

Functional variants of *POC5* identified in patients with idiopathic scoliosis

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Idiopathic scoliosis (IS) is a spine deformity that affects approximately 3% of the population. The underlying causes of IS are not well understood, although there is clear evidence that there is a genetic component to the disease. Genetic mapping studies suggest high genetic heterogeneity, but no IS disease-causing gene has yet been identified. Here, genetic linkage analyses combined with exome sequencing identified a rare missense variant (p.A446T) in the centriolar protein gene *POC5* that cosegregated with the disease in a large family with multiple members affected with IS. Subsequently, the p.A446T variant was found in an additional set of families with IS and in an additional 3 cases of IS. Moreover, *POC5* variant p.A455P was present and linked to IS in one family and another rare *POC5* variant (p.A429V) was identified in an additional 5 cases of IS. In a zebrafish model, expression of any of the 3 human IS-associated *POC5* variant mRNAs resulted in spine deformity, without affecting other skeletal structures. Together, these findings indicate that mutations in the *POC5* gene contribute to the occurrence of IS.

Introduction

Idiopathic scoliosis (IS) is a 3D spinal deformity characterized by lateral curvature of the spine of 10° or more, as measured by Cobb's method, and rotation of the vertebral bodies. The etiology of IS remains unknown, but several studies strongly suggest a contribution from genetic factors (1, 2). Familial aggregation of IS cases was first observed many decades ago (3–5), and the disease is familial in approximately 40% of cases (6, 7). In some of these cases, transmission of the trait appears to be autosomal dominant. However, the results of genome-wide linkage studies in multiplex families were inconclusive, suggesting that IS is genetically heterogeneous (8–10). GWAS in case/control samples were recently used to identify candidate loci for IS susceptibility (11–13); however, functional variants unambiguously causing IS have not yet been identified. In the present study, we sought to refine the search for IS susceptibility loci and to identify the disease-causing gene in a large family with a high prevalence of IS.

Results and Discussion

Using the affected-only method in a large multiplex family with 11 affected individuals and 1 obligate carrier (family F2), we previously mapped a causative IS gene to either chromosome 5q13.3 or

3q12.3 (8). To identify the IS disease-causing gene, we refined the candidate regions for family F2 to chromosome 5q13.3: 73,905,694–79,488,191 (NCBI hg19; Supplemental Figure 1; supplemental material available online with this article; doi:10.1172/JCI77262DS1) and chromosome 3q12.3: 95,083,093–107,153,338 (NCBI hg19; data not shown) and performed whole-exome sequencing on 3 affected individuals from this family.

This provided a sequencing depth per target base of 134 times on average, with at least 10 times for 98.6% of bases (Supplemental Figure 2 and Supplemental Table 1). Variant calling revealed 825 changes (599 single nucleotide variants [SNVs] and 226 indels) in the 3q12.3 and 5q13.3 critical chromosomal regions for these 3 subjects. To identify potentially pathogenic variants, we first excluded synonymous variants or variants located in introns, except for those affecting consensus splice sites. We then parsed a total of 172 variants (Supplemental Table 2) to keep only those that were novel or rare (minor allele frequency [MAF] <5%), which yielded 2 candidate SNVs, one in *GPR128* and the other in *POC5* (Table 1). We excluded the *GPR128* SNV because it did not cosegregate with IS in family F2. The other SNV, c.G1336A, is located in the *POC5* gene (NM_001099271; Supplemental Table 3), where it results in a single amino acid change, p.A446T. The presence of this SNV was then confirmed by Sanger sequencing in all affected members of family F2 (Figure 1). The c.G1336A *POC5* SNV was also observed in 3 probands from 40 additional multiplex IS families and cosegregated with

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Table 1. Criteria used for filtering whole-exome sequencing SNVs and indels

Total variants in all 3 affected individuals combined	272,492
Variants after base quality filtering	237,947
Variants in 3q12.3 and 5q13.3 linkage regions	825 (599 SNVs + 226 indels)
Filtering variants within linkage regions	
Variants present in 3 out of 3 affected individuals	299 (234 SNVs + 65 indels)
Nonsynonymous, indels, and splice-site variants	172
Variants present at MAF < 5% in our control exome data set ($n = 1165$), 1000 Genomes, and dbSNP	2 (<i>GPR128</i> and <i>POC5</i> SNVs)

the disease in all 3 families (F19, F35, and F41, Figure 2A and Supplemental Figure 3, A–C). Cotransmission of this SNV and the disease in families F19, F35, and F41 occurred in 6 meioses (4 meioses in family F19, 1 meiosis in family F35, and 1 meiosis in family F41; Figure 2A). Thus, the probability of a random occurrence of this cosegregation is very low (1 in 64, or ~1.6%). This reinforces the hypothesis that either this SNV or a neighboring DNA modification plays a role in IS. This SNV was also identified in 3/300 chromosomes (1.00%) examined from isolated IS cases (Figure 2A) and was found in 19 chromosomes of 1,268 control individuals of similar ancestry (French, French Canadian, or European, allelic frequency: 19/2536 [~0.75%]).

In addition, a previously unreported *POC5* SNV, c.G1363C (p.A455P), was detected in affected members of IS family F31 (Figure 2B and Supplemental Figure 3D). This SNV was found neither in IS cases C1–C150 nor in control individuals. Another rare *POC5* variant, c.C1286T (p.A429V), was identified in 5/300 chromosomes (~1.65%) from IS patients (Figure 2C and Supplemental Figure 3E), but not in familial IS. This variation is present in 9 chromosomes of control individuals (allelic frequency: 9/2536 [0.34%]). Both the c.G1336A (p.A446T) and c.C1286T (p.A429V) *POC5* SNVs were found more frequently in 191 IS probands than in the controls ($P = 0.0445$ and $P = 0.0273$, respectively; Supple-

mental Table 4). Given that the c.G1336A (p.A446T) *POC5* SNV was found in 4/41 multiplex IS families, we considered the hypothesis that these 4 IS families could be related. We therefore used the Omniexpress SNP array to determine whether there was a founder effect for this SNV. The variant was found to occur on the same 166-kb ancestral haplotype (denoted H), including the whole *POC5* gene and part of the neighboring *ANKDD1B* gene, in all IS patients of this study (Supplemental Figure 4), suggesting that they are remotely related.

To test whether the c.G1336A (p.A446T), c.G1363C (p.A455P), and c.C1286T (p.A429V) *POC5* SNVs are functional or in linkage disequilibrium with another nearby functional DNA modification, we examined the effects of these *POC5* SNVs on skeletal development in zebrafish (*Danio rerio*). Vertebrate spines share remarkable similarities, and given that *POC5* is highly conserved (14) between species and because of the conservation between the zebrafish and human orthologs (58% amino acid similarity), zebrafish should be an accurate model in studying the role of this gene in human IS (15, 16). First, endogenous *pod5* knockdown caused a curly-tailed phenotype; however, embryos with this mutation did not survive past 72 hours post-fertilization (hpf). Coinjection of WT human *POC5* mRNA rescued the phenotype of *pod5* knockdown zebrafish, but not mutated versions of *POC5* mRNA, indicating that the human gene can compensate for the zebrafish gene (Supplemental Figures 5 and 6). To exclude the possibility that this axial phenotype may have resulted from non-specific or mistargeting effects of the morpholino (MO), we also designed and injected zebrafish embryos with a *pod5* splice-blocking morpholino (SBMO). Embryos injected with the *pod5* SBMO also exhibited a curly-tailed phenotype (Supplemental Figure 7). Next, exogenous versions of WT or mutant c.G1336A (p.A446T), c.G1363C (p.A455P), and c.C1286T (p.A429V) human *POC5* mRNAs were expressed in zebrafish at different concentrations (Supplemental Figures 8 and 9). Embryos expressing mutant *POC5* mRNAs, but not WT *POC5* mRNAs, showed an overt axial phenotype after 72 hpf, ranging from mild to severe curvature of the body axis in approximately 50% of injected embryos

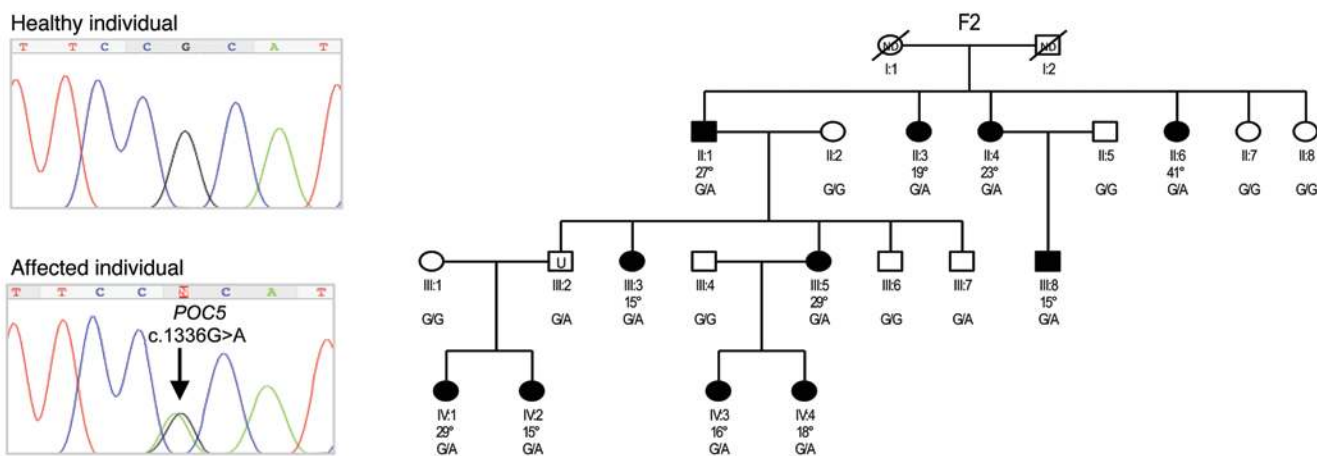


Figure 1. Identification of a rare *POC5* SNV in family F2. Exome sequencing identifies a rare missense SNV in *POC5*, c.1336G>A, listed in dbSNP138 (rs34678567). Sample chromatograms for a healthy individual without this SNV (upper left panel) and for an affected individual with this SNV (lower left panel) are shown. The pedigree of family F2 is shown. Cobb's angle and genotype at the mutated position are indicated below symbols corresponding to individuals. U, uncertain status; ND, status not determined.

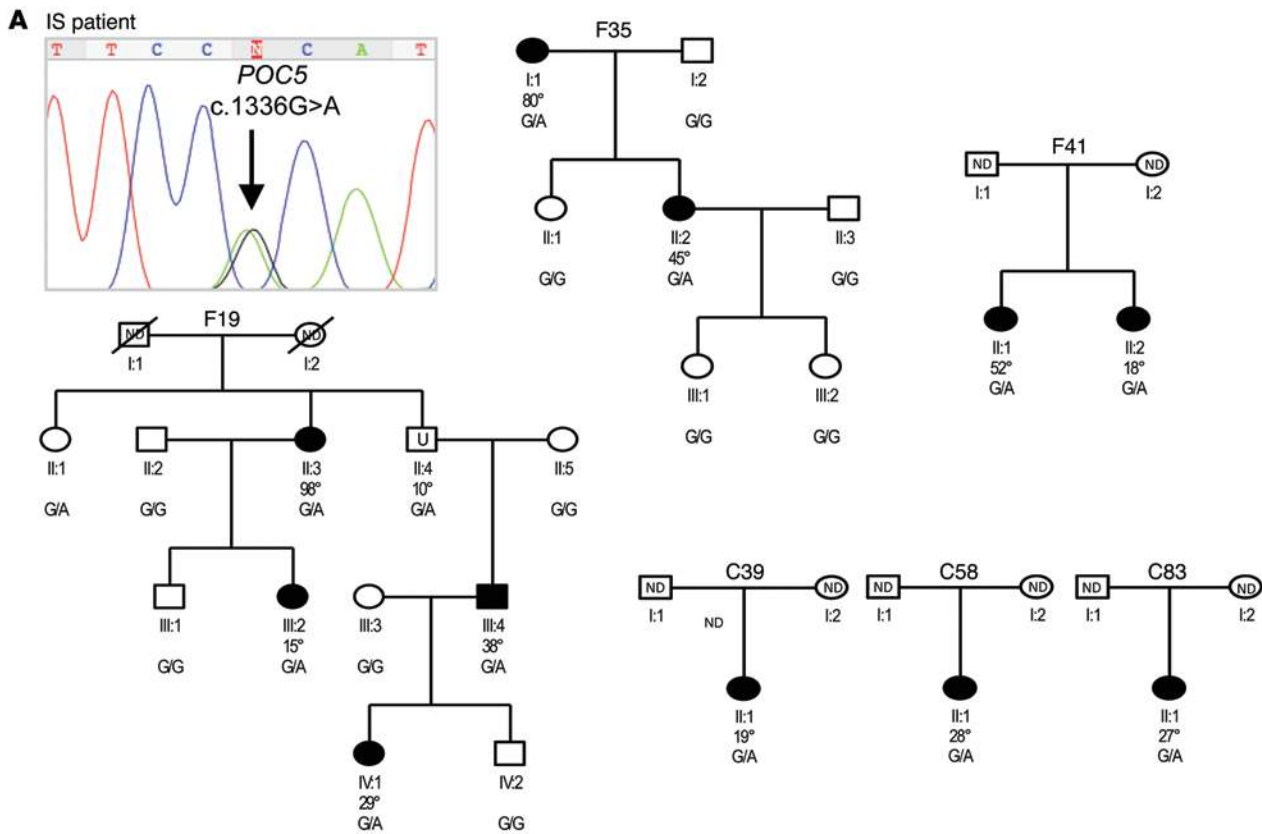


Figure 2. IS families and cases with exon 10 *POC5* SNVs. Forty additional IS families and 150 IS cases were studied. (A) IS families F19, F35, and F41 and IS cases C39, C58, and C83 show the same c.1336G>A *POC5* rare missense SNV listed in dbSNP138 (rs34678567). Sample chromatogram for an IS patient with this SNV is shown. The position of the SNV is indicated by an arrow above the chromatogram. Pedigrees of families and cases are shown. (B) IS family F31 harboring the c.1363G>C *POC5* novel missense SNV and corresponding chromatogram. (C) IS cases C1, C77, C137, C149, and C150 harboring the c.1286C>T *POC5* rare missense SNV listed in dbSNP138 (rs146984380) and corresponding sequence chromatograms. Cobb's angle and genotype at the mutated position are indicated below symbols corresponding to individuals.

($P < 0.001$; Figure 3A). When mut-*POC5* fish reached juvenile stages (50–60 days post-fertilization [dpf]), striking curvature of the fully mineralized vertebral column was observed (Figure 3B), in some instances combined with vertebral rotation (Figure 3B and Supplemental Video 1) reminiscent of the 3D deformation

observed in IS patients. Importantly, no other skeletal or body malformations were observed, including misshapen, missing, or fused vertebrae. The severity of larval phenotypes upon over-expression of mutated *POC5* resulted in spine defects of similar magnitude in juvenile fish.

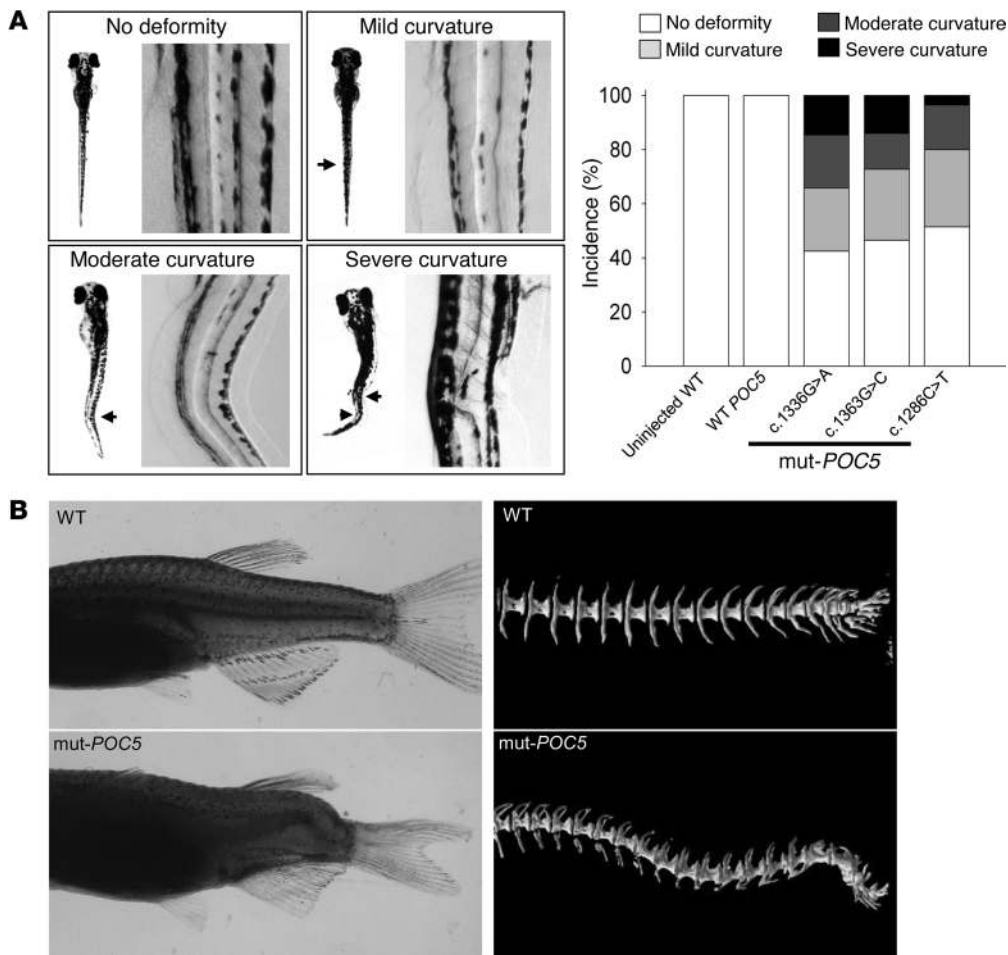


Figure 3. Expression of human *POC5* SNVs leads to scoliosis in zebrafish. Zebrafish expressing mutant *POC5* mRNAs, but not WT *POC5* mRNAs, showed an overt axial phenotype after 72 hpf, ranging from mild to severe curvature of the body axis in approximately 50% of injected embryos ($P < 0.001$, χ^2 test). **(A)** Left panel: mutant zebrafish phenotypes (3–5 dpf). Images illustrating the various degrees of severity of axial spine deformities. Axial spine deformities are indicated by arrows. Right panel: after 3 dpf, embryos injected with 50 ng/ μ l, mut-*POC5* mRNAs c.G1336A ($n = 153$), c.G1363C ($n = 149$), and c.C1286T ($n = 130$) exhibited mild to severe curvature of the body axis compared with noninjected WT ($n = 234$), and WT *POC5*-injected ($n = 122$) embryos. **(B)** Lateral view (left panels) of WT and mut-*POC5* juvenile zebrafish. MicroCT images (right panels) of WT fish showed a fully mineralized normal spine, while mut-*POC5* fish present spine curvature and rotation reminiscent of IS.

To define the spatiotemporal expression of *Poc5* in developing embryos and larvae, we performed whole-mount in situ hybridization (Supplemental Figure 10). *Poc5* was expressed ubiquitously during early somitogenesis. Its expression became restricted to the head and bud region by 24 hpf. By 48 hpf and 72 hpf, its expression became even more confined, to the telencephalon, midbrain, and midbrain-hindbrain boundary. At 72 hpf, expression was particularly strong at the midbrain-hindbrain boundary, a crucial center organizing brain patterning (17). Given that the *poc5* gene is ubiquitously expressed during somitogenesis and that injected embryos show defects in the formation of axial structures, *poc5* could thus play an important role in early aspects of anterior-posterior axis development. These axial defects likely persist and later affect spine formation.

Very little is currently known about the role of *POC5*. In humans, *POC5* localizes to the distal portion of centrioles and is recruited to procentrioles for full centriolar maturation and normal cell-cycle processing (14). This centrosomal protein interacts with centrin (18) and inversin, both involved in cell division, polarity, and motility. Thus, the function of primary cilia and left-right axis determination may be somehow impaired in patients and connected to IS. Interestingly, the 3 functional *POC5* variants result in the switch of an alanine residue (alanine to tyrosine, valine, or proline) and are located in the same region of the gene (NM_001099271, exon 10), suggesting that this region may be functionally significant.

Although phenotypic information regarding IS is not available for individuals in the control cohort of French descent, the penetrance of the disease associated with these SNVs in humans appears to be low for c.G1336A (p.A446T) and c.C1286T (p.A429V), which are present in this cohort at a frequency of 0.75% and 0.34%, respectively. This makes it difficult to explain the high number of affected individuals in families F2 and F19 among carriers of the c.G1336A (p.A446T) SNV (16/20, Figure 1 and Figure 2A). However, a risk-modifying allele, which amplifies disease expression, could also cosegregate in these families. Such a modifier allele may be located on 3q12.3 in family F2, where the same chromosome 3q12.3 haplotype segregates in all 11 affected patients, but not in the unaffected c.G1336A (p.A446T) *POC5* SNV carrier. We are currently attempting to identify this modifier allele in family F2. However, in IS family F19, the putative risk-modifying allele is not located on chromosome 3q12.3, because affected members of this family do not share a common haplotype in the critical 3q12.3 IS interval (data not shown). These data suggest that the modifier allele may be different in the different families where the c.G1336A (p.A446T) *POC5* SNV segregates. If we assume that at least 2 genes are required for disease expression, the combination of 2 rare variants with low marginal penetrance could confer high disease penetrance in carriers. This type of model could explain both the phenotypic and genetic observations in families F2 and F19 and why large multiplex IS families are so rare, as the disease recurrence risk in the sibship of a patient would be less than or equal to one-fourth.

Finally, in the context of this highly heterogeneous disorder with possible digenic inheritance for a subgroup of patients, identifying *POC5* as what we believe to be the first IS-causing gene is a major step toward deciphering the genetic causes of IS. This crucial step will pave the way for future studies to identify additional genes or pathways involved in IS. Further studies of large cohorts of various ethnicities are needed to determine the contribution of *POC5* genetic variants to IS in other populations and to establish the prevalence of these variants in the general population. In the future, drawing a complete picture of the genetic events leading to IS will help with devising preventive strategies and, it is hoped, therapies in this complex disorder.

Methods

Diagnosis of IS. A total of 480 individuals, which includes 330 individuals belonging to 41 multiplex IS families and 150 IS cases, participated in this study (Supplemental Figure 11, A–C). The control population was of similar ancestry and consisted of 1,268 individuals. IS was diagnosed by combining clinical examination and analysis of X-ray images of the spine, and the “affected” status was defined by a Cobb’s angle above or equal to 15° and rotation of vertebrae (see Supplemental Methods and Supplemental Table 5).

Whole-exome capture and sequencing. A potential causal variant in F2 was searched using whole-exome sequencing. See Supplemental Methods for details of exome analysis; see Supplemental Methods and Supplemental Tables 6 and 7 for details on sequencing analyses. Next-generation sequencing results were deposited in the NCBI’s Sequence Read Archive (SRA SRP050379).

Zebrafish experiments. The phenotypic consequences of overexpressing WT and mutated human *POC5* mRNAs in zebrafish were studied. See Supplemental Methods and Supplemental Table 8 for details of zebrafish experiments.

Statistics. A χ^2 test was used to analyze the statistical significance of differences in the zebrafish phenotype distributions between experimental groups. The allelic frequency of each *POC5* SNVs was compared between IS cases and the control population using a 1-tailed Fischer’s exact test. $P < 0.05$ was considered statistically significant.

Study approval. Written informed consent was received from all patients prior to enrollment in the study. Collection and use of patient samples for this study were approved by the ethics committee of the Hospices Civils de Lyon and CHU Sainte-Justine. All zebrafish work was carried out according to Canadian Council on Animal Care (CCAC) guidelines and approved by the local animal care committee.

Further details are available in the Supplemental Methods.

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