Idiopathic scoliosis (IS) affects 3% of children worldwide, yet the mechanisms underlying this developmental model of IS, exhibit defects in ependymal cell cilia development and morphogenesis. Notably, restoration of cilia motility after the onset of scoliosis blocks spinal curve progression. Together, our results indicate a critical role for cilia-driven CSF flow in spine development, implicate irregularities in CSF flow as an underlying biological cause of IS, and suggest that noninvasive therapeutic intervention may prevent severe scoliosis.

Idiopathic scoliosis (IS) is a complex genetic disorder characterized by three-dimensional spinal curvatures, which arise in the absence of observable physiological or anatomical defects. Commonly diagnosed during adolescence, IS can cause disfigurement, reduced respiratory and pulmonary function, and chronic pain. In congenital and neuromuscular forms of scoliosis, spinal curvatures develop from vertebral malformations and/or underlying morbidity of the musculature and nervous system; however, the biological cause of IS has thus far remained unknown. As a result, treatment is limited to managing spinal deformity post-onset, through bracing and/or corrective surgery.

Genome-wide association studies have identified IS-associated polymorphisms in divergent human populations, but phenotypic and genetic variability have made it difficult to define causative mutations. Furthermore, a historical lack of appropriate animal models has confounded our basic understanding of the biology underlying IS. However, teleosts (bony fish) are, like humans, naturally prone to idiopathic spinal curvature, and recent genetic studies have identified faithful zebrafish IS models, providing important insights into the genetic causes of scoliosis as well as a means to functionally validate human IS-associated genetic variants. Notably, zebrafish ptk7 (protein tyrosine kinase-7) mutants present all defining attributes of the human disease, and studies of these mutants have implicated dysregulated Wnt signaling in the pathogenesis of IS.

Ptk7 is an essential regulator of both canonical Wnt-β-catenin and noncanonical Wnt-planar cell polarity (PCP) signaling pathways. Although defects in either pathway are associated with a range of developmental abnormalities, both Wnt-PCP and Wnt-β-catenin signaling have been implicated in the function of cilia (9–11). Cilia are microtubule-based organelles that project into the extracellular space and play critical roles in the perception and integration of environmental signals (12, 13). Although most cell types elaborate short primary cilia, longer motile cilia are present on the surface of specialized cells and generate directional extracellular fluid flow in several contexts.

**Fig. 1.** ptk7 mutant fish exhibit hydrocephalus, EC cilia defects, and spinal curves, all of which are prevented by transgenic reintroduction of ptk7 specifically in motile ciliated cell lineages. (A to C) Representative sagittal SEM images of the brains of a ptk7/+ control [n = 6 (A)], a ptk7 mutant [n = 6 (B)], and a ptk7 mutant expressing Tg(foxj1a::ptk7) [n = 6 (C)], all at 2.5 months of age. The yellow line in (B) demarcates hydrocephalus. Green squares indicate the areas shown in corresponding magnification SEM images (A’ to C’). (D to E) Representative fixed [D] and µCT-rendered [D’ and E’] lateral views of an adult ptk7 mutant [D] and an adult ptk7 mutant expressing Tg(foxj1a::ptk7) [E] and E’]. CCe, corpus cerebelli; CCr, crista cerebellaris; MO, medulla oblongata. Scale bars, 250 μm [(A), (B), and (C)], 10 μm [(A’), (B’), and (C’)], and 5 mm [(D) and (E)].

**Zebrafish models of idiopathic scoliosis link cerebrospinal fluid flow defects to spine curvature**

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Idiopathic scoliosis (IS) affects 3% of children worldwide, yet the mechanisms underlying this spinal deformity remain unknown. Here we show that ptk7 mutant zebrafish, a faithful developmental model of IS, exhibit defects in ependymal cell cilia development and cerebrospinal fluid (CSF) flow. Transgenic reintroduction of Ptk7 in motile ciliated lineages prevents scoliosis in ptk7 mutants, and mutation of multiple independent cilia motility genes yields IS phenotypes. We define a finite developmental window for motile cilium in zebrafish spine morphogenesis. Notably, restoration of cilia motility after the onset of scoliosis blocks spinal curve progression. Together, our results indicate a critical role for cilia-driven CSF flow in spine development, implicate irregularities in CSF flow as an underlying biological cause of IS, and suggest that noninvasive therapeutic intervention may prevent severe scoliosis.

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Ptk7 is an essential regulator of both canonical Wnt-β-catenin and noncanonical Wnt-planar cell polarity (PCP) signaling pathways. Although defects in either pathway are associated with a range of developmental abnormalities, both Wnt-PCP and Wnt-β-catenin signaling have been implicated in the function of cilia (9–11). Cilia are microtubule-based organelles that project into the extracellular space and play critical roles in the perception and integration of environmental signals (12, 13). Although most cell types elaborate short primary cilia, longer motile cilia are present on the surface of specialized cells and generate directional extracellular fluid flow in several contexts.

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Cilia-directed flow within early embryonic organizers breaks left-right (L-R) symmetry in development (14), and cerebrospinal fluid (CSF) flow, which is critical for central nervous system homeostasis (15), is generated by the polarized beating of ependymal cell (EC) cilia lining brain ventricles (16). Abnormal L-R asymmetries and defective CSF flow have been observed in IS patients (17), and an elevated incidence of scoliosis has been documented among primary ciliary dyskinesia patients (18). Therefore, we hypothesized that motile cilia dysfunction may contribute to the etiopathogenesis of IS.

To test this, we first investigated EC cilia structure and function in scoliotic ptk7 mutant zebrafish and sibling ptk7/+ controls. Examination of ptk7/ mutant brain ventricles by scanning electron microscopy (SEM) revealed severe hydrocephalus (Fig. 1, A and B), a phenotype commonly associated with loss of EC cilia function (16). Moreover, whereas a dense network of polarized EC cilia lined the ventral surface of ptk7/+ ventricles, cilia in ptk7 mutant ventricles were sparse and, when present, lacked posterior polarization (Fig. 1, A′ and B′). To directly examine the consequence of EC cilia defects, we tracked fluorescent microsphere movement across the ventral surface of the rhombencephalic ventricle (Fig. 2A). Dynamic anterior-to-posterior flow was observed across the ventricle of ptk7/+ brains (Fig. 2, B and G, and movie S1). In contrast, although some movement was observed during particle tracking in ptk7 mutants, microspheres exhibited irregular trajectories and significantly reduced speeds (Fig. 2, C and G, and movie S2). These results demonstrate abnormal CSF flow within the ventricular system of scoliotic ptk7 mutants and are consistent with a role for EC motile cilia defects in the etiology of IS.

To investigate whether scoliosis specifically results from motile cilia dysfunction, we assessed potential amelioration of ptk7 mutant spinal curves through transgenic reintroduction of wild-type Ptk7 in motile ciliated cell lineages only. The transcription factor Foxj1a is a master regulator of motile ciliaogenesis (19). We therefore cloned and characterized a foxj1a enhancer element that specifically drives transgene (Tg) expression in all known sites of motile cilia formation, as demonstrated in multiple foxj1a::eGFP transgenic lines (eGFP, enhanced green fluorescent protein) (fig. S1, A to D, and movie S3). Along the trunk of juvenile animals, Tg(foxj1a::eGFP) expression was predominantly restricted to midline structures of the brain and spinal cord. We next generated four independent foxj1a::ptk7 stable transgenic lines (fig. S1E) and found that the presence of Tg(foxj1a::ptk7) restored EC cilia and CSF flow in ptk7 mutant fish and prevented hydrocephalus from manifesting (Fig. 1, C and C′; Fig. 2, D and G; and movie S4). Importantly, spinal curve formation, assessed by microcomputed tomography (μCT), was also fully suppressed by the transgenes (n = 59; Fig. 1, D to E′), showing that scoliosis in mutants is specifically caused by Ptk7 dysfunction in motile ciliated lineages.
If cilia motility defects contribute to IS pathogenesis, then ccdc40 (20), ccdc151 (21), dyx1c1 (22), and c21orf59 (23, 24) mutations, which all disrupt cilia motility, should lead to the development of scoliosis. However, aberrant cilia motility causes a characteristic suite of embryonic phenotypes that usually result in death by 1 to 2 weeks of development (20, 21), precluding analysis of adolescent spine formation. To circumvent this early lethality, we used two strategies. First, we took advantage of the c21orf59 temperature-sensitive mutation m304, here called c21orf59TS (24). At 30°C (a restrictive temperature), c21orf59TS mutant embryos exhibited abnormal cilia motility and associated developmental defects (Fig. 3, A and B). However, at 25°C (a permissive temperature), c21orf59TS embryos retained cilia motility and could develop normally (Fig. 3C). c21orf59TS mutants that were raised at 25°C for 5 days to prevent embryonic defects and then shifted to 30°C resembled wild-type zebrafish for 5 days to prevent embryonic defects and then shifted to 30°C to 30°C at 19 dpf (A), 24 dpf (B), 29 dpf (C), and 34 dpf (D). (E) Quantification of scoliosis phenotypes in temperature-shifted mutants, observed at 6 months post-fertilization (numbers are percentages). (F) Schematic of restrictive (30°C) to permissive (25°C) temperature shift experiments performed with c21orf59TS mutant embryos. (G and H) Lateral images of a juvenile sib control (G) and a c21orf59TS mutant (H), both kept at 30°C, show curve initiation in mutants by 18 dpf. (I) Representative µCT image of an 80-dpf unshifted c21orf59TS mutant. (J and K) Representative µCT images of 80-dpf c21orf59TS mutants shifted from 30°C to 25°C at 18 dpf [n = 6 (J)] or at 23 dpf [n = 6 (K)]. Scale bars, 5 mm [(A) to (D)] and 2 mm [(G) and (H)].

These experiments further demonstrate a post-embryonic requirement for motile cilia function during spine development, including embryonic lethality (fig. S3, C to F), in agreement with gene knockdown studies (22). dyx1c1 mutants injected with wild-type mRNA to prevent embryonic defects developed severe three-dimensional spinal curvatures in the absence of congenital vertebral malformations (Fig. 3J; fig. S2, A and B; and fig. S4). Furthermore, ccdc151 and ccdc40 mutant embryos that were phenotypically normal in embryonic stages (owing to wild-type mRNA injection) also developed late-onset spinal curves that model IS (Fig. 3, K to L). Our demonstration that mutations in four different genes, each of which has been shown to disrupt cilia motility, all yield similar adolescent spinal curve phenotypes provides strong evidence that motile cilia dysfunction represents the underlying cell-biological cause of IS in these models.

These experiments further demonstrate a post-embryonic requirement for motile cilia in spine morphogenesis. Transient knockdown of Dxy1c1 or Ccdc151 through only the first 3 to 4 days of embryogenesis [by injection of translation-blocking antisense morpholino oligonucleotides (MOs)] did not result in adolescent spinal curvatures, despite the fact that MO-injected embryos phenocopied genetic mutants during early embryogenesis (fig. S5). To define the critical developmental window for motile cilia function in the etiopathogenesis of IS, we performed a series of temperature shift experiments using the c21orf59TS mutant allele. c21orf59TS mutant embryos were raised at 25°C for at least 5 days (to prevent embryonic phenotypes), transferred to a restrictive temperature (30°C) at defined incremental stages of development, and screened for spinal curvatures at sexual maturity (Fig. 4, A to D). c21orf59TS mutants that were shifted to restrictive temperatures at 19 days post-fertilization (dpf) all developed severe spinal curves by 5 weeks of age (Fig. 4, A and E). In contrast, c21orf59TS mutants that were shifted to 30°C at 24 and 29 dpf exhibited milder spinal curvatures (Fig. 4, B, C, and E), whereas c21orf59TS mutants that were shifted to 30°C at 34 dpf displayed no signs of scoliosis through the adult stages (Fig. 4, D and E). These results indicate a finite and temporally defined requirement for motile cilia function during spine morphogenesis. This time interval correlates with documented periods of accelerated adolescent growth (4), when spinal curves typically manifest in IS.

Last, to determine whether restoration of motile cilia function can prevent severe spinal curvature progression after the onset of scoliosis, we performed restrictive-to-permissive temperature shifts at defined time points. c21orf59TS mutant embryos were first raised at 25°C until 7 dpf to allow normal embryonic development, transferred to 30°C until the onset of spinal curve formation, and then returned to permissive temperatures at incremental stages of spinal curve progression (Fig. 4, F to H). Restoration of motile cilia activity at the onset of scoliosis blocked spinal curve progression (Fig. 4, J to K). This provides a proof-of-principle that the development of severe IS spinal curvatures can be managed without invasive surgical manipulation.

The data presented here demonstrate that cilia motility is required for zebrafish spine morphogenesis. Given the acute hydrocephalus and EC cilia defects observed in pk7 mutants, the predominant expression of foxj1a transgenes throughout the brain and spinal cord of juvenile animals, and the severe CSF flow defects observed across zebrafish IS models, we suggest that irregularities in CSF flow represent the underlying cell-biological cause of IS. Several observations support this model: (i) Disruption of CSF activity via Kaolin injection into the subarachnoid space can cause scoliosis in both dog and rabbit models (25, 26), and (ii) scoliosis is highly prevalent in multiple human conditions associated with obstructed CSF flow, including Chiari malformation, syringomyelia, and myelomeningoceles (27-29).

Our data explain these observations and further imply an evolutionarily conserved role for CSF flow in spine morphogenesis, thus warranting reexamination of the anatomy, physiology, and genetics of CSF flow in cases of human IS. Downstream of CSF flow, molecular mechanisms influencing spine morphogenesis remain to be determined but could involve multiple gene products that have been previously associated with IS [e.g., potential motile cilia functions for the centriolar protein POC5 (6) or chondrocyte-specific activation of GPR126 (30)]. Ultimately, our demonstration that severe spinal curvatures can be prevented with the restoration of motile cilia activity may have important therapeutic ramifications; pharmaceutical manipulation of the production and/or downstream interpretation of CSF signals could potentially stop severe
Cancer

The histone H3.3K36M mutation reprograms the epigenome of chondroblastomas

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More than 90% of chondroblastomas contain a heterozygous mutation replacing lysine-36 with methionine-36 (K36M) in the histone H3 variant H3.3. Here we show that H3K36 methylation is reduced globally in human chondroblastomas and in chondrocytes harboring the same genetic mutation, due to inhibition of at least two H3K36 methyltransferases, MMSET and SETD2, by the H3K36M mutant proteins. Genes with altered expression as well as H3K36 di- and trimethylation in H3.3K36M cells are enriched in cancer pathways. In addition, H3K36M chondrocytes exhibit several hallmarks of cancer cells, including increased ability to form colonies, resistance to apoptosis, and defects in differentiation. Thus, H3K36M proteins reprogram the H3K36 methylation landscape and contribute to tumorigenesis, in part through altering the expression of cancer-associated genes.

H3K36me, or histone H3 trimethylation at lysine 36, is essential for cell proliferation, differentiation, and proper genome integrity. In human chondroblastoma (CHB), one of the most common benign bone tumors, a single amino acid variation (G34W) in the histone variant H3.3 is recurrently observed. This mutation, which encodes H3.3K36M, disrupts the histone H3 catalytic domain and de novo methyltransferases, leading to global loss of H3K36me2 and H3K36me3. While studies have shown that H3K36me2 is a key epigenetic mark for stem cell proliferation, little is known about how this mutation reprograms the epigenome of CHB. To understand how the H3.3K36M mutant protein affects the epigenome, we used the CRISPR/Cas9 system to introduce the H3.3K36M mutation into human chondroblastoma cells. Our results show that the H3.3K36M mutation promotes tumorigenesis of this poorly studied tumor in mice.

ENDNOTE

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REFERENCES AND NOTES


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SUPPLEMENTARY MATERIALS

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Materials and Methods

Figs. S1 to S5

References (31–33)

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Zebrafish models of idiopathic scoliosis link cerebrospinal fluid flow defects to spine curvature


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Altered fluid flow causes curved spine

Adolescent idiopathic scoliosis is characterized by three-dimensional spinal curves and affects 3% of the world's children. However, the biological basis of this condition is unclear. Grimes et al. studied zebrafish models that likewise display a curved spine. Defects in the formation and function of motile cilia in the central nervous system perturbed the flow of cerebrospinal fluid (CSF), leading to abnormal spinal curvatures as the fish grew. Curves could be partially rescued by restoring CSF flow, suggesting potential therapeutic strategies if the same mechanism is shared in humans. Science, this issue p. 1341