Scoliosis Traced to Problems in Spinal Fluid Flow

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Caption: Normal zebrafish (top left) and a normal skeleton (bottom left); zebrafish with scoliosis (top right) and an abnormal scoliotic skeleton (bottom right).
Credit: Grimes DT, Boswell CW, Morante NF, Henkelman RM.

Many of us may remember undergoing a simple screening test in school to look for abnormal curvatures of the spine. The condition known as adolescent idiopathic scoliosis (IS) affects 3 percent of children, typically showing up in the tween or early teen years when kids are growing rapidly. While scoliosis can occur due to physical defects in bones or muscles, more often the C- or S-shaped spinal curves develop for unknown reasons. Because the basic biological mechanisms of IS have been poorly understood, treatment to prevent further progression and potentially painful disfigurement has been limited to restrictive braces or corrective surgery.

Now, in work involving zebrafish models of IS, a team of NIH-funded researchers and their colleagues report a surprising discovery that suggests it may be possible to develop more precisely targeted therapeutics to reduce or even prevent scoliosis. The team's experiments have, for the first time, shown that mutation of a gene associated with spinal curvature in both zebrafish and humans has its effect by altering the function of the tiny hair-like projections, known as cilia, that line the spinal cord. Without the cilia's normal, beating movements, the fluid that bathes the brain and spinal cord doesn't flow properly, and zebrafish develop abnormal spinal curves that look much like those seen in kids with scoliosis. However, when the researchers used genetic engineering to correct such mutations and thereby restore normal cilia function and flow of cerebral spinal fluid (CSF), the zebrafish did not develop spinal curvature.

Zebrafish are normally found in tropical freshwater and are a favorite research model to study vertebrate development. They are also an especially good model for studying IS.

The latest study, published recently in the journal *Science*, involved a research team led by Brian Ciruna of the University of Toronto and The Hospital for Sick Children, Toronto, and Rebecca Burdine of Princeton University, Princeton, NJ. The team was interested in zebrafish with mutations in the *ptk7* gene, which encodes the enzyme protein-tyrosine kinase-7 [1].

Ciruna's lab had earlier shown that mutations in the *ptk7* gene could lead to spinal curvature [2]. While *ptk7* was already known to play a role in embryonic development, it wasn't clear how the gene, when altered, might lead to scoliosis in adolescence. In the latest work, the researchers examined the brains and spinal cords of the *ptk7*-mutant fish in search of clues to their earlier findings. They found something unexpected: the motile cilia normally found at the surface of the brain and lining the spinal cord were unusually sparse. Many of the cilia that were present were also positioned incorrectly.

Those mutant fish developed a curved spine and a brain-swelling condition known as hydrocephalus, which is also associated with defects in motile cilia. While the researchers did not directly measure the movement of those defective cilia, they injected tiny fluorescent beads into the fish and saw had almost no CSF flow. That finding was especially intriguing in light of reports of defective CSF flow in people with scoliosis.

To find out whether those problems in the cilia were responsible for the scoliosis, the researchers restored the normal gene function specifically in the cells that produce cilia. And indeed, with Ptk7 and motile cilia restored, the hydrocephalus and scoliosis no longer developed in the zebrafish, as CSF began to flow normally.

To provide even more evidence of a link between cilia and scoliosis, the researchers introduced other mutations in four genes known to play a role in cilia development and function. They found that zebrafish carrying any of those mutant genes also developed scoliosis.

In a clever series of experiments exploring the timing of scoliosis development, the researchers took advantage of a temperature-sensitive mutation in *c21orf59*, a gene that the Burdine lab recently showed is essential for cilia function [3]. The mutation allowed them to switch cilia's movement on and off simply by changing the temperature of the water in the zebrafish tanks. When zebrafish carrying the mutation were kept at 25° Celsius (77° Fahrenheit), the protein product of the gene functioned normally, allowing cilia to work. When those same fish were kept at a temperature of 30° Celsius (86° Fahrenheit), the mutant protein no longer folded correctly, causing cilia to malfunction.

The researchers initially raised zebrafish embryos at 25° Celsius to allow normal development. They then moved the fish to a tank set to 30° Celsius after 19, 24, 29, or 34 days, causing the flow of CSF to slow as the cilia stopped functioning normally. Those studies found that fish were susceptible to developing spinal curvatures when motile cilia stopped working properly at 19 days, a developmental stage corresponding to adolescence in humans.

Fish moved to the warmer tank just a few days later developed only mild curves. Those shifted to the warmer temperature at 34 days showed no scoliosis at all. Taken together, these findings provide evidence that scoliosis might ultimately be treated or prevented with therapeutics designed to encourage normal cilia function and CSF flow.

In the meantime, there is plenty still left to learn. It's not yet clear precisely how CSF flow influences spinal development in adolescence. The researchers suggest that the rapid growth during adolescence may increase the risk of developing asymmetries, and changes in CSF flow might allow the body to detect and correct them. When CSF doesn't flow normally, those checks and balances may break down.

Scoliosis is known to be highly prevalent in people with several health conditions associated with obstructed CSF flow. This, together with the new evidence, implies an evolutionarily conserved role for CSF in the development of the spine in fish and humans. They also suggests it would be useful to reexamine CSF flow in children with scoliosis. More broadly, these new findings show once again the value of animal models for learning about human disease.

References:

[1] Zebrafish models of idiopathic scoliosis link cerebrospinal fluid flow defects to spine curvature. Grimes DT, Boswell CW, Morante NF, Henkelman RM, Burdine RD, Ciruna B. Science. 2016 Jun 10;352 (6291):1341-1344.

[2] ptk7 mutant zebrafish models of congenital and idiopathic scoliosis implicate dysregulated Wnt signaling in disease. Hayes M, Gao X, Yu LX, Paria N, Henkelman RM, Wise CA, Ciruna B. Nat Commun. 2014 Sep 3;5:4777.

[3] c21orf59/kurly controls both cilia motility and polarization. Jaffe KM, Grimes DT, Schottenfeld-Roames J, Werner ME, Ku TS, Kim SK, Pelliccia JL, Morante NF, Mitchell BJ, Burdine RD. Cell Rep. 2016 Mar 1; 14 (8):1831-1839.

Links:

Questions and Answers about Scoliosis in Children and Adolescents (NIAMS/NIH)

Burdine Lab (Princeton University, Princeton, NJ)

Brian Ciruna (The Hospital for Sick Children, Toronto)

NIH Support: National Institute of Child Health and Human Development

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