver*

Kandice Swindle  
*University of Colorado, Denver*

Robin M. Baschal  
*Children's Hospital Colorado*

Katherine Gowan  
*University of Colorado, Denver*

*See next page for additional authors*

Follow this and additional works at: [https://digitalcommons.wustl.edu/open_access_pubs](https://digitalcommons.wustl.edu/open_access_pubs)

**Recommended Citation**

Baschal, Erin E.; Wethey, Cambria I.; Swindle, Kandice; Baschal, Robin M.; Gowan, Katherine; Tang, Nelson L.S.; Alvarado, David M.; Haller, Gabe E.; Dobbs, Matthew B.; Taylor, Matthew R.G.; Gurnett, Christina A.; Jones, Kenneth L.; and Miller, Nancy H., "Exome sequencing identifies a rare HSPG2 variant associated with familial idiopathic scoliosis." *G3*. 5,2. 167-174. (2015).  
https://digitalcommons.wustl.edu/open_access_pubs/4501

This Open Access Publication is brought to you for free and open access by Digital Commons@Becker. It has been accepted for inclusion in Open Access Publications by an authorized administrator of Digital Commons@Becker. For more information, please contact vanam@wustl.edu.
Exome Sequencing Identifies a Rare HSPG2 Variant Associated with Familial Idiopathic Scoliosis

Erin E. Baschal,* Cambria I. Wethey,* Kandice Swindle,* Robin M. Baschal,† Katherine Gowan,‡ Nelson L.S. Tang,§ David M. Alvarado,** Gabe E. Haller,** Matthew B. Dobbs,**† Matthew R.G. Taylor,**† Christina A. Gurnett,**† Kenneth L. Jones,§ and Nancy H. Miller*†,‡

†Department of Orthopedics, University of Colorado Denver Anschutz Medical Campus, Aurora, Colorado 80045, ‡Musculoskeletal Research Center, Children’s Hospital Colorado, Aurora, Colorado 80045, §Department of Biochemistry, University of Colorado Denver Anschutz Medical Campus, Aurora, Colorado 80045, **Department of Chemical Pathology and Li Ka Shing Institute of Health Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong SAR, ††Department of Orthopaedic Surgery, Washington University School of Medicine, St. Louis, Missouri 63130, ‡‡Saint Louis Shriners Hospital for Children, St. Louis, Missouri 63131, ††Department of Cardiology, University of Colorado Denver Anschutz Medical Campus, Aurora, Colorado 80045, and §§Department of Neurology, Washington University School of Medicine, St. Louis, Missouri 63130

ABSTRACT  Idiopathic scoliosis occurs in 3% of individuals and has an unknown etiology. The objective of this study was to identify rare variants that contribute to the etiology of idiopathic scoliosis by using exome sequencing in a multigenerational family with idiopathic scoliosis. Exome sequencing was completed for three members of this multigenerational family with idiopathic scoliosis, resulting in the identification of a variant in the HSPG2 gene as a potential contributor to the phenotype. The HSPG2 gene was sequenced in a separate cohort of 100 unrelated individuals affected with idiopathic scoliosis and also was examined in an independent idiopathic scoliosis population. The exome sequencing and subsequent bioinformatics filtering resulted in 16 potentially damaging and rare coding variants. One of these variants, p.Asn786Ser, is located in the HSPG2 gene. The variant p.Asn786Ser also is overrepresented in a larger cohort of idiopathic scoliosis cases compared with a control population (P = 0.024). Furthermore, we identified additional rare HSPG2 variants that are predicted to be damaging in two independent cohorts of individuals with idiopathic scoliosis. The HSPG2 gene encodes for a ubiquitous multifunctional protein within the extracellular matrix in which loss of function mutation are known to result in a musculoskeletal phenotype in both mouse and humans. Based on these results, we conclude that rare variants in the HSPG2 gene potentially contribute to the idiopathic scoliosis phenotype in a subset of patients with idiopathic scoliosis. Further studies must be completed to confirm the effect of the HSPG2 gene on the idiopathic scoliosis phenotype.

KEYWORDS HSPG2 perlecan idiopathic scoliosis exome sequencing

Idiopathic scoliosis (IS) [MIM: %181800] is a common inherited disorder of the immature skeleton, affecting 3% of the pediatric population, with girls characteristically more severely affected than boys (Asher and Burton 2006). Clinically, IS is defined as a structural lateral spinal curvature of ≥10° with a rotatory component, documented by radiographic analysis and occurring in otherwise-normal children. Current treatment modalities are limited to observation, physical therapy, bracing, and surgery. Significant curves in adolescence can lead to severe curve progression in adulthood. In an effort to prevent severe progression and deformity as an adult, these adolescents may elect to undergo an operative spinal instrumentation and fusion, a costly procedure with life-long implications. These methods focus on the physical symptoms and cosmetic deformity but fail to address the need for preventative options.

The familial nature of IS is well established, but the mode of inheritance is not clear (Cowell et al. 1972; Riseborough and Wynnedavies 1973; Bonaiti et al. 1976; Czeizel et al. 1978; Tang et al. 2012). It is unlikely that IS families are homogeneous with respect to their
genetic architecture but represent a spectrum of inheritance patterns ranging from simple Mendelian to complex polygenic. Concordance rates have been reported between 0.73 and 0.92 for monozygotic twins and between 0.36 and 0.63 for dizygotic twins (Kesling and Reinker 1997; Inoue et al. 1998; Andersen et al. 2007). Despite clear evidence for the importance of genetics in the susceptibility to IS, limited progress has been made in the identification of genes that are causative or may influence disease susceptibility, variation, or progression. The current literature includes multiple studies that have used linkage and association analyses. Genome-wide linkage analyses have effectively localized candidate regions; however, progress has been burdened by heterogeneity both within and between study populations and by insufficient statistical power (Wise et al. 2000; Chan et al. 2002; Salehi et al. 2002; Justice et al. 2003; Miller et al. 2005, 2006; Alden et al. 2006; Ocaka et al. 2008; Gurnett et al. 2009; Raggio et al. 2009; Clough et al. 2010; Marosy et al. 2010; Edery et al. 2011). Association studies, including genome-wide association studies (GWAS), have implicated select genes, including CHD7, LBX1, and GPR126 (Gao et al. 2007; Sharma et al. 2011; Takahashi et al. 2011; Kou et al. 2013). A recent study has completed exome sequencing for an IS cohort and identified rare variants in the fibrillin genes, FBN1 and FBN2, which were associated with curve severity (Buchan et al. 2014).

In the current study, we completed exome sequencing for a multigenerational family with familial IS, with the goal of identifying rare genetic variants that contribute to the IS phenotype.

MATERIAL AND METHODS

Subjects

Denver IS population: For the Denver IS population, written informed consent was obtained from study subjects who were enrolled in accordance with protocols approved by the Johns Hopkins School of Medicine Institutional Review Board and the University of Colorado Denver Institutional Review Board (Colorado Multiple Institutional Review Board, Study Approval #06-1161 and 07-0417). A diagnosis of IS required clinical/family history and physical examination consistent with a spinal curvature in the coronal plane, a standing anteroposterior curvature by the Cobb method with pedicle rotation, and no congenital deformity or other existing genetic or structural abnormality. Individuals were classified as having familial IS if they had at least one first-degree relative affected with IS (Justice et al. 2003; Miller et al. 2005). The majority of individuals in our study are likely to exhibit an autosomal dominant mode of inheritance (Miller et al. 2005, 2012). We collected blood samples from all participants and extracted genomic DNA according to standard phenol-chloroform purification protocols (Sambrook et al. 1989; Moore and Dowhan 2002) or using the QIAGEN Gentra Puregene Blood Kit.

St. Louis IS population: As described by Buchan et al. (2014), IS patients in the St. Louis dataset were recruited from St. Louis Children’s Hospital and St. Louis Shriners Hospital for Children. All patients had scoliosis of unknown etiology with spinal curves measuring ≥10°. Patients with developmental delay, multiple congenital anomalies, or known underlying medical disorders (e.g., Ehlers-Danlos syndrome, Marfan syndrome) were excluded. We selected 140 unrelated IS cases of European ancestry with severe deformity (spinal curves measuring ≥40°) for the exome sequencing screen. DNA was collected from affected probands after we obtained informed consent.

Control populations: Comparisons with control populations used either the 1000 Genomes population (European ancestry, N = 379) (Abecasis et al. 2012) or the National Heart, Lung, and Blood Institute (NHLBI) Exome Sequencing Project (ES) European ancestry subset (EA-ESP), version ESP6500SI-V2, N = 4300.

Exome sequencing

Denver exome sequencing: A multigenerational family with European ancestry was selected for exome sequencing, with DNA samples available on five affected and three unaffected individuals (two of the unaffected individuals are marry-in relatives). This family was selected based on the number of affected family members with severe curves and the relative pedigree-distance between affected family members. Exome capture was performed on 1 μg of genomic DNA from three affected individuals (IV:1, IV:6, and II:4) in this family using the Illumina TruSeq Exome Capture kit. Samples were sequenced with a 2x100bp run at the Illumina HiSeq 2000 at the University of Colorado Denver Genomics and Microarray Core Facility.

St. Louis exome sequencing: For the St. Louis IS dataset, exon enrichment was performed using the SureSelect Human All Exon 38Mb and 50Mb kits (Agilent Technologies, Santa Clara, CA) or the TruSeq Exome Enrichment kit (Illumina, San Diego, CA) at the Washington University Genome Technology Access Center, as described previously (Buchan et al. 2014).

Bioinformatics filtering

Exome sequencing reads were mapped to the reference human genome sequence (hg19) with large-scale alignment software (GSNAP) (Wu and Nacu 2010). Sequence calls for variants (single nucleotide variants (SNVs), insertions/deletions) were performed using the Broad Institute’s Genome Analysis Toolkit (GATK) (McKenna et al. 2010; Depristo et al. 2011). The resulting variants were filtered in a systematic way. The program ANNOVAR was used to filter the variants by cross referencing various genetic variation databases (e.g., 1000 genomes database, NHLBI Exome Sequencing Project 6500 dataset, etc), allowing us to extract information about variant frequencies (if previously reported) and location within genes (Wang et al. 2010). Variants were restricted to those that (1) had no more than a 1% frequency in the phase 1 1000 genomes data (Abecasis et al. 2012), and 2) altered the coding sequence (nonsense, splice-site, missense and insertion/deletion). The nonsense and missense variants were subsequently cross-referenced to the dbNSFP database to determine whether the resulting changes to the protein are predicted to be damaging (see Supporting Information, File S1) (Liu et al. 2011). In addition, we required at least 5X sequence coverage at any given position to call a genotype. For the multigenerational family, we required that all three individuals share the variant if they had a genotype at that position.

For the St. Louis dataset, next-generation sequencing reads were aligned to hg19 human reference sequence and variants were called and annotated, as described by Buchan et al. (2014).

Sanger sequencing

The variants that remained after the filtering process were confirmed by Sanger sequencing. The variants identified through exome sequencing were tested in the original three affected individuals who underwent exome sequencing and five additional relatives (two affected and three unaffected). As discussed in the Results section, one variant
(chromosome 6, 168376925, in HGC6.3) had a high frequency in the EA-ESP population and therefore was not Sanger sequenced.

Primers were designed using the program Primer3 (Koressaar and Remm 2007; Untergasser et al. 2012). Primer sequences can be found in Table S1. Sanger sequencing was completed at either the University of Colorado Denver Barbara Davis Center for Childhood Diabetes or at Beckman Coulter Genomics. Details of PCR conditions and Sanger sequencing protocols are located in File S1.

Sanger sequences were analyzed using CodonCode software (CodonCode Corporation, Centerville, MA), and figures were generated using Sequencher (GeneCodes Corporation, Ann Arbor, MI).

**HSPG2 sequencing**

Exons from the HSPG2 gene (97 exons, 14369 base pair mRNA, 4391 amino acids) were sequenced in 100 unrelated individuals with IS. All individuals had a curvature of >10° with a range of 18–119°), and were predominantly of European ancestry. To decrease sequencing costs, sequencing was completed using 25 pools of four individuals each. An Illumina MiSeq 2x250bp run was performed at the University of Colorado Denver DNA Diagnostic Laboratory. The HSPG2 libraries were enriched using long-range polymerase chain reaction (PCR), resulting in approximately 2.5 to 10 kb fragments with a Takara long range PCR kit (Clontech). PCR conditions followed manufacturer’s recommendations. Primer sequences can be found in Table S1. For high GC regions, betaine was used in the PCR. A GENios FL (Tecan) microplate reader using SYBR green was used to quantify the libraries. The library was generated using a Nextera XT DNA sample kit (FC-121-1031; Illumina) and Nextera Index kit (FC-121-1011; Illumina).

Bioinformatics filtering was completed using the same protocol as described earlier. The filtering process resulted in 14 potentially damaging and rare HSPG2 variants in these 100 individuals. The 14 variants were Sanger sequenced in each of the four individuals within the pool to confirm the variant and identify the individual within the pool that carried the variant. The Sanger sequencing protocol was the same as described for the multigenerational family. We were unable to confirm an HSPG2 variant at position 22263709 (p.Met1Thr) due to problems with the PCR-amplification process. This variant was removed from all further analyses, resulting in a total of 13 rare variants in the HSPG2 gene.

The median sequencing coverage across the HSPG2 gene was 172X, with a range of 45X to 349X. Exons 27–42 had lower coverage in multiple pools, where the median coverage was 68X across these 16 exons. Due to these concerns with sequence quality, we chose to Sanger sequence all 100 IS individuals for all 13 variants to confirm the variant frequency in our population.

**Statistical comparisons**

The Fisher’s exact test (two-sided) was used to calculate the P-values for comparisons between the IS population(s) and the 1000 Genomes European population or the EA-ESP, with α = 0.05. Mulator 2.0, beta-31 was used to generate and check all nucleotide and protein positions (Wildeman et al. 2008), and positions were verified using MutationTaster and the public version of the Human Gene Mutation Database (HGMD) (Schwarz et al. 2014; Stenson et al. 2014).

**RESULTS**

**Exome sequencing identifies an HSPG2 variant in an IS family**

We studied a multigenerational family affected with familial IS and of European ancestry (Figure 1A). Affected individuals were diagnosed with familial IS based on radiographic findings of a spinal curvature of ≥10°. The proband, individual III:2, was diagnosed at 13 years of age with a right thoracic curve and a left lumbar curve of 60° and 40°, respectively, and underwent posterior spinal instrumentation and fusion. The transmission pattern in this family suggested a Mendelian mode of inheritance, for which exome sequencing is ideally suited. Exome sequencing, followed by variant detection and filtering, was completed for three individuals in this family. Sequencing coverage was 64× overall, with IV:1, IV:6, II:4 at 97X, 82X, and 22X respectively. Filtering resulted in a list of 16 variants that were at a frequency of <1% in the 1000 Genomes data, were predicted to be damaging (according to the criteria defined in the Materials and Methods section and File S1), and were insertions/deletions, nonsense, missense, or splice-site changes. In addition, to be considered for further study, variants were required to be present in all three affected individuals in the family.

After the filtering process, 16 potentially damaging and rare variants were identified in this family (Table 1 and Table S2). One variant was removed due to an excessive frequency in the EA-ESP dataset (in HGC6.3, 23% frequency in EA-ESP). For each of the remaining 15 variants, Sanger sequencing was completed for the individuals who originally underwent exome sequencing and for additional relatives with DNA samples [two affected and three unaffected (two of the unaffected individuals were marry-in relatives)]. Sanger sequencing was unable to replicate the original result for 1 of the 15 variants (OPRD1) in the original three exome sequenced individuals (Table S3).

After filtering and Sanger sequencing, there were 14 variants of interest (Table 1). Therefore, we surveyed the literature for mouse and human phenotypic associations. Previously reported mouse phenotypes for loss-of-function mutations in 13 of the 14 genes produced either phenotypes not clearly associated with musculoskeletal disease or had no evident phenotype. Those 13 genes also have no reported association with a human musculoskeletal phenotype.

The single remaining variant is NM_005529.5:p.Asn786Ser, a heterozygous missense variant in the HSPG2 gene (MIM *142461) on chromosome 1p36. All of the affected individuals in this family are heterozygous for this variant (CT genotype) (Figure 1B). Of the three unaffected individuals, two are homozygous for the reference allele (TT genotype) and one is heterozygous (IV:2, CT genotype). The presence of the CT genotype in an unaffected male may be due to incomplete penetrance of the IS phenotype.

HSPG2 encodes for heparan sulfate proteoglycan 2, or perlecan. Perlecan is a key component of basement membranes and is strongly associated with musculoskeletal development, both in mouse and in human (Costell et al. 1999; Nicole et al. 2000; Arikawa-Hirasawa et al. 2001b; Stum et al. 2006; Rodgers et al. 2007), making it an excellent candidate gene for IS.

**Sequencing of the HSPG2 gene in 100 individuals with IS**

Seeking to further our knowledge of the importance of the HSPG2 gene in IS, we designed an HSPG2 sequencing project. Exons from the HSPG2 gene (97 exons, 14369 base pair mRNA, 4391 amino acids) were sequenced in 100 unrelated individuals with IS (Denver dataset, curvature range 18–119°) with a custom targeted gene approach on an Illumina MiSeq platform. After variant filtering using the bioinformatic pipeline described previously, we found 14 potentially damaging and rare HSPG2 variants. Again, variants were defined as potentially damaging according to our criteria defined in the Materials and Methods section and File S1, and rare if the frequency was <1% in the 1000 Genomes database. We were unable to confirm one variant with
Sanger sequencing, and it was consequently removed from further analyses (see the section Materials and Methods). Each variant had a frequency of 1% in the 1000 Genomes database, and 3 of the 13 identified variants were unique (not previously identified in the 1000 genomes data or in the NHLBI ESP; Table 2 and Table S4). All 13 variants were tested by Sanger sequencing in all 100 individuals to assess variant frequencies in our IS population. Including one individual from our multigenerational family (IV:1), we had a total of 101 individuals with 20 occurrences of HSPG2 variants. One individual had two HSPG2 variants. One of the 13 variants was the same variant previously identified from the exome sequencing of our multigenerational IS family (p. Asn786Ser). This specific variant was heterozygous in 3 of the 100 additional sequenced individuals. In the 1000 Genomes data, this variant was only found in four individuals, all male (three from the European subset and one from the Puerto Rican subset). This difference is significant, with the CT genotype present in 4 of 101 IS individuals compared with 77/4300 EA-ESP, \( P = 0.046, \text{OR} = 2.5, 95\% \text{CI} 0.95-6.0 \). Furthermore, an additional 11 potentially damaging variants in HSPG2 were discovered that were not previously identified in the Denver IS group, for a total of 18 HSPG2 variants in the St. Louis dataset.

In summary, in the Denver IS dataset, we identified 13 potentially damaging and rare variants in the HSPG2 gene, with 20 occurrences in 101 individuals with IS. This finding provides further support that HSPG2 may have an important role in IS etiology.

### Further investigation of the HSPG2 finding in an independent IS population

Next, we investigated HSPG2 damaging variants in the exome sequencing data from an independent group of 140 IS individuals (St. Louis dataset). We identified 7 of the original 13 Denver HSPG2 variants in this St. Louis dataset, including p.Asn786Ser. The CT genotype for this variant is present in six individuals in the St. Louis dataset (compared with 77/4300 EA-ESP, \( P = 0.046, \text{OR} = 2.5, 95\% \text{CI} 0.95-6.0 \)). Furthermore, an additional 11 potentially damaging variants in HSPG2 were discovered that were not previously identified in the Denver IS group, for a total of 18 HSPG2 variants in the St. Louis dataset.

---

**Figure 1** (A) Pedigree for the multigenerational family with IS. Individuals IV:1, IV:6, and II:4 underwent exome sequencing. Sanger sequencing was completed for those three individuals in addition to the affected individuals III:2 and III:6 and unaffected individuals III:1, III:7, and IV:2. Lumbar and thoracic curves are abbreviated with L and T, respectively. Double curves are represented by a slash (right thoracic/left lumbar), and triple curves are represented with three slashes (left high thoracic/right thoracic/left lumbar). CT or TT is the individual genotype at HSPG2 position p. Asn786Ser. A “?” represents individuals that have not been examined, so their scoliosis status is unknown. (B) Chromatograms for Variant p. Asn786Ser. Chromatograms for p.Asn786Ser are shown for two individuals from the multigenerational family (III:6, affected, and III:7, unaffected). The variant p.Asn786Ser, in the HSPG2 gene, is heterozygous in the affected individuals within the family, but not in the unaffected individuals (with the exception of IV:2, as discussed in the section Results).
dataset (Table 2 and Table S4). We tested all 18 variants by Sanger sequencing and found that three low-quality variants did not conform, resulting in 15 verified HSPG2 variants from the St. Louis dataset. One individual had two HSPG2 variants.

Collectively, in the combined Denver-St. Louis IS population, the data for the p.Asn786Ser variant are compelling. There are 10 IS individuals with a CT genotype of 241 individuals total. We compared this with 77 of 4300 in the EA-ESP population and found that the CT genotype is significantly enriched in the combined IS population ($P = 0.024$, OR 2.4, 95% CI 1.1–4.8).

Overall, 21 potentially damaging and rare variants in HSPG2 were identified in the combined Denver-St. Louis IS population, with 48 occurrences in 241 individuals. The variants are distributed across the HSPG2 gene (Figure 2).

| Chr Position | Gene | rs # | Ref | Var | HGVS Protein Position | Variant Type | 1000 Genomes Frequency | EA-ESP Frequency |
|--------------|------|------|-----|-----|-----------------------|--------------|------------------------|------------------|
| Chr1 22205601 | HSPG2 | rs143736974 | T | C | p.N786S | Missense | 0.0018 | 0.009 |
| Chr1 22333346 | EPHB2 | rs28936395 | G | A | p.D679N | Missense | 0.0023 | 0.0071 |
| Chr1 28060600 | FAM76A | rs147811051 | G | A | p.R121H | Missense | 0.00081 | 
| Chr1 29189481 | OPRD1 | rs370180812 | G | T | p.A269S | Missense | 0.00017 | 
| Chr1 45524416 | ZSWIM5 | rs149382804 | G | A | p.R373W | Missense | 0.00046 | 0.0014 |
| Chr1 51873967 | EGF2 | rs16753384 | G | A | p.S438L | Missense | 0.0064 | 0.022 |
| Chr4 77662160 | SHROOM3 | rs368255851 | A | T | p.D945V | Missense | 0.00012 | 
| Chr6 43483718 | YIPF3 | rs18512691 | GCAT | G | p.D65del | Nonframeshift deletion | 0.0095 |
| Chr1 168376925 | HGC6.3 | rs35001101 | G | GT | p.T137Hfs*7 | Frameshift insertion | 0.0024 |
| Chr7 139281560 | HIPK2 | T | TCTGCCG | p.Q873_T874insRQ | Nonframeshift insertion | |
| Chr7 149473614 | SSPO | rs117048984 | A | G | p.Y77C | Missense | 0.0047 | 0.0016 |
| Chr7 150938427 | CHF2 | rs144589067 | G | A | p.A1182V | Missense | 0.00087 | 0.0022 |
| Chr1 132055190 | ADCY8 | rs75246765 | C | A | p.A204S | Missense | 0.0006 | 0.016 |
| Chr8 133883671 | TG | rs114322847 | C | T | p.P1118L | Missense | 0.00087 | 0.022 |
| Chr10 112570222 | RBM20 | rs18512691 | G | A | p.S49L | Missense | 0.00097 | 0.0097 |
| ChrX 132092485 | HS6ST2 | C | T | p.G4254S | Missense | 1 | 0.0021 |
| Chr1 109929303 | EGF2 | rs149382804 | C | T | p.P292L | Missense | 0.00097 | 0.0097 |
| Chr1 22205601 | HSPG2 | rs143736974 | T | C | p.N786S | Missense | 0.0018 | 0.009 |
| Chr1 22333346 | EPHB2 | rs28936395 | G | A | p.D679N | Missense | 0.0023 | 0.0071 |
| Chr1 28060600 | FAM76A | rs147811051 | G | A | p.R121H | Missense | 0.00081 | 
| Chr1 29189481 | OPRD1 | rs370180812 | G | T | p.A269S | Missense | 0.00017 | 
| Chr1 45524416 | ZSWIM5 | rs149382804 | G | A | p.R373W | Missense | 0.00046 | 0.0014 |
| Chr1 51873967 | EGF2 | rs16753384 | G | A | p.S438L | Missense | 0.0064 | 0.022 |
| Chr4 77662160 | SHROOM3 | rs368255851 | A | T | p.D945V | Missense | 0.00012 | 
| Chr6 43483718 | YIPF3 | rs18512691 | GCAT | G | p.D65del | Nonframeshift deletion | 0.0095 |
| Chr1 168376925 | HGC6.3 | rs35001101 | G | GT | p.T137Hfs*7 | Frameshift insertion | 0.0024 |
| Chr7 139281560 | HIPK2 | T | TCTGCCG | p.Q873_T874insRQ | Nonframeshift insertion | |
| Chr7 149473614 | SSPO | rs117048984 | A | G | p.Y77C | Missense | 0.0047 | 0.0016 |
| Chr7 150938427 | CHF2 | rs144589067 | G | A | p.A1182V | Missense | 0.00087 | 0.0022 |
| Chr8 132055190 | ADCY8 | rs75246765 | C | A | p.A204S | Missense | 0.0006 | 0.016 |
| Chr8 133883671 | TG | rs114322847 | C | T | p.P1118L | Missense | 0.00087 | 0.022 |
| Chr10 112570222 | RBM20 | rs18512691 | G | A | p.S49L | Missense | 0.00097 | 0.0097 |

**Table 1** Rare variants (MAF 0.01)\(^a\) identified in our multigenerational family with IS\(^b\)

**Table 2** HSPG2 rare variants (MAF <0.01)\(^a\) identified in individuals with IS\(^b\)

---

*MAF, minor allele frequency; IS, idiopathic scoliosis; Chr, chromosome; Ref, reference allele; Var, variant allele; EA-ESP, European American Exome Sequencing Project.

\(^a\) The original MAF filter used the 1000 genomes frequency.

\(^b\) Additional details for each variant are provided in Table S2.

\(^c\) Variant in the OPARD1 gene was not present in all of the affected individuals in the family and was removed from further analyses.

\(^d\) Variant in the HGC6.3 gene was removed from further analyses due to the high frequency in the EA-ESP population.
DIscussIOn

We performed exome sequencing for a multigenerational family with familial IS and identified a potentially damaging and rare variant in the HSPG2 gene. Our previous linkage results were reviewed for potential correlation with the location of newly identified variants (Miller et al. 2005, 2006; Alden et al. 2006; Clough et al. 2010; Marosy et al. 2010). When this particular family was analyzed individually, marginal linkage signals were noted at nine loci (Miller, unpublished data, 2014). One of these loci, located on chromosome 1 (19.48.3 Mb), contains the HSPG2 gene. These ancillary linkage data provide additional evidence that a variant within HSPG2 potentially contributes to the IS phenotype in this family.

Upon further sequencing of the HSPG2 gene in an additional 100 individuals with IS, we identified 13 potentially damaging and rare variants in HSPG2, with 20 occurrences in 101 individuals. Finally, using exome sequencing data from a second IS population, we found 15 variants in HSPG2. Combined, we found 21 rare variants with 48 occurrences in 241 individuals with IS. Specifically, p.Asn786Ser was present in 10 individuals in our dataset and is enriched in these two IS populations when compared with the EA-ESP dataset (P = 0.024, OR 2.4).

HSPG2 encodes for heparan sulfate proteoglycan 2, or perlecan. Perlecan is a modular proteoglycan consisting of five structural core domains to which long chains of glycosaminoglycans (GAGs: heparan sulfate or chondroitin sulfate) are attached (Figure 2) (Murdoch et al. 1992). Domain I, the N-terminal domain, is unique to perlecan and has three attachment sites for heparan sulfate/chondroitin sulfate chains. Domain II is homologous to low-density lipoprotein (LDL) receptor sites. Domain III consists of multiple modules homologous to laminin alpha chains and laminin epidermal growth factor-like (EGF-like) repeats and is divided into three subdomains (III-1, III-2, III-3) (Schulze et al. 1996). Fibroblast growth factor-7 (FGF-7), platelet-derived growth factor (PDGF), and von Willebrand A-domain related protein (WARP) are known to bind subdomains III-1, III-2, and III-2, respectively (Gohring et al. 1998; Mongiat et al. 2000; Allen et al. 2006). The largest domain, domain IV, contains multiple immunoglobulin G-like (IgG-like) repeats similar to neural cell adhesion molecules. Domain V, at the C-terminus of the protein, has homology to the globular domain of laminin and contains a GAG attachment and EGF-like repeats. This domain interacts with cell-surface integrins (α2β1), forming additional complexes linking the extracellular matrix (ECM) with the cell, and is responsible for perlecan self-assembly (Bix et al. 2004).

Perlecan stabilizes the ECM and is localized to basement membranes, vascular structures, cartilage, and osteogenic tissues (Costell et al. 1999). This protein is abundantly expressed by skeletal myofibers and is incorporated into the basal lamina of both the synaptic and extrasynaptic regions within the neuromuscular junction (NMJ) (Singhal and Martin 2011). Perlecan binds multiple proteins and signaling molecules (including FBN1 [previously implicated in IS (Buchan et al. 2014)], FGF-7, latent transforming growth factor-beta binding protein-2 (LTBP-2)), indicating it has a role as an ECM signaling scaffold with an ability to participate in cellular proliferation, differentiation, and migration (Arikawa-Hirasawa et al. 1999; Farach-Carson and Carson 2007; Hayes et al. 2011; Hayes et al. 2014). Perlecan is strongly associated with musculoskeletal development because it is essential for endochondral growth, regeneration, and both avascular cartilage and skeletal development, making HSPG2 an excellent gene candidate for IS.

Classically, pathogenic mutations in the FBN1 gene result in Marfan syndrome, a multisystem disorder that includes the musculoskeletal abnormality of scoliosis. Early investigations noted FBN1 abnormalities in the paraspinous tissues and fibroblasts of select individuals with severe IS (Miller et al. 1996). Most recently, Buchan et al. (2014) identified rare variants in the FBN1 gene as risk factors for severe spinal deformity in individuals with IS (Buchan et al. 2014). Collectively, these findings and the known colocalization of perlecan and FBN1 within paraspinal tissues make HSPG2 an excellent candidate gene for IS pathogenesis.

Mutations within the HSPG2 gene have been associated with two human diseases transmitted in an autosomal recessive pattern of inheritance, Schwartz-Jampel syndrome, type 1 (SJS1) [MIM: #255800] and dyssegmental dysplasia Silverman-Handmaker type (DDSH) [MIM: #224410]. Individuals with SJS1 are characterized by a skeletal dysplasia, including kyphoscoliosis, joint contractures, and myotonia resulting in a fixed facial expression and prolonged muscle contractions (Nicolet et al. 2000; Stum et al. 2006). SJS1 mutations occur throughout the HSPG2 gene and are not concentrated in a specific exon or domain (Table S5) (Nicolet et al. 2000; Arikawa-Hirasawa et al. 2002; Stum et al. 2006; Rodgers et al. 2007). In SJS1, the mutated proteins have varying degrees of functionality. Animal models have shown site-specific mutations of the gene, which result in down-regulation of perlecan at the transcriptional level (Rodgers et al. 2007). In contrast, DDSH is a more severe phenotype than SJS1 and is considered lethal (Arikawa-Hirasawa et al. 2001b). The causative mutational events for DDSH are functional null mutations, resulting in a truncated perlecan protein core that is not secreted (Table S5) (Arikawa-Hirasawa et al. 2001a). This is similar to the Hspg2 homozygous knockout mouse, which exhibits significant kyphoscoliosis and skeletal defects (Costell et al. 1999). Thus, homozygous HSPG2 mutations in humans and mice display a more severe phenotype with an earlier onset compared with the adolescent-onset FIS phenotype in individuals with heterozygous HSPG2 variants. This finding suggests that haploinsufficiency may result in a later onset and progressive disease, as observed in FIS.

Similar to SJS1 mutational events, the variants we identified in IS individuals are spread out across the HSPG2 gene. Our original variant (p.Asn786Ser) is located in domain III-1, which is known to bind
We completed a test of variant burden across the entire HSPG2 gene, comparing the occurrence of potentially damaging and rare variants in individuals with IS to the EA-ESP control population. We used a Z test for population proportions to compare the number of variant occurrences in the two populations. Using the overall combined dataset, we found there were 48 occurrences in 241 individuals with IS compared with 720 occurrences in 4300 individuals in the EA-ESP dataset ($P = 0.20$). If we remove the variants in perlecan domain IV from both datasets, because domain IV is highly repetitive and the functions are more likely to be redundant within the gene, there are 31 occurrences in 241 individuals with IS compared with 385 occurrences in 4300 individuals in the EA-ESP dataset ($P = 0.040$). This marginally significant result increases our confidence that variants in HSPG2 contribute to the IS phenotype, but sequencing in additional individuals with IS will be needed to draw strong conclusions.

IS, defined as a lateral spinal curvature of at least 10°, is present in 3% of the general population and can be undetected within many individuals. Thus, a variant that contributes to the IS phenotype could be expected to be present in a control population at a reasonably high frequency. In addition, IS is characterized by incomplete penetrance, so not all individuals who carry a variant are expected to have clinical disease, as seen with individual IV-2, who is unaffected but is heterozygous at the variant of interest (p.Asn786Ser). Finally, we do not expect that one gene is causative for IS in all individuals. We believe that HSPG2 may contribute to the IS phenotype in a subset of individuals with IS but that other genes may be required to modify the disease and result in clinical/identifiable disease (i.e., another variant in a different gene may also be required to have a larger/progressive curvature). Future studies should include both sequencing the HSPG2 gene in larger IS cohorts and performing functional studies of the identified variants to provide confirmation that the variants contribute to the IS phenotype.

In summary, IS is a complex disease. Genetic findings have been hampered by incomplete penetrance, subclinical disease in control populations, unknown modes of inheritance, and genetic heterogeneity. Additionally, IS may be a polygenic disease, even within families. By demonstrating the presence of the p.Asn786Ser variant in all affected individuals in a multigenerational family, with incomplete penetrance in one unaffected individual, and enrichment of this variant in two independent cohorts of IS patients, we now have evidence that rare variants in the HSPG2 gene potentially contribute to IS susceptibility.

**ACKNOWLEDGMENTS**

We thank the many people who contributed to this project: Bifeng Gao and Katrina Diener at the University of Colorado Denver Genomics and Microarray Core Facility, Randall Wong at the Barbara Davis Center for Childhood Diabetes Molecular Biology Core Facility, Hongyan Wang and Binbin Li at Fudan University, Steve Sperber and Yong Shi at the University of Colorado Denver DNA Diagnostic Laboratory for their work on sequencing the HSPG2 gene, and Rebecca O’Rourke for her analysis of the HSPG2 sequencing results. Grant funding was provided by the LARRK Foundation, Rose Brown Endowment Funds, Children’s Hospital of Colorado Research Institute, and by research grants from Shriners Hospital for Children, Children’s Discovery Institute of Washington University, St Louis Children’s Hospital, and the University of Missouri Spinal Cord Injuries Research Program. Our research used the REDCap database, which is supported by NIH/NCCR Colorado CTSI Grant ULI TR000154. We thank the NHLBI GO Exome Sequencing Project and its ongoing studies which produced and provided exome variant calls for comparison: the Lung GO Sequencing Project (HL-102923), the WHI Sequencing Project (HL-102924), the Broad GO Sequencing Project (HL-102925), the Seattle GO Sequencing Project (HL-102926) and the Heart GO Sequencing Project (HL-103010).

**LITERATURE CITED**

Abecasis, R. G., A. Auton, L. D. Brooks, M. A. DePristo, R. M. Durbin et al., 2012 An integrated map of genetic variation from 1,092 human genomes. Nature 491: 56–65.

Alden, K. J., B. Marosy, N. Naegwu, C. M. Justice, A. F. Wilson et al., 2006 Idiopathic scoliosis: identification of candidate regions on chromosome 19p13. Spine 31: 1815–1819.

Allen, J. M., J. F. Bateman, U. Hansen, R. Wilson, P. Bruckner et al., 2006 WARP is a novel multimeric component of the chondrocyte pericellular matrix that interacts with perlecan. J. Biol. Chem. 281: 7341–7349.

Andersen, M. O., K. Thomsen, and K. O. Kyvistik, 2007 Adolescent idiopathic scoliosis in twins: a population-based study. Spine 32: 927–930.

Arikawa-Hirasawa, E., H. Watanabe, H. Takami, J. R. Hassell, and Y. Yamada, 1999 Perlecan is essential for cartilage and cecal development. Nat. Genet. 23: 354–358.

Arikawa-Hirasawa, E., W. R. Wilcox, A. H. Le, N. Silverman, P. Govindraj et al., 2001a Dyssegmental dysplasia, Silverman-Handmaker type, is caused by functional null mutations of the perlecan gene. Nat. Genet. 27: 431–434.

Arikawa-Hirasawa, E., W. R. Wilcox, and Y. Yamada, 2001b Dyssegmental dysplasia, Silverman-Handmaker type: unexpected role of perlecan in cartilage development. Am. J. Med. Genet. 106: 254–257.

Arikawa-Hirasawa, E., A. H. Le, I. Nishino, I. Nonaka, N. C. Ho et al., 2002 Structural and functional mutations of the perlecan gene cause Schwartz-Jampel syndrome, with myotonic myopathy and chondrodysplasia. Am. J. Hum. Genet. 70: 1368–1375.

Armstrong, G. W., N. B. Livermore, 3rd, N. Suzuki, and J. G. Armstrong, 1982 Nonstandard vertebral rotation in scoliosis screening patients. Its prevalence and relation to the clinical deformity. Spine 7: 50–54.

Asher, M. A., and D. C. Burton, 2006 Adolescent idiopathic scoliosis: natural history and long term treatment effects. Scoliosis 1: 2.

Bix, G., J. Fu, E. M. Gonzalez, L. Macro, A. Barker et al., 2004 Endorepellin causes endothelial cell disassembly of actin cytoskeleton and focal adhesions through alpha2beta1 integrin. J. Cell Biol. 166: 97–109.

Bonaïti, C., J. Feingold, M. L. Briard, F. Lapeyre, P. Rigault et al., 2004 Genetics of idiopathic scoliosis [in French]. Helv. Paediatr. Acta 51: 229–240.

Buchan, J. G., D. M. Alvarado, G. E. Haller, C. Cruchaga, M. B. Harms et al., 2014 Rare variants in FBN1 and FBN2 are associated with severe adolescent idiopathic scoliosis. Hum. Mol. Genet. 23: 5271–5282.

Chan, V., G. C. Fong, K. D. Luk, B. Yip, M. K. Lee et al., 2002 A genetic locus for adolescent idiopathic scoliosis linked to chromosome 19p13.3. Am. J. Hum. Genet. 71: 401–406.

Clough, M., C. M. Justice, B. Marosy, and N. H. Miller, 2010 Males with familial idiopathic scoliosis: a distinct phenotypic subgroup. Spine 35: 162–168.

Costell, M., E. Gustafsson, A. Azzodi, M. Morgelin, W. Bloch et al., 1999 Perlecan maintains the integrity of cartilage and some basement membranes. J. Cell Biol. 147: 1109–1122.

Cowell, H. R., J. N. Hall, and G. D. MacEwen, 1972 Genetic aspects of idiopathic scoliosis: a distinct phenotypic subgroup. Spine 35: 162–168.

DePristo, M. A., E. Banks, R. Poplin, K. V. Garimella, J. R. Maguire et al., 2011 A framework for variation discovery and genotyping using next-generation DNA sequencing data. Nat. Genet. 43: 491–498.
