




# Zebrafish: an important model for understanding scoliosis

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## Abstract

Scoliosis is a common spinal deformity that considerably affects the physical and psychological health of patients. Studies have shown that genetic factors play an important role in scoliosis. However, its etiopathogenesis remain unclear, partially because of the genetic heterogeneity of scoliosis and the lack of appropriate model systems. Recently, the development of efficient gene editing methods and high-throughput sequencing technology has made it possible to explore the underlying pathological mechanisms of scoliosis. Owing to their susceptibility for developing scoliosis and high genetic homology with human, zebrafish are increasingly being used as a model for scoliosis in developmental biology, genetics, and clinical medicine. Here, we summarize the recent advances in scoliosis research on zebrafish and discuss the prospects of using zebrafish as a scoliosis model.

**Keywords** Scoliosis · Zebrafish · Cilia · Cerebrospinal fluid flow · Urotensin signaling · Reissner's fiber

## Introduction

The spine is the most typical characteristic of vertebrates and is essential for survival and reproduction. Composed of alternating vertebrae and intervertebral disks, the vertebrate spine extends from the head to the pelvis and is fastened

by robust spinal ligaments and tendons [1]. In addition to its role as a protector of the spinal cord inside it, the spine also helps support weight and maintain posture during body movement. The most common spinal disorder in humans is spinal curvature, known as scoliosis, which was first described by the Greek physician Hippocrates (460–370 BC) [2]. Conceptually, scoliosis is characterized by a lateral spinal curvature greater than 10°, as measured by the Cobb angle on X-ray images and can be divided into several subtypes including congenital, syndromic and idiopathic scoliosis [3–6]. Scoliosis is a disorder that affects the health of a large population of children and adolescents (2–3%) and has gradually become a major public health concern [3, 7].

Scoliosis has been studied for centuries. Although genetic factors have been suggested to play a vital role, its etiology and pathogenesis still remain unclear. Recent studies using zebrafish have demonstrated the advantages of using model organisms to examine the biological origins and mechanisms of scoliosis [8–13]. In humans, natural biomechanical strains, such as gravity, loads on the spine and are thought to contribute to scoliosis [14, 15]. The mouse and rat, the two most widely used mammalian models, are quadruped animals with biological forces perpendicular to the direction of the spine, in sharp contrast to that in humans, who have bipedal gait, thus rendering it a less useful model for scoliosis unless using a bipedal model through amputation of the forelimbs [16–18]. By

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contrast, the teleost fish are prone to develop scoliosis under both natural and laboratory conditions, making them another candidate organism for modeling human scoliosis [19, 20].

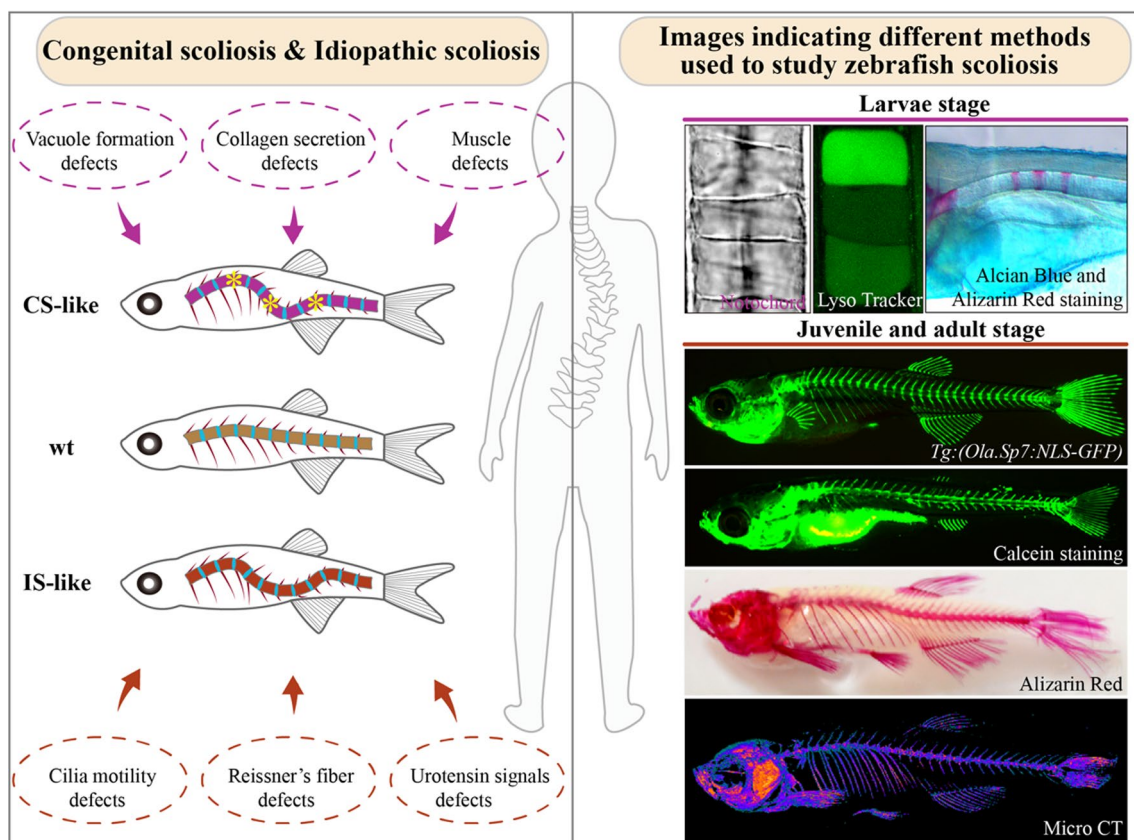
Zebrafish have several advantages for modeling human scoliosis. First, the morphology and structure of the spine are similar in zebrafish and humans, and there is a high degree of genetic conservation between them [5, 8, 21–23]. Second, it is hypothesized that the mechanical force generated during swimming is loaded on the spine in a manner similar to that in humans [20]. Zebrafish embryos are transparent and develop ex utero, which is convenient for monitoring spine development. Moreover, unlike mice, which have complex genetic operation, zebrafish are suitable for high-throughput screening through forward and reverse genetics [24–27]. Finally, various bone staining and imaging techniques, together with micro-CT and transgenic analysis, have been developed, making it easier to visualize the development of the zebrafish spine directly and effectively [28] (Fig. 1).

Recently, important breakthroughs have been made in understanding the mechanisms underlying scoliosis in zebrafish. In this review, we will first introduce scoliosis and briefly discuss the disadvantages of current animal models. Next, we will focus on the progress of current research and discuss the potential molecular mechanisms of scoliosis in zebrafish. Finally, we will discuss future directions for scoliosis studies in zebrafish models.

## Scoliosis in humans

### Congenital scoliosis

Congenital scoliosis (CS) is a deformity of the spine caused by vertebral body and rib deformity. Although termed “congenital scoliosis”, some CS patients only display a deformity of the spine at birth, while spinal curvature develops later during growth [29, 30]. The incidence of CS is approximately 1/1000 in the general population. CS may be caused



**Fig. 1** Zebrafish as a vertebrate model for scoliosis. Left: Different types of scoliosis and their potential causes from zebrafish studies. Scoliosis due to neuromuscular defects is also illustrated as CS like group. Asterisks indicate the abnormally developed vertebrae in CS-like zebrafish mutants. Right: Various bone staining and imaging methods used to evaluate skeleton development in zebrafish. At lar-

vae stages, the notochord and vacuoles can be easily visualized via bright-field image (top left) or LysoTracker dye staining (top middle). Skeletal development in zebrafish can be visualized via alcian blue-Alizarin red double staining (top right). At juvenile or adult stages, skeleton development can be visualized via transgenic labeling, calcein staining, Alizarin red staining or micro-CT

by defective vertebral segmentation involving fusion of hemivertebrae, wedges, and vertebrae and can occur throughout the spine. CS can occur in isolation or as part of other congenital syndromes, and genetic factors contribute substantially to its development [31, 32]. For instance, several studies have shown that abnormal Notch signaling is involved in the formation of CS [33–36]. However, the mechanisms underlying these signaling pathways remain unclear.

Compared with CS, which is directly related to defects in spinal development, neuromuscular scoliosis is usually a secondary scoliosis due to developmental defects in the nervous system and muscles [37]. It may develop as the result of cerebral palsy, spinal muscular atrophy, Duchenne muscular dystrophy, myelomeningocele, or Friedreich ataxia [38, 39]. In addition, similar to CS, neuromuscular scoliosis usually develops more severe spinal deformities than idiopathic scoliosis (IS) [37].

### Idiopathic scoliosis

IS, the most common type of disease in spinal curvature, accounts for approximately 80% of scoliosis cases and affects around 4% of the population [40]. The term “idiopathic” indicates its etiology is still unknown [7]. Since IS usually occurs during adolescence (10–18 years of age), it is also referred to as AIS [40]. IS is characterized by a three-dimensional rotation of the spine without clear vertebral abnormalities or significant physical defects. Compared with CS, IS usually occurs in relatively healthy individuals; however, the exact reason and mechanisms are still unclear. Several hypotheses have been proposed regarding IS pathogenesis, including defects in the development of the central nervous system, skeletal spinal growth, bone metabolism, and biomechanics [40–42]. Osteopenia and abnormal bone metabolism have been shown to be related to IS in patients, as well as in a mouse model [43–45]. Hormones may also influence the pathogenesis and progression of IS, as women usually have a higher incidence of scoliosis and suffer from more severe spinal curvature than men [40, 46]. Genetic factors play essential roles in IS progression. For instance, concordance rates are higher among monozygotic twins (as high as 70%) than in dizygotic twins (36%) [47]. Only a small number of genes have been reported to be associated with AIS using genome-wide association studies and exome sequencing, including ladybird homeobox 1 (LBX1), G protein-coupled receptor G6 (GPR126), centriolar protein (POC5), A-kinase anchoring protein 2 (AKAP2), chromosome domain helicase DNA binding protein 7 (CHD7) and planar cell polarity protein Vang-like protein 1 (VANGL1) [12, 48–54]. However, these genes are involved in a wide range of biological processes, and the mechanisms underlying scoliosis remain to be determined.

### Animal models of scoliosis

Because humans are upright walking vertebrates, the function and load of the human spine in adapting to the environment and daily life may differ from that of other vertebrates. Some studies have suggested that an upright posture may contribute to the development of scoliosis, especially IS [15, 17]. It is believed that the unique spatial structure of the upright human spine–pelvis complex and the resulting biomechanical load patterns make it easier to rotate than the spine of other animals. In fact, there is a small amount of intrinsic rotation even in healthy individuals. Development of rotation that exceeds a certain threshold and induces the development of scoliosis depends on several factors, including gene expression and individual lifestyle [40].

Mice are the most commonly used animal models in biomedical research. The mouse spine is physiologically and anatomically comparable to the human spine. In addition, micro-CT and X-ray microscopy are easily performed to analyze the mouse spinal structure. Nevertheless, it is difficult to obtain IS models in mice or other quadrupeds, which may be due to the fact that the mechanical load on the spine in mice are different from those seen in humans [55]. By amputating rodent forelimbs and domesticating them by holding food in high places to mimic the mechanics of the human spine, researchers have been able to create mouse models of scoliosis. In these models, differences in leptin, osteopontin, and calmodulin levels were associated with the severity and progression of scoliosis [56–58]. However, whether these models can mimic the actual conditions of scoliosis remains controversial [16]. Pinealectomy has also been used to induce scoliosis in chickens, suggesting that disruption of the endocrine system and melatonin deficiency may induce IS [59, 60]. However, the intervertebral joints in chickens are histologically different from the human intervertebral disk, making human AIS modeling difficult [61]. Notably, many of these procedures were aimed at recreating human scoliosis rather than studying its pathogenesis [16].

The overall structure of the vertebrate spine is evolutionarily conserved [8]. Recent studies have shown that spinal curvature is the most common natural deformity of bony fish and scoliosis can be easily observed in teleost [19, 20]. It is thought that the spinal load of bony fish during swimming is similar to that of humans, which may give fish an innate advantage over traditional quadrupeds in modeling scoliosis [8, 20]. Many features of human IS have been identified in curved guppies [62]. However, the lack of genomic and genetic resources of the guppy hinders the identification and functional study of genes responsible for scoliosis.

Zebrafish, a vertebrate animal with abundant genetic resources and complete genomic information, has recently

become a powerful model for scoliosis (Fig. 1). The spinal morphology and vertebral structure of zebrafish are similar to those of humans [5, 8, 21–23]. Zebrafish scoliosis models have been successfully established using both *N*-ethyl-*N*-nitrosourea (ENU) mutagenesis and reverse genetic methods [9, 24, 63, 64]. At present, with the rapid development of gene editing and sequencing technologies, zebrafish are providing new insights into the biology of scoliosis.

## Scoliosis in zebrafish

### Zebrafish model of CS

CS is usually caused by defects in vertebral development. Despite the difference in spinal formation and segmentation between humans and zebrafish [5], zebrafish can still be used to model the characteristics of CS, such as severe vertebral defects with fusion and disorganized neural and vascular arches [65, 66]. In most cases, CS occurs due to the disruption of notochord formation and maintenance in zebrafish (Fig. 1; Table 1) [66–69]. Therefore, the study of embryonic notochord development can provide new insights into the molecular mechanisms related to CS.

In zebrafish larvae, the notochord also serves as a scaffold to provide rigidity and flexibility to the body axis during movement [70]. In teleost, the notochord is a transitional

form required for the formation of spinal and vertebral structures. In zebrafish larvae, the notochord mainly consists of vacuolated cells and peri-notochordal sheath cell epithelium, which can secrete collagen as well as other extracellular matrix to form a peri-notochordal basement membrane sheath that envelops the notochord [71]. The volume expansion of vacuolated cells is essential for axial elongation in zebrafish, and defects of this process can result in shortening of the embryonic axis [67, 68, 72, 73]. The vacuolated cells of the teleost are defined by a rigid fluid-filled vacuole. The formation and maintenance of vacuoles are regulated by dual serine/threonine and tyrosine protein kinase (Dstyk) activity [67]. Dstyk mutants display abnormal vacuoles, which lead to a reduced volume of vacuolated cells and short embryonic axes [66, 67]. Furthermore, vacuolar development defects result in notochord deformation, together with late-onset scoliosis, due to vertebral bone growth defects [66, 67] (Fig. 2).

Maintenance of the notochord also depends on the function of the peri-notochordal sheath cell epithelium. Excess secretion of collagen proteins, such as those in morphants of the polycystic kidney genes *pkd1a/b* and *pkd2*, causes dorsal overbending of the notochord [74, 75]. Pioneering work in zebrafish mutant screening identified many mutants characterized by abnormal notochord development [76]. One of these mutants, *leviathan*, was later linked to a mutation of *col8a1a* gene. The *leviathan* mutant displayed a folded notochord at the larval stage

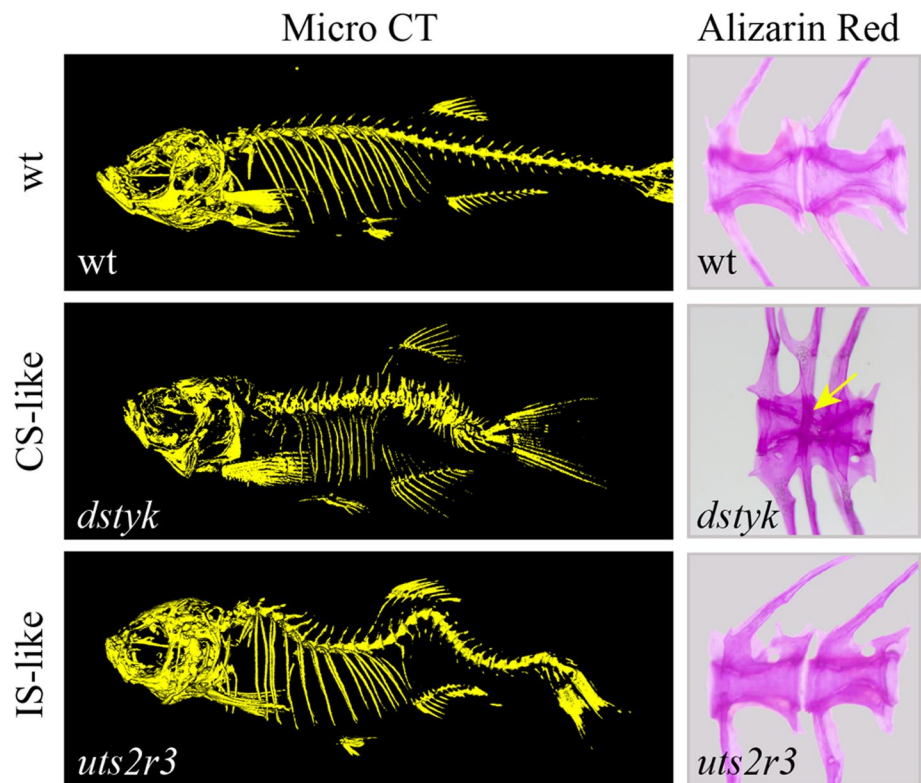
**Table 1** Zebrafish mutants resembling congenital scoliosis

Gene	Function	Type of scoliosis	References
<i>dstyk</i>	Notochord vacuole development	CS-like	[66, 67]
<i>col8a1a</i>	Extracellular matrix	CS-like	[69]
<i>tbx6</i>	Somite development	CS-like	[77]
<i>her1<sup>-/-</sup>; her7<sup>-/-</sup></i>	Somite segmentation	CS-like	[77]
<i>myadml2l</i>	Possible skeletal system development	CS-like	[64]
<i>colla1a</i>	Extracellular matrix	CS-like	[64]
<i>colla2</i>	Extracellular matrix	CS-like	[64]
<i>cmn</i>	Extracellular matrix	CS-like	[64]
<i>colla1b</i>	Extracellular matrix	CS-like	[64]
<i>myhz2</i>	Skeletal muscle fiber development	CS-like	[64]
<i>col2a1a</i>	Extracellular matrix	CS-like	[64]
<i>gim</i>	Undefined	CS-like	[24]
<i>dwa</i>	Undefined	CS-like	[24]
<i>dur</i>	Undefined	CS-like	[24]
<i>meox1</i>	Somite and skeletal system development	CS-like	[65]
<i>bhu</i>	Undefined	ND*	[24]
<i>nkx3.2</i>	Axial and limb skeletogenesis	ND*	[161]
<i>abcc6a</i>	Membrane transporter, osteogenesis	ND*	[162]
<i>stat3</i>	Signal transduction	ND*	[163]

\*ND not detected. The original paper reported scoliosis in these mutants, while no further information was given



**Fig. 2** Phenotypes of CS-like and IS-like zebrafish mutants. Left, Micro-CT images showing the morphology of spine in wild type, *dstyk* mutant (CS-like) and *uts2r3* mutant (IS-like). Alizarin red staining images of the vertebral body in different mutants were shown on the right. Arrow indicates the fusion between two adjacent vertebrae in *dstyk* mutants. Images of *dstyk* mutants courtesy of Xianding Sun and Lin Chen



and was CS-like with vertebral malformations in adulthood [69]. In *leviathan* mutants, notochord curvature is caused by the loss of type VIII collagen in the notochord extracellular matrix, which further affects the position of osteoblasts and causes vertebral defects during later development [69]. In addition, ENU mutagenesis screening also identified several CS-like mutants due to defects in genes related to collagen proteins and extracellular matrix [64]. These results suggest that abnormal extracellular matrix secretion may be one of the key factors affecting CS.

In addition to scoliosis due to notochord defects, several zebrafish scoliosis models harboring defects in the nervous system and muscle have recently been reported (Table 1). Early defects in somitogenesis due to mutations in somite clock genes or myosin heavy chain genes lead to scoliosis during later development [77, 78]. Notably, abnormal notochord development was also observed in these mutants, suggesting that scoliosis may develop through a combination of muscle and notochord defects [78]. Similarly, tissue-specific knockdown of *smn1* in motor neurons induced severe late-onset scoliosis, resembling neuromuscular scoliosis [79]. Although the underlying mechanisms remain to be elucidated, these scoliosis zebrafish models open new avenues for studying CS and neuromuscular scoliosis.

### Zebrafish model of IS

Compared to CS, IS is more complicated due to the lack of apparent causes. Exome sequencing analysis of the family population with IS revealed that some mutation sites were related to cilia, microtubule skeletons, extracellular matrices and muscles [12, 48, 52, 80–86]. Recently, a potential relationship between cilia and scoliosis was suggested using a zebrafish model. These studies showed that zebrafish cilia mutants will develop late-onset scoliosis with no apparent vertebral deformity, resembling IS [9, 63, 84, 87] (Fig. 2; Table 2).

Cilia are hair-like organelles that protrude from the surface of eukaryotic cells and can be classified as primary (immotile) and motile cilia based on their motility properties. The beating of motile cilia can either propel the movement of unicellular organisms or drive fluid flow in metazoan animals. By contrast, primary cilia can function as cellular antennas to mediate the transfer of chemical or physical signals between the cell and its environment [88–90]. Ciliary dysfunction can cause several debilitating genetic disorders, termed ciliopathies [91–93]. For example, defects in motile cilia are the main cause of primary ciliary dyskinesia (PCD), a rare autosomal recessive ciliopathy [94–97].

**Table 2** Zebrafish mutants resembling idiopathic scoliosis

Gene	Function	Type of scoliosis	References
<i>ptk7</i>	Regulator of Wnt signaling	IS-like	[11]
<i>sspo</i>	Matricellular protein, Reissner fiber component	IS-like	[134]
<i>uts2r3</i>	Urotensin-II receptor	IS-like	[105]
<i>ccdc151</i>	Cilia motility	IS-like	[63]
<i>ccdc40</i>	Cilia motility	IS-like	[63]
<i>dnaaf4</i>	Cilia motility	IS-like	[63]
<i>cfap298</i>	Cilia motility	IS-like	[63]
<i>kif6</i>	Cilia biogenesis	IS-like	[9]
<i>kif7</i>	Cilia biogenesis	IS-like	[85]
<i>tll11</i>	Modification of ciliary tubulin	IS-like	[84]
<i>armac9</i>	Cilia biogenesis	IS-like	[131, 164]
<i>mapk7</i>	Osteogenesis	IS-like	[10, 150]
<i>slc39a8</i>	Cationic transport	IS-like	[165]
<i>dnah10</i>	Cilia motility	IS-like	[166]
<i>poc5(OE)**</i>	Cilia biogenesis	IS-like	[12]
<i>lbx1b(OE)**</i>	Wnt/planar cell polarity signaling	IS/CS-like	[50]
<i>cep290</i>	Centriole formation	ND*	[129]
<i>bbs1</i>	Cilia biogenesis	ND*	[130]
<i>bbs5</i>	Cilia biogenesis	ND*	[122]
<i>adams9</i>	Cleavage of the extracellular matrix, cilia biogenesis	ND*	[24]
<i>falkor</i>	Undefined	ND*	[24]
<i>foxc1a<sup>+</sup>; foxc1b<sup>-/-</sup></i>	Transcription factor	ND*	[167]

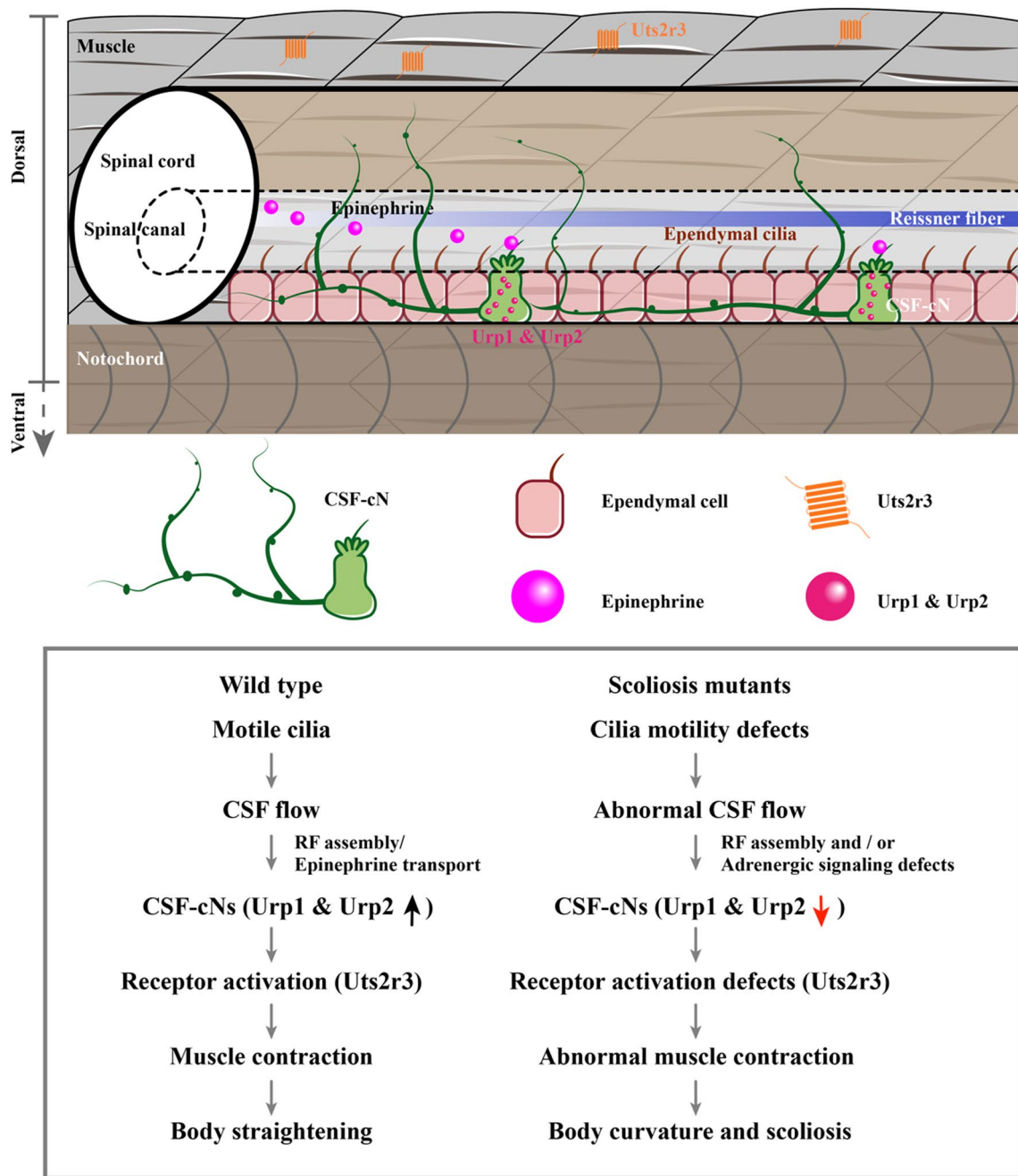
\*ND, not detected. The original paper reported scoliosis in these mutants, while no further information was given. Most of these genes are cilia related, it is likely that these mutants display IS-like phenotype.

\*\*These scoliosis phenotypes were generated through an overexpression (OE) of mutant mRNA (*poc5*) or wild type transgene (*lbx1b*)

### Defects in ciliogenesis cause body curvature in zebrafish larvae

In zebrafish larvae, cilia are present in many tissues, including the Kupffer's vesicle, olfactory epithelia, pronephric ducts, ependymal epithelium, and sperm [90, 98]. Defects in ciliogenesis often lead to ventral body curvature in zebrafish, and most ciliary mutants fail to survive into adulthood [99, 100]. The development of a curved body axis in zebrafish cilia mutants has long been an enigma. Recently, our research group and others have uncovered the essential role of cilia-driven cerebrospinal fluid (CSF) in zebrafish body axis development. CSF is a water-like fluid found in the brain and spinal cord and is vital for homeostasis of the central nervous system. Unlike humans, ciliary beating is the dominant factor for CSF circulation in the brain ventricles and central canal of zebrafish larvae [101–103]. Interestingly, the severity of motile cilia defects and curvature of the body axis were correlated with transport efficiency of the spinal cord CSF flow, suggesting a close link between CSF and axial development in zebrafish [101–105].

CSF signals are sensed, at least partially, by CSF-contacting neurons (CSF-cNs) located on the floor plate of the central canal in zebrafish (Fig. 3). CSF-cNs are ciliated and GABAergic sensory neurons found in most vertebrates [106–108]. Urotensin neuropeptides are a special type of neuropeptides that can be secreted from CSF-cNs [109] (Fig. 3). These neuropeptides are widespread in vertebrates and perform a variety of functions, including the constriction and relaxation of blood vessels and sleep regulation [110–113]. Urp1 and Urp2 are the two major urotensins expressed in CSF-cNs in zebrafish larvae [109]. The adrenergic signals from the CSF are essential for the expression of the Urp1 and Urp2 neuropeptides [105, 114, 115]. Upon activation, these neuropeptides may be secreted from the CSF-cNs and further bind to their receptor (Uts2r3, also known as Uts2ra) in the dorsal muscle fibers [105, 114] (Fig. 3). This process plays an important role in straightening the body axis during embryonic development and knocking down either urotensin genes (*urp1* and *urp2*), receptor genes (*uts2r3*), or adrenergic receptor genes (*adrb1/adrb2b*), will lead to ventral body curvature [105, 114, 115].



**Fig. 3** Model illustrating the Urotensin signaling pathway during body axis development of zebrafish. The beating of ependymal motile cilia drives CSF flow, which mediates the transmission of epinephrine signals and the assembly of Reissner’s fiber. The adrenergic signals activate the expression of Urotensin neuropeptides in the CSF-cNs

(Urp1 and Urp2). Finally, the urotensin neuropeptides may further activate the Uts2r3 receptor localized to the dorsal muscle fibers and promote body straightening. Abnormalities in the Urotensin signaling pathway will induce either body curvature in larvae or scoliosis in adult zebrafish

Moreover, injection of synthesized Urp peptides or epinephrine treatment can rescue axial bending defects in cilia-related mutants [105, 114]. These data suggest that motile cilia play an important role in the maintenance of the body axis morphology during embryonic development in zebrafish (Fig. 3). Interestingly, the urotensin

signaling pathway seems to be conserved in regulating body axis development in bony vertebrates, as disruption of Utr4, the homolog of Uts2r3, also led to body curvature in *Xenopus* [116].

### Ciliary defects cause scoliosis in adult zebrafish

Recently, CSF flow defects have been linked to scoliosis in adult fish [63]. In the brain ventricles, CSF flow is directed by the coordinated beating of motile cilia of multiciliated ependymal cells. The beating direction of these cilia is controlled by cellular polarity, which includes rotational polarity of the basal foot orientation, translational polarity of cilia distribution, and tissue-level polarity of the relative cilia positions within different cells [117, 118]. The establishment of these polarities depends on the core PCP proteins, including VANGL, Frizzled, Dishevelled, and Prickle [119–121]. Since beating of motile cilia contributes substantially to CSF flow, it is not surprising that defects in cilia motility cause scoliosis in zebrafish. For instance, zebrafish mutants with cilia motility defects develop late-onset scoliosis [9, 63, 87, 122]. Protein tyrosine kinase 7 (PTK7), a catalytically defective receptor protein tyrosine kinase involved in the Wnt/PCP signaling pathway, is the causative gene for scoliosis [11, 123]. Interestingly, zebrafish *ptk7* mutants also display strong scoliosis during development [63, 124]. Further studies showed that zebrafish Ptk7 is essential for coordinated ciliary beating and PCP polarity of ependymal cells, defects of which cause abnormal CSF flow, severe hydrocephalus, and scoliosis [63]. Restoring the function of Ptk7 in motile ciliated cells can successfully rescue defects in spinal morphogenesis. Moreover, *ptk7* mutants exhibited abnormal immune responses within the spinal cord, suggesting that neuroinflammation is a possible trigger for scoliosis. Treatment with anti-inflammatory drugs such as acetylsalicylic acid (aspirin) alleviated the severity of scoliosis, providing a potential strategy for the prevention and treatment of scoliosis [125]. Finally, mutations in PCD-related genes also induce scoliosis in zebrafish [63]. These data highlight the importance of cilia-driven CSF flow in the spinal development of zebrafish and suggest that disturbances or blockages of CSF flow are potential contributing factors of scoliosis. In line with this, several studies have consistently reported that the prevalence of scoliosis is elevated in patients with PCD [96, 126]. In addition, genetic variations in PCD-related genes have been detected in patients with clinical scoliosis [127].

Interestingly, defects in other ciliogenesis-related genes also resulted in scoliosis in zebrafish (Table 2). For example, mutations of *Cep290*, *Cc2d2a*, *Bbs1*, *Bbs5*, *Kif6*, *Kif7*, *Ttll11* and *Armc9* all lead to scoliosis in zebrafish [9, 84, 85, 122, 128–131]. Mutations in *KIF6/7* are also related to scoliosis in humans [9, 85]. These genes are not related to cilia motility but are involved in ciliary functions. Moreover, most cilia are grossly normal in zebrafish mutants [85, 128, 130]. These data imply that, in addition to its role as a driving force for CSF flow, the signal transduction function of primary cilia may also contribute to the development of

the zebrafish spine. However, the underlying mechanisms remain unknown.

### Scoliosis due to Reissner's fiber (RF) defects

Defects in the assembly of RF have also been shown to cause scoliosis in zebrafish [132–135]. RF was originally described in lampreys and later shown to be widely present in vertebrates [136, 137]. In zebrafish, RF is localized from the posterior ventricles of the brain to the caudal tip of the spinal cord [138]. RF is assembled through the aggregation of the subcommissural organ (SCO)-spondin protein, which is secreted from the SCO of the brain into the ventricular CSF [136, 139]. Although RF has long been thought to promote CSF flow and prevent hydrocephalus formation [140], its function remains largely unknown. In zebrafish, RF is suspended in the CSF and exist throughout the central canal [132, 135]. Interestingly, SCO-spondin mutants exhibited body curvature at larval stages and developed progressive scoliosis during later growth [133–135], pointing to a critical role of RF during axial development. Notably, the absence of RF did not affect ciliary beating or CSF flow in the brain ventricle and spinal canal [132]. By contrast, coordinated beating of the floor plate cilia is essential for RF assembly [132, 135].

RF interacts with monoamines and catecholamines, which may participate in the transmission of neural signals in body axis development [141]. Soluble SCO-spondin can be used as a regulator of synaptic growth, thus promoting neurogenesis [142–144]. Similarly, RF is closely associated with CSF-cNs in the spinal cord, which may aid transmission of CSF epinephrine signals [105, 141, 145]. The apical cilia and microvilli of CSF-cNs may interact directly with RF in the spinal canal to receive these signals. Further analysis showed that the loss of RF reduced spontaneous calcium activity in CSF-cNs [102, 114]. The stimulation of calcium activity requires the presence of polycystic kidney disease 2-like 1 (PKD2L1), a mechanosensitive channel protein of the TRP superfamily [146]. Moreover, *pkd2l1* mutants also exhibit scoliosis during later development [102]. These studies suggest a relationship between cilia motility, CSF flow, RF assembly, and the physiology of CSF-cNs. Nevertheless, it remains unclear how defects in CSF-cNs result in abnormal spinal development in scoliosis mutants.

### Scoliosis due to defects downstream of CSF-cNs

One of the critical factors controlling body axis development in zebrafish larvae is the urotensin neuropeptide, which is mainly secreted from CSF-cNs. These neuropeptides are essential for the body straightening of early zebrafish larvae through activating downstream Urotensin receptor *Uts2r3* (Fig. 3). Strikingly, *uts2r3* mutants also exhibited severe



scoliosis in adults, similar to those mutants with ciliary motility defects [105] (Fig. 2). These data suggest that, in parallel with its role during body axis straightening at larval stages, urotensin signals also participate in the regulation of spinal development during later stages. In SCO-spondin scoliosis mutants, the expression of these neuropeptides is compromised in CSF-cNs. Interestingly, epinephrine treatment or overexpression of *Urp2* in CSF-cNs could rescue axis bending in RF-defective mutants [114, 133, 134], suggesting that RF regulates body axis straightening through urotensin signals. Importantly, scoliosis is common in patients with Parkinson's disease, characterized by defects in dopamine production, which further demonstrates the role of epinephrine signals in scoliosis [147, 148].

The urotensin receptor is mainly expressed in the dorsal muscle fiber cells of zebrafish larvae, as suggested by the expression of green fluorescent protein (GFP) driven by *uts2r3* promoter. Unfortunately, GFP expression is lost in adult fish (our unpublished data), making it unclear whether the asymmetric distribution of this receptor is still present. Moreover, it is still unclear how the abnormal function of this receptor in muscle cells causes spinal curvature. Interestingly, a recent study of human patients with scoliosis identified defects in *UTS2R*, the homolog of zebrafish *Uts2r3*, strongly implying that urotensin signals may participate in the regulation of spine development [149].

### Diversity of factors causing IS

Scoliosis is thought to be attributable to many factors, including hormonal dysregulation, genetic defects, and environmental exposure. Zebrafish can also serve as a candidate model for investigating these conditions. The prevalence and severity of scoliosis are higher in females, which was also demonstrated in zebrafish *ptk7* mutants, making zebrafish an alternative model for studying the role of hormones in scoliosis [11, 46]. Mitogen-activated protein kinase 7 (MAPK7), also known as extracellular-signal-regulated kinase 5 (ERK5), belongs to the family of conventional MAPKs. Recently, a *MAPK7* variant was identified in a three-generation family affected by AIS, and zebrafish *mapk7* mutants also exhibited severe scoliosis [10, 150]. ERK5 plays various roles during cell proliferation and migration, which are essential for osteoblast differentiation. These data suggest that osteogenic defects may also be associated with IS in both zebrafish and humans.

Scoliosis is a common phenotype in zebrafish larvae treated with toxic compounds [151–154]. Noticeably, most of the treatments caused spinal curvature together with other defects, including pericardial edema, heartbeat defects, and craniofacial malformation at larval stages, making it unfeasible to investigate vertebral defects at later stages due to high mortality. Interestingly, one study showed that exposure

to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in juvenile zebrafish caused scoliosis in adult fish. Strikingly, scoliosis was also common in the next two generations (F1 and F2 offspring), indicating that TCDD exposure causes genetic defects in zebrafish spine development [155, 156].

### Perspectives

Recent studies in zebrafish models have made significant contributions to our understanding of the mechanisms underlying scoliosis. Owing to their strong reproductive capacity, zebrafish are suitable for high-throughput forward genetic screening (ENU mutagenesis screening) to identify potential genes responsible for scoliosis [24, 64]. In addition, current gene editing techniques, such as CRISPR/Cas9, have made it possible to efficiently and economically target the zebrafish genome to produce mutants based on information from clinical screening for scoliosis. Reverse screening for scoliosis mutants can also help to identify pathogenic variants from DNA sequencing of scoliosis patients.

Finally, caution should also be taken when investigating scoliosis using zebrafish. The SCO of the human fetus failed to immunoreact with any RF-associated antibodies, making the presence of RF in humans controversial [157]. In humans, CSF flow in the ventricle and spinal canal is mainly driven by arterial pulsation and respiration [158], which differs from the cilia-driven pattern of CSF in zebrafish. Moreover, the mechanisms of spinal formation and segmentation in zebrafish differ slightly from those in humans [5]. Despite these findings, clinical data have suggested a conserved role of the ciliary pathway during scoliosis progression between humans and zebrafish [96, 127, 147, 149].

In summary, zebrafish provide an effective model for elucidating the mechanisms underlying scoliosis. Recent studies have shown that ciliary defects contribute to scoliosis by disrupting the physiological roles of muscle fibers, implying that IS may be associated with neuromuscular defects. Exploring the underlying mechanisms of IS in neuromuscular related defects should be a focus of future studies. The mechanisms underlying spatial multidirectional curvature (left–right and dorsal–ventral curvature) in IS-like zebrafish mutants remain unknown. Future studies on axon guidance and differential activation of CSF-cNs may help resolve this query. Moreover, it is still unknown how urotensin neuropeptides are secreted from CSF-cNs, which may provide key information for understanding spinal curvature formation. Finally, the mechanism by which muscular dysplasia leads to scoliosis remain to be determined. Similarly, the regulatory mechanisms underlying scoliosis and *Uts2r3* malfunction in zebrafish muscle fibers need to be further investigated. Recently, it has been suggested that asymmetrical paraspinal muscle activation may be associated with AIS [159, 160],

which provides new insights into neuromuscular function during spine development. Zebrafish will offer an effective platform for investigating these processes.

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## Declarations

**Conflict of interest** The authors declare no relevant competing interests to disclose.

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