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Review

The Application of Zebrafish Model in the Study of Idiopathic Scoliosis

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Abstract: Idiopathic scoliosis (IS) is an unexplained dysplasia of the spine that can occur at any age, with idiopathic scoliosis making up the largest proportion of the total population worldwide, approximately 2-3%. Scoliosis is not just a cosmetic defect, but the development of the spinal deformity can lead to cardiovascular and respiratory lesions, often resulting in serious health problems. Treatment of the condition usually involves major surgery, which is both a physical and financial burden for patients. In order to clarify the aetiology of IS and provide a theoretical basis for new diagnostic and treatment methods IS, it is important to use different animal models for experiments. Zebrafish is an emerging model animal with a short reproductive cycle, minimal breeding expenses, and other beneficial traits including *in vitro* fertilisation, *in vitro* development, and embryo transparency. As a result, genetic alteration and observation are simpler than with traditional model animals. This study examines the history of animal models for IS research, focuses on the benefits and drawbacks of zebrafish as an IS model and the advances it provides to IS research, and anticipates zebrafish application prospects in IS research.

Keywords: adolescent idiopathic scoliosis; animal model; zebrafish; cilia; cerebrospinal fluid

1. Concept of scoliosis

Scoliosis is a spinal deformity disorder characterised by lateral spine bending, sagittal physiological bending, and vertebral rotation [1]. Scoliosis is classified into three types based on its aetiology: idiopathic scoliosis (IS), which refers to various unexplained spinal dysplasias, congenital scoliosis (CS), which is a lateral curvature of the spine that occurs before birth, and neuromuscular scoliosis (NS), a type of spinal curvature resulting from disease or injury affecting the nerves, muscles or other neuromuscular systems [2]. Of these, the most common is IS, and this article will mainly discuss scolioses caused by genetic factors, specifically IS.

Adolescent IS (AIS) has a prevalence ranging from 0.47% to 5.2% [3] and is thought to afflict 2-3% of the global population [4]. Pathological symptoms of this spinal condition, which primarily affects children and adolescents, include spinal S-shaped deformity, rib swelling, and chest curvature [5]. AIS not only impacts the appearance of human body flaws, but also the incorrect growth of the spine can create serious problems, such as cardiovascular and respiratory disorders, which often lead to severe impairment and greatly reduce the quality of life of patients. Currently, the primary method of treating AIS is follow-up observation, and when the deformity becomes severe, surgery is used to heal big wounds. This method places a significant financial and physical load on patients and their families. According to statistics, female AIS patients are more than male. According to survey data from various populations, the ratio between the two ranges from 1.5: 1 to 3: 1 [4]. A great deal of research is currently being carried out on scoliosis, especially IS, regarding its pathogenesis, pathophysiological changes, and the apparent link of the disease to genetic and environmental

factors. The precise pathogenesis, however, remains unclear, making it unable to offer a useful guide for the disease's early prevention and therapy.

2. Research progress on the pathogenesis of scoliosis

The pathogenesis of scoliosis has been studied for more than a century. Early researchers thought that spinal deformity was primarily caused by biomechanical factors, such as imbalanced spinal muscle activity [6], incorrect posture [6,7], or excessive rib growth [8], and not by the development of the spine itself, but rather by other tissues, organs, and even personal behaviour. This theory does not apply to many AIS patients, but it can be used to explain a small percentage of scoliosis instances. In 1959, Thillard discovered that chickens would develop scoliosis if their pineal glands were removed [9]. Based on this, the theory that melatonin is the cause of scoliosis is proposed. This experiment was replicated with bipedal mice after the 1990s, and identical results were observed [10,11]. However, in other experimental species such as hamsters, rabbits, and primates, alterations in melatonin did not promote scoliosis [10,12]. Most crucially, pineal damage in humans did not result in the development of scoliosis [13].

Simultaneously, based on various scoliosis hypotheses embraced by themselves, combined with pathological features and gene functions, the researchers selected some genes, such as connective tissue protein genes [14], bone formation and bone metabolism genes [15], pineal gland hormone genes [13], and puberty and growth genes [16]. These genes were examined and validated in animals such as mice and chickens, but no conclusive pathogenic gene responsible for scoliosis was identified in these experiments.

With the confirmation of the heritability of AIS [17] and the introduction of genome wide association study (GWAS), researchers have carried out many GWAS studies on scoliosis in different ethnic groups, and mapped some loci related to AIS susceptibility in chromosomes 12p, 18q and 19p13 [18–20]. Genes such as *LBX1*, *TPH1*, *SER1* and *matrilin-1* (*MATN1*) were involved [21–24], and *LBX1* was verified in zebrafish and mice.

Afterwards, Hayes *et al.* created a *ptk7a* zebrafish model to investigate the gene's role in Wnt signaling and observed IS-like phenotypes in zygotic homozygotes, which prompted them to examine human samples for pathogenic variants in *PTK7* [25]. Based on this finding, researchers carried out further studies using zebrafish models and proposed a hypothesis for the pathogenic mechanism of IS, that is, abnormal cilia development disrupts the normal flow of cerebrospinal fluid, thereby trigger IS [26]. A subsequent study substantiated the involvement of *PTK7* in a sizable cohort of Chinese IS patients [27].

3. Animal models for IS research

A good animal model can help people perform in-depth study on the mechanisms of human diseases and reach more trustworthy results. The selection of animal models has always been a challenge in IS research. Unlike other diseases, IS is related to the stress of the spine when humans walk upright, hence its animal model must simulate these spinal force characteristics. The choice of animal models for IS has changed from quadrupeds to 'natural' bipeds, to 'artificial' bipeds, and then to fish. In the early days of scoliosis research, researchers used four-legged animals such as calves [28], goats [29], rabbits [30], and rats [31] for explorations. The axial load and dorsal shear stress on the spine of quadruped animals, on the other hand, are significantly different from those of upright humans. In subsequent IS studies, quadruped mice were also directly used as models [32], but this is entirely based on cost and efficiency considerations, and it was difficult to propose convincing hypotheses and theories in these studies, so quadrupeds are not suitable models for IS research.

IS bipedal animal models are now split into two categories: 'natural' bipedal animals and 'artificial' bipedal animals: 'natural' bipedal animals include chickens and primates. As previously stated, the melatonin theory is based on a chicken experimental model. However, compared to non-avian vertebrates, chickens' spines have a completely different anatomical structure [33]. Only 2 intervertebral spaces (6-7 and 7-8 vertebrae) have clearly visible intervertebral discs, which are movable parts, and the spine only has 8 thoracic vertebrae and no fused lumbar vertebrae [34].

Although chickens are bipedal, their thoracic and lumbar vertebrae are still primarily horizontal, unlike how the human spine is affected by gravity [35]. Additionally, this makes it challenging for other bipedal animals, like primates [12] and rabbits [10], to reproduce the results reported in chickens.

Among the 'natural' bipedal species, primates are phylogenetically closest to humans, and most primates walk upright or semi-upright. Their spines are also anatomically similar to those of humans. These characteristics are very suitable for IS research. However, primates cannot be used extensively in the investigation of IS mechanism due to their expensive cost and lengthy generation cycle.

To study IS using bipeds, scientists have created "artificial" bipedal animal models. Cut off the forefoot of quadruped animals, such as bipedal rats and mice, then arrange food on a high spot to create this form of animal model [11]. The scientific significance of this model is demonstrated by the fact that bipedal rats and mice in several experimental models displayed a higher incidence of scoliosis than the matching quadruped animal model [11]. It should be pointed out that if the relevant tissues and cells of a species have developed a special molecular regulatory mechanism to adapt to an upright spine during long-term evolution (which is very likely), then even if quadrupeds are forcibly reversed to bipedalism, thus changing the load direction of the spine from horizontal to vertical, there will still be a substantial difference in the adaptation mechanism between the models and the target species.

In order to find an animal model more suitable for studying the mechanism of IS, the researchers compared the stress patterns of the human and fish spines. The spine of a teleost fish is not loaded horizontally due to buoyancy [33]. When swimming ahead, it is more likely to experience longitudinal water pressure, which is extremely comparable to the vertical stress features of the human spine. Interestingly, scoliosis is a prevalent ailment in bony fish, unlike in terrestrial mammals [33,36]. Further study has revealed that humans and fish share many common causes of vertebral system defects, including genetic factors, dysregulation of physiological processes (e.g. calcium regulation), developmental abnormalities (e.g. fusion of vertebrae) and infection by pathogens (viruses or parasites), demonstrating the homology of spinal deformities, and suggesting the viability of employing fish as a model for human scoliosis research [33]. Zebrafish, as the most extensively used model fish, is unquestionably the chosen fish IS model.

4. The advantages and disadvantages of zebrafish as a scoliosis research model

Although each lineage of teleosts and mammals has acquired unique traits or lost specific skeletal elements during long-term evolution, the vertebrate skeleton is a highly conserved organ system, and some distinctive features of the skeleton are related to early fish embryos and adults, making small teleost fish such as zebrafish an excellent animal model for studying human skeletal diseases [37]. There are three clear advantages to using zebrafish for genetic research: 1. The fish is small, easy to feeding, and the breeding cost is low; 2. Through *in vitro* fertilisation and reproduction, both fertilised eggs and embryos have optical transparency, which is convenient for observation and application of gene editing and other research techniques; 3. Artificial fertilisation is simple to operate, and the number of offspring is large, which is very conducive to the construction and screening of pure lines. Because of these benefits, zebrafish are becoming increasingly popular in medical research. The benefits of zebrafish animal models for IS study go beyond that.

The spine architecture and vertebral structure of zebrafish are quite similar to those of humans from a physiological standpoint. The notochord is the spine's centrosome, and the spinal cord travels through the neural arch [38]. The vertebrae are arranged uniformly from front to back, with bone bows on the dorsal and ventral sides [36]. Furthermore, the precaudal vertebrae of the zebrafish are associated with the rib segments, exhibiting the same natural lordosis as bipedal species such as humans [36]. Although important developmental changes, such as connections to fins and the Weberian apparatus for cranial linkage, must be considered when modelling the establishment of the spine [39]. But the basic structure of the zebrafish and human spines is well conserved [36]. The Weberian apparatus is a chain of tiny bones called Weberian ossicles that connect the swim bladder

to the inner ear. This mechanism enhances the fish's ability to hear by amplifying sounds and sensing vibrations in the water [40].

From the perspective of molecular genetics, human and zebrafish coding genes are highly related, with approximately 71% of human protein-coding genes having one or more zebrafish orthologues and 47% having a one-to-one relationship [41]. The basic skeletal components, development, and essential developmental genes of the skeleton are conserved across a wide range of vertebrates, from teleosts to mammals[42,43]. Although developmental networks may differ between species, the core functions of these networks are often conserved, even across phyla. This has made it possible to study gene function between distantly related vertebrates such as teleost fish and mammals, and it is why the zebrafish can be used as a model to study human skeletal diseases [37].

Many genes and pathways function in the zebrafish vertebrae and surrounding tissues, and are conserved across species. For example, the Hedgehog (Hh) signaling pathway is essential for the development of the spine and surrounding tissues, and one of the most studied genes in this pathway, sonic hedgehog (*Shh*), is conserved in all vertebrates [44]. The Wnt signaling pathway, which is involved in some key developmental processes such as the formation of the body axis, vertebrae, somites, and neural crest, has many components that are conserved from zebrafish to humans[45]. The aforementioned *ptk7* mutation is associated with CS and IS via dysregulation of the Wnt signaling pathway [25].

It should be mentioned that throughout its evolutionary history, teleost fish like zebrafish have undergone a fish-specific genome-wide doubling event. Zebrafish possesses more copies of several coding genes than humans and mice do, despite the fact that some of the genes obtained via doubling were quickly lost [46]. For instance, both zebrafish orthologs *runx2a* and *runx2b* share similarities with the human *RUNX2* gene [47]. Although this feature of zebrafish increases the functional redundancy of the genome, it also increases the difficulty of the operation, as several homologous genes must be knocked out or silenced at the same time when testing gene function. In the case of non-lethal gene knockouts, multiple copies mean that more experimental procedures are required to obtain samples with complete inactivation of the target gene for subsequent research, and may also raise the issue of potential functional differences between different copies. This is clearly a disadvantage for the study of human disease mechanisms using zebrafish as a model. The retention of homologous genes in zebrafish, however, frequently enables the subtle functional examination of such genes [47], without the need to apply a number of genetic approaches to accomplish conditional knockout as in mice. Therefore, the fact that zebrafish have multiple copies of genes also has positive aspects that can be exploited in research.

Zebrafish have been used as an animal model for many skeletal disease studies in light of their potential for studying human skeletal diseases [48,49], and IS studies have also gotten some extremely instructive results using zebrafish models.

5. Two uses of zebrafish model in IS research

The purpose or use of zebrafish models in IS research can be divided into two categories: functional verification, in which zebrafish is subjected to gene editing experiments such as CRISPR/Cas9 to verify candidate pathogenic genes obtained from human IS samples; and mechanism exploration, in which zebrafish with scoliosis phenotype is directly used as the research object to explore the mechanism of IS occurrence.

In the first category of studies, candidate genes can be identified through three main approaches: genome-wide or whole-exome association analysis of large groups of disease and control individuals; locating gene loci from families with scoliosis phenotype in some individuals; or identifying genes with abnormal sequences from a single patient sample (Table 1). The choice of approach depends largely on the availability of suitable sample groups. In fact, the difficulty of obtaining familial data and the ease of obtaining random groups determined the high proportion of GWAS research. Due to the higher prevalence of idiopathic scoliosis in females and the fact that women are prone to more severe deformities, most of these studies have intentionally selected severely curved female

individuals as the disease group to locate candidate genes. GWAS are commonly used, with female individuals with severe spinal curvature often selected as the disease group to identify candidate genes. Studies based on a single scoliosis case tend to introduce a large number of false positive candidate genes, so only those that have been validated by animal models are likely to be accepted and published. Therefore, this type of research has the lowest proportion. Validation in model organisms is essential for human-derived genes to be of value for future research. For instance, the candidate genes *GPR126* [50], *POC5* [51], and *LBX1* [52], identified through multiple population association analyses, have been validated for their association with IS through zebrafish knockout experiments. Other genes, such as *BNC2* [53], *PAX1* [54], *MAPK7* [55], *TLL1* [56] and *PTK7* [57], have also been confirmed through gene knockout experiments in model animals, mainly using zebrafish [25,51–54,56,58] (Table 2).

Table 1. Human-derived candidate genes for spinal deformity.

Related diseases	Gene	Sample source	Year	Reference
AIS	<i>TPH1</i>	103 AIS cases and 108 controls (Chinese Han population)	2008	[59]
	<i>DSCAM</i>	419 AIS families	2011	[60]
	<i>CNTNAP2</i>			
	<i>GPR126</i>	1,819 AIS cases and 25,939 controls	2013	[61]
	<i>CHL1</i>	500 AIS cases and 500 controls (10-18 years old females in a Han Chinese population)	2014	[62]
	<i>BNC2</i>	2,109 AIS cases and 11,140 controls	2015	[63]
	<i>AJAP1</i>			
	<i>PAX3</i>	4,317 AIS cases and 6,016 controls (Chinese Han population)	2015	[64]
	<i>EPHA4</i>			
	<i>BCL-2</i>			
		620 AIS cases and 1,287 controls (female Caucasian)	2015	[65]
	<i>LBX1</i>	667 AIS cases and 901 controls (French-Canadian population)	2018	[66]
	<i>TBX1</i>	79,211 individuals (Japanese population)	2019	[67]
	<i>DSE</i>			
	<i>FTO</i>			
	<i>BOC</i>			
	<i>HSPG2</i>	1752 AIS cases and 1584 controls (China)	2019	[68]
	<i>FBN1 and FBN2</i>	952 AIS cases and 1499 controls	2019	[69]
		2432 AIS cases and 2292 controls (Chinese population)	2018	[70]
	<i>POC5</i>	French-Canadian and British families and sporadic cases	2021	[71]
	<i>COL8A2</i>	23 Multigeneration Idiopathic Scoliosis Families	2021	[72]
	<i>COL4A3</i>			
	<i>COL6A5</i>			
	<i>COL27A1</i>			
	<i>COL7A1</i>			
	<i>COL21A1</i>			
	<i>COL9A2</i>			
	<i>COL9A3</i>			
	<i>COL4A6</i>			
	<i>HSPG2</i>			
	<i>FBN2</i>			
	<i>ADAMTSL2</i>			
	<i>LTBP4</i>	302 AIS cases and 818 controls	2022	[73]
	<i>TTN</i>			
	<i>CLCN1</i>	11 AIS cases (Taiwan)	2022	[74]
	<i>SOX8</i>			

	<i>ROBO3</i>	135 AIS cases and 267 controls	2023	[75]
	<i>PAX1</i>	3,102 individuals	2015	[76]
IS	<i>CELSR2</i>	1739 IS cases and 1812 controls (Swedish-Danish)	2017	[77]
	<i>KIF7</i>	3 IS cases in a multigenerational family	2020	[78]
		161 CS cases and 166 controls		
CS	<i>TBX6</i>	(PUMCH in China, Oct. 2010 – Jun. 2014)	2018	[79]
PSCS		GSE11854 expression dataset		
	<i>COL27A1</i>	associated with somite formation in the GEO database	2022	[80]
PCD	<i>DNAAF2</i>	patients from two consanguineous families	2021	[81]
OI	<i>FKBP10</i>	Patients diagnosed with OI	2023	[82]
MFS	<i>FBN1</i>	a child with Marfan syndrome	2023	[83]
CDD	<i>SP7</i>	2 CDD cases from a large consanguineous family	2023	[84]

AIS: adolescent idiopathic scoliosis. IS: Idiopathic scoliosis, CS: congenital scoliosis, PSCS: poor segmental congenital scoliosis, PCD: primary ciliary dyskinesia, OI: Osteogenesis imperfecta, MFS: Marfan syndrome, CDD: Craniodiaphyseal dysplasia, PUMCH: Peking Union Medical College Hospital.

However, identifying and verifying candidate genes is only the first step towards understanding the pathogenesis of IS. Firstly, mutations in these genes affect only a small proportion of individuals with IS. For instance, even genes such as *GPR126*, which have been identified in several ethnic groups by GWAS, can only explain a limited number of cases. Secondly, proposing one or more hypotheses that can reasonably connect these genes, which may not be related to existing human knowledge, is a challenge for researchers' creativity. Finally, the GWAS study of IS may have reached its bottleneck period, as researchers have compared different races, increased the sample size to almost 100,000 individuals, and still failed to identify further explanatory clues. As a result, the proportion of such research in exploring the mechanism of IS has gradually declined over the past five years.

The second type of research involves obtaining zebrafish with a scoliosis phenotype, which can be achieved mainly in two ways: serendipitous discovery and screening for induced mutations. It has occasionally been discovered in investigations with different goals that knocking down particular genes in zebrafish can lead to IS. For instance, scientists studying the human developmental disorder cilia disease discovered that zebrafish with the *cc2d2a* gene mutation displayed scoliosis phenotype, and scientists studying primary ciliary motor dysfunction discovered that zebrafish with the *c21orf59*, *ccdc40*, *ccdc151*, and *dyx1c1* mutations also displayed juvenile or late-onset scoliosis, similar to AIS [85–88] (Table 2).

Table 2. Model biological verification for diseases with spinal deformity.

Related diseases		Gene	Animal model validation	Year	Reference
Reissner Fiber	AIS spine curvature	<i>scospondin</i>	zebrafish	2018, 2020	[89–91]
		<i>dnah10</i>	zebrafish	2022	[92]
		<i>camel</i>	zebrafish	2021	[93]

	IS	<i>vangl2</i>	zebrafish	2022	[94]
		<i>scospondin</i>	zebrafish	2020	[95]
		<i>katnb1</i>	zebrafish	2022	[96]
		<i>ccdc57</i>	zebrafish	2023	[97]
cilia	AIS	<i>rpgrip1l</i>	zebrafish	2019	[98]
		<i>cfap298</i>	zebrafish	2021	[99]
		<i>dnah10</i>	zebrafish	2022	[100]
		<i>ccdc57</i>	zebrafish	2023	[101]
	IS	<i>ttll11</i>	zebrafish	2021	[102]
		<i>katnb1</i>	zebrafish	2022	[103]
		<i>ccdc57</i>	zebrafish	2023	[104]
	CS, IS	<i>ptk7</i>	zebrafish	2014, 2016	[26,105]
	IS, CSF stasis	<i>kif6</i>	mouse, zebrafish	2014, 2018	[106,107]
		<i>ccdc40</i>	zebrafish	2011	[108]
	PCD	<i>dyx1c1</i>	mouse, zebrafish	2013	[109]
		<i>ccdc151</i>	mouse, zebrafish	2014	[110]
		<i>zmynd10</i>	medaka, zebrafish	2017, 2018	[111,112]
	ciliopathies	<i>c21orf59</i>	zebrafish	2013	[113]
		<i>cep290</i>	zebrafish	2019, 2022	[114,115]
	ciliopathy syndromes	<i>bbs-5</i>	<i>C. elegans</i> , mouse,	2022	[116]
		<i>nphp-4</i>	zebrafish		
others	AIS	<i>gpr126</i>	zebrafish	2013	[117]
			mouse	2019	[118]
		<i>bnc2</i>	zebrafish	2015	[119]
		<i>mapk7</i>	zebrafish	2018	[120]
		<i>lbx1</i>	mouse, zebrafish	2016, 2022, 2023	[121–123]
	CS	<i>uncx</i>	zebrafish	2023	[124]
		<i>Foxo4</i>	rat	2019	[125]
		<i>poc5</i>	zebrafish	2015	[126]
	IS	<i>kif7</i>	zebrafish	2021	[127]
		<i>ppp2r3b</i>	zebrafish	2023	[128]
	PCD	<i>zmynd10</i>			
		<i>kif3b</i>	zebrafish	2018	[129]
		<i>uts2ra</i>			
	thoracic aortic aneurysm	<i>col1a2</i>			
		<i>col5a1</i>	zebrafish	2022	[130]
		<i>col5a2</i>			
		<i>fbn1</i>			

	<i>pkd2l1</i>	zebrafish	2018	[131]
spine curvature	<i>urp1</i>			
	<i>urp2</i>	zebrafish	2022, 2023	[132,133]

AIS: adolescent idiopathic scoliosis, IS: Idiopathic scoliosis, CS: congenital scoliosis, PCD: primary ciliary dyskinesia.

Furthermore, a random mutant zebrafish population can also be created to screen individuals with a heritable scoliosis trait. In 2020, Sun *et al.* obtained a mutant zebrafish line with congenital scoliosis-like vertebral malformations through ENU mutagenesis screening. [134]. They also discovered and validated the related gene *dstyk* in the ENU-induced family, and its protein product DSTYK is found in the late endosomal / lysosomal compartment and contributes in lysosome production in mammalian cells. This gene's knockout enhances mTORC1-dependent TFEB nuclear translocation, which inhibits notochord vacuole and lysosomal production, resulting in vacuole deficits in notochord cells during notochord development and vertebral malformations in *dstyk* mutant zebrafish. In turn, inhibiting mTORC1 activity can repair the *dstyk* mutant's abnormalities in notochord vacuole biosynthesis and scoliosis[134]. In the same year, Bagwell *et al.* conducted a study demonstrating that mutations in *dstyk* cause notochord vacuole fragmentation and a severe congenital scoliosis-like phenotype in zebrafish [135].

The second type of study can only provide possible evidence for the molecular mechanism of spinal dysplasia and does not exhibit a direct correlation between IS and actual cases. Therefore, combining the candidate genes of AIS patients obtained from the first type of research with the results of the second type of research can provide potential hypotheses. Through innovative approaches and using zebrafish as a model, these hypotheses can be fully tested and confirmed, which may be the most practical approach to discover the underlying mechanism of IS. In the following sections, we will describe in detail the research involving the *PTK7* gene, which hypothesizes that cilia damage and abnormal cerebrospinal fluid flow contribute to IS, as well as other related studies using this combination of techniques.

6. The relationship between cilia and IS was studied by zebrafish model

Researchers generated maternal-zygotic and zygotic mutant zebrafish strains of protein tyrosine kinase-7 (*ptk7*). The former had congenital scoliosis, while the latter had an idiopathic scoliosis phenotype that was highly comparable to that of humans [25,57] . Su *et al.* also confirmed the involvement of PTK7 in IS [27]. Cilia function is intimately connected to the classical β -catenin and planar cell polarity (PCP) Wnt signalling pathways, which Ptk7 is a key regulator of. Organelles called cilia can be seen sticking out from the surface of eukaryotic cells. They can be classified as primary (non-moving) cilia, and moving cilia based on how they move. The movement of single-cell organisms or the fluid of metazoan animals, such as cerebrospinal fluid (CSF), can be facilitated by the action of moving cilia [136–138]. It is worth mentioning that scoliosis phenotypes have been seen in several investigations of cilia-related genes [139–142], and scoliosis patients have CSF flow abnormalities. CSF is a watery fluid found in the brain and spinal cord that is necessary for central nervous system homeostasis. Based on the preceding evidence, the researchers hypothesised that the lack of zebrafish *ptk7* produced cilia malfunction, which resulted in CSF flow abnormalities and scoliosis. To test this idea, researchers used the transcription factor Foxj1 [143], which is the major enhancer of human motor cilia development, to create *Tg (foxj1a:: ptk7)* zebrafish. Ptk7 malfunction in the motor cilia lineage resulted in brain motor cilia development problems and aberrant CSF flow, according to the findings. And *foxj1* overexpression can reverse scoliosis caused by *ptk7* deletion while also allowing normal development of motor cilia and CSF flow [144]. The researchers also created zebrafish strains with conditional knockouts of other genes essential for the development of motor cilia, like *c21or f59*, and mutant will also exhibit the same phenotypes as *ptk7* mutant zebrafish in order to further demonstrate that the abnormal development of motor cilia can cause scoliosis [144].

Later research has further supported the impact of cilia abnormalities on zebrafish axial development (Figure 1). For instance, the cilia's structure is influenced by the tubulin tyrosine ligase-like 11 (*TTL11*), whose loss also causes scoliosis in zebrafish [145]. The deletion of *CCDC57* protein, which has an effect on the assembly of cilia, also causes the zebrafish mutants displaying ventral curvature at the embryonic stage [146,147]. Li *et al* established a *ccdc57* knockout zebrafish strain with frameshift mutations. The team's research results showed that the dysfunction of *ccdc57* can disrupt the coordinated beating of multiple cilia bundles in brain ventricle, change the cilia beat pattern, and result in scoliosis in zebrafish [147]. Additionally, Wang *et al* performed exome sequencing analysis on 195 AIS patients in southern China and discovered the genes *dnaaf1* and *zmynd10* that are related to ciliary dynein assembly and that also exhibit scoliosis phenotypes in zebrafish when any one of them is knocked out [148].

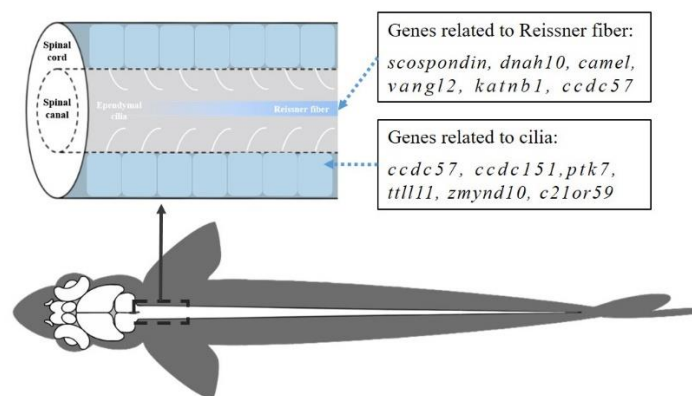


Figure 1. Schematic diagram of the structure of the zebrafish spinal cord.

How does CSF influence the development of the zebrafish body axis? The discovery of the cilia-CSF-scoliosis relationship is a significant advancement in understanding the mechanism of scoliosis formation. Urotensin-related peptide (Urp), which is present in CSF, has been linked to scoliosis in zebrafish, according to study from Zhang *et al*. Zebrafish adults who have Urp receptor mutations can develop severe scoliosis [149]. To investigate whether the urotensin signaling pathway is also involved in the regulation of spinal curvature in human scoliosis patients, bilateral paravertebral muscle tissue was collected from scoliosis patients during surgery. The researchers compared the expression of urotensin signaling pathway genes in paravertebral muscle tissue obtained from the convex and concave sides of the spinal curvature sites. It was found that the expression of *UTS2R* was significantly higher in convex muscle tissue than in concave muscle tissue [146].

Reissner fibre (RF) has received attention from other researchers (Figure 1). Most notochords contain RF, although its function is unknown. It is a protein filament released at the mesencephalic-diencephalic (M-D) junction by specialised ependymal cells. It is positioned in the centre of the notochord canal filled with CSF. It continues to the notochord canal's caudal end and decomposed here, the byproduct enters the CSF and blood [150]. Recent research has revealed that RF in zebrafish is in a very dynamic balance of secretion and breakdown [151]. The glycoprotein SCO-spondin, which is highly conserved in notochords, is the major composed of RF [152]. It is a big multi-domain extracellular matrix molecule that is primarily involved in cell adhesion and axon guidance [150]. Cantaut-Belarif *et al*. (2018) created a *scospondin* gene knockout line in zebrafish and discovered that RF was required for zebrafish body axis expansion. The cilia and CSF flow were normal in RF-deficient zebrafish, but the juveniles displayed an evident scoliosis phenotype [153]. Later, the researchers created a zebrafish strain producing *scospondin* with a missense mutation. In early embryos, this SCO-spondin protein can ensure the assembly and extension of RF, but as the embryo develops, RF increasingly disintegrates, and zebrafish embryos also exhibit the phenotypic of scoliosis [154]. The previously mentioned deletion of the *ccdc57* not only results in abnormal cilia movement, but also causes significant thinning, discontinuity or deletion of RF in adult zebrafish mutants of *ccdc57*. The results of these studies once again demonstrated the importance of RF

integrity for the formation of the zebrafish spine. Additionally, scientists have discovered that CSF-contacting neurons (CSF-cNs) in the spinal cord need RF to perceive the body axis' curvature [155].

7. Conclusions and perspectives

Zebrafish is a well-established model organism with several benefits. It is quite practical to observe and modify its embryos genetically. Imaging and bone staining both have established technologies. More importantly, its similarity to human spine pressure during exercise and conservation with human bone formation are of unique value for the research of IS. The relevance of the zebrafish model is exemplified by the fact that many potential pathogenic genes identified in human patients through GWAS analysis have been confirmed in zebrafish.

As a result, zebrafish have taken the position of other animals as the primary animal model for research on the pathophysiology of scoliosis. Recently, scientists have discovered a strong correlation between cilia-driven CSF flow and scoliosis using zebrafish as a model. They later discovered that the presence of urotensin in CSF fluid might influence how the spine bends dorsally and ventrally, and that RF breakdown can result in scoliosis. We now have a much better knowledge of how scoliosis develops because to these recent discoveries.

However, there are still numerous issues and connections that need to be researched in the study of the scoliosis mechanism. A large number of genes detected in human IS patients cannot be explained by CSF flow and RF abnormalities (Table 2). The SCO, which is a synthesizing organ for the RF, atrophies shortly after human birth, resulting in the cessation of SCO-spondin synthesis. Even in the zebrafish cerebrospinal fluid-RF model, there are still many aspects that require further investigation [156]. For instance, are there additional substances in cerebral fluid, besides urotensin, that influence the geometry of the body axis? What substances does the body utilise to control how the body axis is shaped? What part does RF play in the formation of the spine? How do RF and CSF-cNs work together to detect axis curvature? To fully explore and study all of these issues, researchers must fully utilise zebrafish and other animal models.

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