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GENETICS

Genetic animal modeling for idiopathic scoliosis research: history and considerations

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Abstract

Purpose Idiopathic scoliosis (IS) is defined as a structural lateral spinal curvature ≥ 10° in otherwise healthy children and is the most common pediatric spinal deformity. IS is known to have a strong genetic component; however, the underlying etiology is still largely unknown. Animal models have been used historically to both understand and develop treatments for human disease, including within the context of IS. This intended audience for this review is clinicians in the fields of musculoskeletal surgery and research.

Methods In this review article, we synthesize current literature of genetic animal models of IS and introduce considerations for researchers.

Results Due to complex genetic and unique biomechanical factors (i.e., bipedalism) hypothesized to contribute to IS in humans, scoliosis is a difficult condition to replicate in model organisms.

Conclusion We advocate careful selection of animal models based on the scientific question and introduce gaps and limitations in the current literature. We advocate future research efforts to include animal models with multiple characterized genetic or environmental perturbations to reflect current understanding of the human condition.

Keywords Idiopathic scoliosis · Animal models · Spine · Genetics · Review · CRISPR/Cas9

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Abbreviations

IS	Idiopathic scoliosis
GWAS	Genome-wide association study
QTL	Quantitative trait locus

Introduction

Idiopathic scoliosis (IS) is a structural lateral curvature of the spine ≥ 10° with a rotatory component [1] that affects 2–3% of otherwise healthy children across most populations [2, 3]. Current therapeutic options are limited to bracing, surgery, and physical therapy, with spinal fusion surgery being the only treatment option for severe progressive curvatures. IS runs in families and is believed to have a significant genetic component [4–13], with multiple genetic risk loci identified including those in or near *ADGRG6/GPR126* [14–19] and *LBX1* [17, 20–32]. However, IS etiology is still poorly understood, despite decades of research. The genetic and phenotypic complexity of IS [33], difficulty in obtaining musculoskeletal tissue samples, and unique biomechanical [34] and hormonal factors hypothesized to contribute to IS

in humans have all made IS a challenging disease to understand. Nevertheless, dozens of animal models of scoliosis have been created, helping to bridge gaps in understanding of the disease.

Animal models of scoliosis have been developed through environmental, surgical, and genetic methodologies, which are used most often today. Genetic regions of interest may be disrupted within an animal model to determine its function in axial development; these mutations may be induced constitutively (permanent gene disruption across cell types) or conditionally (tissue-specific).

In this review, we provide a brief overview of the history of animal modeling and considerations for choosing a species as a model for scoliosis research, followed by an overview of genetic animal modeling used in IS research to date. The intended audience for this review is clinician scientists in the fields of musculoskeletal research and orthopedic surgeons seeking updated information regarding the development of scoliotic animal models for genetic research. We build upon previous IS animal model reviews [35–39] and conclude by noting gaps in the current literature and providing recommendations for future research efforts.

A brief history of comparative medicine

Comparative medicine is based on the principle that animals share phenotypic and genetic traits with humans, and that discoveries made within animals can gain understanding of human disease. Although observational animal research was recorded as early as the sixth century B.C. [40], the birth of comparative anatomy largely occurred during the Renaissance, notably through Andreas Vesalius' 1543 *De Humani Corporis Fabrica* (The Structure of the Human Body) [41]. Centuries later, Charles Darwin's *On the Origin of Species* (1859) and Gregor Mendel's work on the basic laws of heritability (1865) established genetic relationships between individuals and species, providing a foundation for comparative medicine. By 1902, William Castle had bred mouse (*Mus musculus*) lines for research purposes, followed by Clarence Little's creation of the genetically homogenous strain of dilute brown non-agouti (dba) mice [42]. Through the last century, animal and cell culture models have greatly improved our understanding of cellular structures and biochemical interactions underlying human physiological processes.

Today, animal models are crucial for basic and translational research efforts. Significant pre-clinical data, usually including validation of safety and efficacy in animal models, are required by the Food and Drug Administration prior to initiating clinical trials for pharmaceuticals, medical devices, and biologics. The first step in such efforts is creating an appropriate animal model to mirror the human condition; this ensures that (1) the pathology of the condition is understood and (2) there is a model in which potential clinical treatments can be evaluated. To date, IS research efforts using animal models have largely aimed to understand IS etiology. The following section introduces considerations for scoliosis researchers selecting an animal model.

Considerations for selecting an animal model

Researchers must carefully examine how closely the animal models the human condition, which may include the underlying pathophysiology and genetics of the organism, similarity of phenotypic endpoints to the human, and the similarity of the animal and human disease [43, 44]. Key factors include: (1) relevance of the model to the scientific question, (2) cost and feasibility, (3) resources and reagents compatible with the species, and (4) ethical considerations for the use of the species for research, as detailed below:

(1) Relevance of the model to the scientific question: Animals selected for research must be similar enough to the human condition or system to provide relevant data. When designing genetic studies, it is important to examine genetic homology between the animal and human, as genes under study may be duplicated, deleted, or significantly divergent between the two species. Animal models should be evaluated for three types of *validity*, which indicates the strength in which research findings translate to the human condition [44]:

- *Face validity* indicates shared phenotypic or symptomatic manifestations of the clinical condition under study, where ideally the disease condition occurs naturally in the model.
- *Construct validity* is the degree of underlying biology shared with the human condition, which can include similarity of the target organ or structure. Advances of molecular genetics now allow study of genetic variation and molecular pathways at the individual nucleotide level within animal models, adding a significant level of specificity to the study of the human condition when the mutational variants are known.
- *Predictive validity* indicates a model's similar response to therapeutic agents as in humans. Treatment responses even between mammalian systems can differ remarkably in certain disease contexts [45–49].

Proper evaluation of validity is necessary for researchers to interpret data and address its limitations, thus ensuring more effective clinical translation of the research findings.

(2) Cost and feasibility: Popular animal systems used in musculoskeletal research vary widely in cost of procurement

and upkeep. A complete evaluation of costs associated with an animal model ensures that care for the animals is budgeted for.

(3) Available resources and reagents: Common animal models (i.e., mouse) have a greater array of resources and commercially available reagents, including well-annotated genomes. Reagents designed for use in one animal species (e.g., antibodies) may not react to others. Custom-made reagents can be created, albeit through an increased cost [50].

(4) Ethical considerations: Federal, national and institutional scientific boards require animal research to be evaluated on the basis of ethics, including justification and documentation of minimization efforts for animal suffering. A review of ethical considerations specifically for musculoskeletal research has previously been published by Allen et al. [51].

Popular animal models for IS research

Table 1 provides an overview of pros and cons associated with different research models for musculoskeletal research, as well as common research applications. Differences in spinal anatomy between popular animal models have been reviewed previously [52–57].

Rodents: Mouse (*Mus musculus*) is the most popular mammalian animal model for the study of multiple human disorders, including disorders of the musculoskeletal system [40, 58]. The mouse genome is approximately 85% identical to the genome of *Homo sapiens* [59]. Mice have cheaper husbandry costs compared to other mammals (i.e., rats, rabbits), and have a wide selection of available reagents, including antibodies and assays, and the genomes of commonly used strains are well annotated. Rats (*Rattus*) are also popular models for human disease [60–62] and in some situations have some unique advantages as a model for research. The larger body size allows for more sophisticated surgical approaches and physiological measurements. Additionally, the rat has proven to be an excellent model for human spinal cord injury, with a response process closely mimicking that of the human [63–65].

Despite the genetic, hormonal and developmental similarities of mouse to human, mice are quadruped, presenting significant biomechanical differences to human and, it has been proposed, a resistance to developing scoliosis spontaneously [36].

Zebrafish: The zebrafish (*Danio rerio)* is a popular animal model with several advantages including a well-defined genome, easily manipulated and transparent embryos, fast generation times, and low husbandry costs compared to mammals [66–68]. Zebrafish embryos contain a ciliated organ (Kupffer's vesicle), thought to be responsible for the determination of left/right body axis, and an appropriate model for investigating ciliopathies [69]. Numerous resources and reagents are available including transgenic lines in which developing structures during early embryonic stages can be visualized. Zebrafish International Resource Consortium (ZIRC) is a federally supported zebrafish database providing resources for researchers.

Zebrafish have been a popular animal model in scoliosis research, thoroughly reviewed by Grimes et al. [70]. Bony fish present unique advantages for scoliosis research, as they naturally develop a degenerative scoliosis with advanced age, as well as other spontaneous spinal curvatures upon environmental or genetic disturbances [38, 71, 72]. The cranial-to-caudal biomechanical load placed on the spine of the zebrafish swimming through viscous water has been hypothesized to mimic the biomechanical load on the human spine [71]. Although yet proven, bony fish appear to be more susceptible to mechanical strain of the spine, spinal curve initiation, and severe curve progression compared to quadrupeds [38].

Despite these advantages, key differences in the physiology of the zebrafish must be considered when translating research findings to humans. The Reissner fiber, a proteinaceous strand that runs along the spinal column of most vertebrates, has been implicated in onset of scoliosis-like curvatures in several zebrafish models [73, 74], but has not yet been demonstrated as present in humans (see *Bony Fishes*). Hormonal differences between fish and humans are substantial, some of which have been implicated in IS development [75] (i.e., leptin [76, 77], melatonin [78–82], estrogen [80, 83])**.**

Avian models: Some of the earliest models of scoliosis were created in avians, including the pinealectomized chicken [84–88]. Avians are bipedal when on land and thus present some biomechanical similarities to humans. The removal of the pineal gland created a three-dimensional spinal deformity that was then compared morphologically to that of human IS. Multiple concerns including postural mechanical forces, surgical specificity, lack of consistent reproducibility, and endocrine metabolic differences between the human and avian species have led to question the relevance of the avian models.

Non-human primates: Non-human primates have only been used as scoliosis models within a few studies, likely due to their expense and unclear utility as a scoliosis model. In the 1970s, researchers used a rib excision technique, which had previously been used to induce scoliosis in rabbits [89], to induce scoliosis in thirteen baboons (*Papio papio, Papio hamadryas*). However, no animals developed scoliosis [90]. This technique again failed to produce scoliosis in monkeys (species not specified) a decade later [91]. Surgical attempts to produce scoliosis in Rhesus macaques (*Macaca mulatta*) via sacrospinalus muscle resection [92] or excision of the intercostal nerves and erector spinae muscle

Table 1 Summary of popular animal models for scoliosis research and their most frequent experimental uses

Model type	Popular species	Research applications	Major advantages	Major disadvantages
Rodent	Mouse, rat, hamster, guinea pig, chinchilla	Systemic, genetic research, basic genetic and protein characterizations, musculoskeletal disorders, cancer, toxicology, pharmaceutical testing, diet and microbiome research, infectious diseases, chronic diseases	Mammalian, 80% overall genetic homology to human, ease of genetic manipulation, fast reproduction rates, cheapest mammalian model, largest genomic and experimental resources, very well-annotated genome, established genetic lineages, simple husbandry needs, most widely used models for biological research	Sex hormones must be accounted for, breeding more complex, surgery required to access gestating offspring, more expensive than many non-mammalian models, some major differences to human (i.e., immune response, inflammation, neurological disease, other), susceptible to infectious disease
Mid- to large mammals	Sheep, pig, horse, dog	Systemic, genetic, and musculoskeletal disorders; cardiovascular research, regenerative medicine	Mammalian, genetic homology to human, skeletal system may be closer to human, larger tissues more amenable to surgical manipulations	Expensive, complex breeding, fewer genetic and experimental resources, significant social and behavioral considerations, possible aggression between individuals
Non-human primate	Rhesus or cynomolgus macaque, chimpanzee, marmoset	Reproductive research, virology, immunology, brain research, neurophysiology, pharmaceutical and medical device testing, infectious diseases, social and behavioral research	Closest physiology to human; 90–98% genetic homology to human (depending on species); hormonal, immunologic, developmental, and behavioral characteristics similar to human	Very expensive, significant outbred genetic variation, ethical considerations, highly specialized facilities required, only permitted for specific research applications, complex social considerations, specialized veterinary experience required
Avian	Chicken, duck, zebra finch, pigeon, European starling	Virology, craniofacial development, embryology, cancer, parasitology, aging, retinal disease, vaccine production, learning and memory	Large eggs, bipedal, ease of surgical manipulation, natural mutant lines, warm blooded	Non-mammalian, significant skeletal differences to human, variable social and breeding considerations, sensitive respiratory system to toxins, easily stressed
Bony fishes (teleosts)	Zebrafish, medaka, guppy	Neurology, genetic screening, cell biology, toxicology, bone biology, craniofacial development	Transparent embryos, exterior development, rapid embryonic development, inexpensive, easy husbandry and breeding, large clutch sizes, less susceptible to disease, easy shipment/exchange of genetic lines, natural scoliosis, abundant genetic resources, transgenic fluorescent marker lines	Non-mammalian, significant physiological and biomechanical differences to human, genetic redundancy

were also largely unsuccessful [90]. Pinealectomy, which was used to induce scoliosis across multiple studies in avians and smaller mammals [37], also failed to induce scoliosis in any macaques [93].

The reasons for the failures to produce an idiopathic-like scoliosis in non-human primates are unclear, although differences in biomechanical loading and posture are certain to play a role [90]. One study compared spinal muscle composition in humans to Rhesus macaques (*Macaca mulatta*), the most common non-human primate model, to determine the animal's suitability as an experimental scoliosis model [94]. After significant differences in superficial muscle tissue composition were found, the authors were not able to recommend Rhesus macaques as a model for scoliosis, although the deep tissue muscles appeared similar. Interestingly, degenerative scoliosis appears to be common among older captive macaques [95]. In one study, 46.6% of captive macaques displayed some degree of degenerative scoliosis ($n = 58$, age 0–27 years), which was their most frequently observed osteopathology [96]. The failures of surgical techniques to produce a scoliosis curvature highlight the importance of differences between species when it comes to the spinal column and scoliosis. Further research with non-human primates may be needed to help elucidate the pathogenesis of human scoliosis.

Other models: Larger mammals, including rabbits and pigs, have been used for decades in musculoskeletal research conditions including osteoarthritis [97] and osteoporosis [98]. Two recent publications focus on the mini pig as a model of scoliosis, the first created a scoliotic deformity through a surgical posterior medial costotransversectomy [99], and the second through the use of a torsional tension device [100]. Both of these models rely on the biomechanical aspects of the growing spine to induce a scoliotic deformity, and potentially aid us in understanding growth modulation techniques of the spine. Larger mammalian models more closely resemble humans in terms of the genome, endocrine systems, and bone characteristics.

Genetic animal models of IS

Idiopathic scoliosis (IS) research progress has been hindered by the lack of developmentally appropriate animal models to aid in understanding the complex biomechanics of the growing human spine. Some have proposed that the unique structural–mechanical challenges of bipedalism on the spinal column are key factors driving IS, due to the relatively few observations of scoliosis in non-bipedal animals [101–103]. Despite these challenges, researchers have developed numerous animal models to inform our understanding of scoliosis pathology including birds, mammals, and bony fishes. Figure 1 provides examples of several genetic animal models

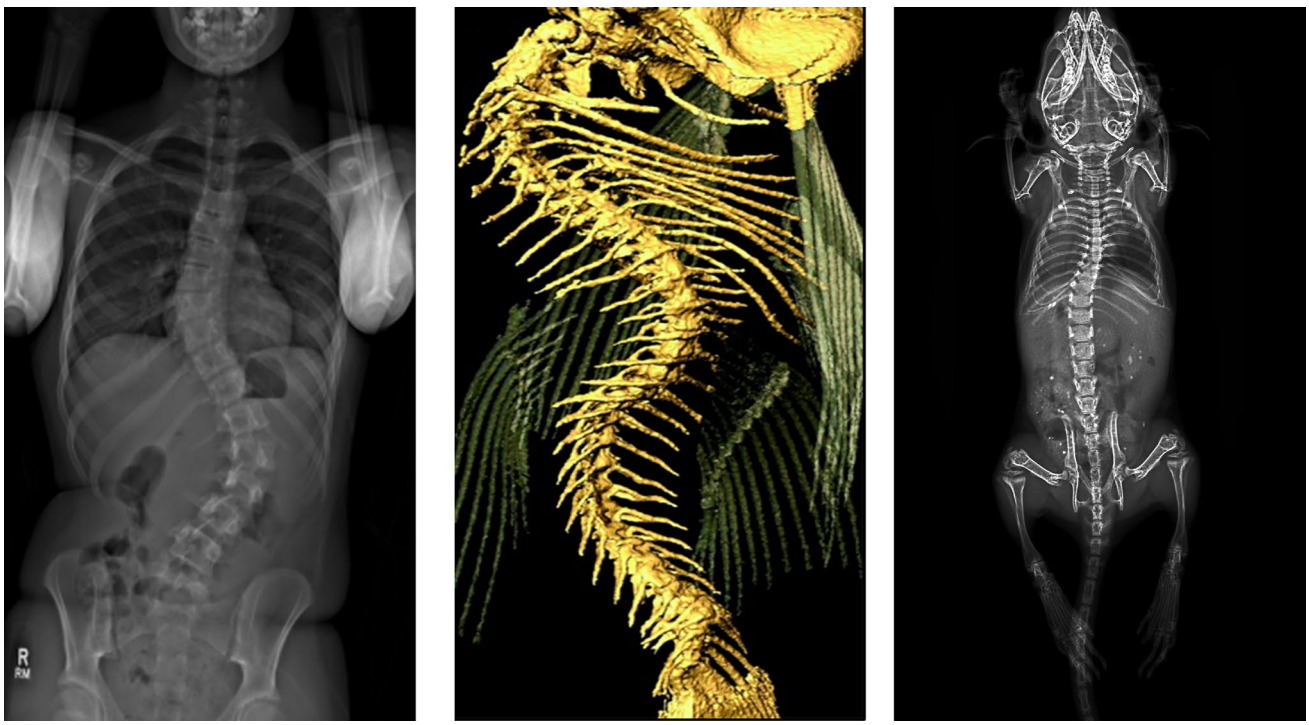

Fig. 1 Examples of genetic animal models of scoliosis. Left: radiograph of adult female with scoliosis. Middle: adult *ptk7* mutant zebrafish. Right: adult female homozygous conditional *Adgrg6* mutant mouse

that exhibit abnormal axial spinal curvatures and are commonly used for research.

Early animal models of scoliosis involved a surgical pinealectomy, performed in chickens, rats, and mice, resulting in some animals developing spinal curvatures [37]. As the pineal gland secretes melatonin, this led to an interest in the melatonin pathway as related to the etiology of IS (reviewed by Girardo et al. [81]). Recently, a bipedal mouse model of scoliosis deficient in melatonin was found to have reduced incidences of scoliosis, as well as improved bone mineral density, after treatment with exogenous melatonin [104].

The concept of bipedalism as a factor contributing to spinal imbalance and curvature led to another surgical technique involving the amputation of the forelimbs in rats and mice, creating an artificial model of bipedalism [101, 105]. In rabbit and mouse resection of the lower ribs within the thoracic cavity was also found to result in spinal curvatures [89].

Spontaneous or environmentally induced models of scoliosis have been observed. A thoracic scoliosis developed spontaneously in a line of Japanese white rabbits; this line was later found to have high serum melatonin [106]; however, a genetic cause to date has not been pinpointed. Recently, a stranded minke whale was observed with a severe scoliotic spine, which was believed to be initiated by blunt traumatic injury to two vertebrae followed by compensatory biomechanical, compensatory spinal curvatures [107].

Recent animal models of scoliosis have largely been created using genetics techniques, including CRISPR–Cas9, the Cre–Lox system, and transient models modifying RNA expression. Conditional genetics allow the researcher to specifically ablate or alter gene expression in defined tissues or a specific tissue lineage both spatially and temporally. By targeting a specific tissue lineage, one can study the mutational effects on the pathophysiology of a specific tissue/organ as related to the disease of interest [108]. These techniques are extremely useful to circumvent embryonic lethality of a particular mutation or variant [108]. The availability of tools for the Cre–Lox system and CRISPR–Cas9 is particularly robust in mouse, while CRISPR–Cas9 or antisense morpholino oligonucleotides are the current standard for genetic approaches in the zebrafish model system. Comprehensive summaries of genetic scoliosis models to date are provided in Table 2 (mammalian models) and Table 3 (non-mammalian models).

Despite being quadruped, the murine nervous, endocrine, and musculoskeletal systems share significant similarities with humans compared to non-mammalian models. One of the first genetic murine models of scoliosis reported was in a *Gdf5*;*Gdf6* (Growth Differentiating Factor-5, -6) double mutant, which developed spinal curvature as well as defects in skeletal patterning and joint tissues [109]. After discovering a translocation affecting *CHD2* (Chromodomain DNA Helicase Protein 2) in a patient with scoliosis and developmental delay, a heterozygous *Chd2* ± mouse was created showing marked spinal kyphosis, growth retardation, and reduced body fat [110]. Mice homozygous for a deletion in *Fgfr*3 (Fibroblast Growth Factor Receptor 3) developed kyphoscoliosis at 2 months of age, which was then partially corrected with the bone anabolic agent PTHrP-1–34 [111], suggesting that bone metabolism has a role in scoliosis, a concept that has been implicated in select populations of human AIS patients [112–116].

Use of conditional genetics has pinpointed the role of cartilaginous tissues during the development of scoliosis in mice. A gain-of-function *Npr2* (Natriuretic Peptide Receptor 2) mutation expressed specifically in chondrocyte lineages expressing *Col11a2* (Collagen Type XI Alpha 2 Chain) was discovered in a family with scoliosis, tall stature, and macrodactyly, which then caused bony overgrowth and severe kyphosis in mouse [117]. Chondrocyte-specific deletion of *Ptpn11* (Tyrosine-protein phosphatase non-receptor type 11, encoding Shp2) at 4 weeks of age in mice led to juvenile scoliosis whereas constitutive deletion led to severe skeletal defects, including scoliosis [118]. Chondrocyte-specific knockdown of *Sox9*, an essential transcription factor for chondrocyte differentiation, was found to cause multiple musculoskeletal and developmental abnormalities in mice, including severe kyphosis [119]. Osteochondroprogenitor-specific depletion of *Gpr126/Adgrg6* (Adhesion G Protein-Coupled Receptor G6), variants in which were implicated in IS by multiple GWAS [14–19], was found to cause a late-onset scoliosis in mouse, as well as an abnormal sternum phenotype similar to pectus excavatum [120]. Deletion of *Prmt5* (Protein Arginine Methyltransferase 5) in the same osteochondroprogenitor cell lineages in mouse was found to produce early-onset scoliosis [121].

Clinical investigations have shown a potential proprioceptive mechanosensory deficit in IS individuals, thus leading to study of this regulatory system in murine models. Murine mutants null for *Runx3* (Runt-related Transcription Factor 3) and *Egr3* (Early Growth Response 3) both exhibited a scoliosis phenotype. A third mouse model with selective loss of *Piezo2* (Piezo Type Mechanosensitive Ion Channel Component 2) in proprioceptive neurons also led to spinal kyphosis and scoliosis, again indicating the potential role of the proprioceptive system in maintaining spinal alignment [122].

Bony fishes: Fish models, first championed by the *curveback* guppy, which spontaneously developed isolated spinal curvatures, was one of the first established genetic models of scoliosis and was used to suggest that scoliosis was not exclusive to bipedalism [38, 71, 123].

The authors later identified a major quantitative trait locus (QTL) in the *curveback* guppy containing over 100

Table 2 Summary of mammalian genetic models of scoliosis

Gpr126 conditional mutant	Mouse	Genetic	Conditional knockout in osteochondroprogenitor cells (*col2cre*), Cre–LoxP	Scoliosis	Rib cage malformation, failure of midline fusion within IVD	Thoracic scoliosis, late onset	Yes	*Hum Mol Genet,* 2015 [120]
Shp2 conditional mutant	Mouse	Genetic	Conditional knockout in osteochondroprogenitor cells (*col2cre*), Cre–LoxP	Scoliosis	Growth plate defects	Thoracic and lumbar scoliosis, kyphosis	No	*Spine*, 2013 [118]
Chd2/+ heterozygous mutant	Mouse	Genetic	Constitutive knockout, Embryonic Stem Cell GeneTrap	Lordokyphosis	Syndactyly, lack of eye development, growth retardation, low body fat		Yes	*Am J Med Genet* 2008 [110]
Gdf5/6 double mutant	Mouse	Genetic	Constitutive knockout	Scoliosis	Joint fusion, inner ear defects, connective tissue defects		No	*Developmental Biology*, 2003 [109]
Col11a2::NPR2 mutant	Mouse	Genetic	Conditional transgene in chondrocytes	Severe kyphosis	Growth plate defects		No	*PLoS ONE, 2012* [117]
Fgfr3-/- mutant	Mouse	Genetic	Constitutive knockout	Scoliosis and kyphosis	Overgrowth of long bones, osteopenia, inner ear defects		No	*Frontiers in Endochrinology*, 2015 [111]
Lordoscoliotic rabbit	Rabbit	Genetic	Spontaneous mutation	Lordoscoliosis			No	*Spine, 2003* [106]
Prmt5 conditional mutant	Mouse	Genetic	Conditional knockout in osteochondroprogenitor cells (col2), Cre–LoxP	Scoliosis	Impaired hypertrophic chondrocyte differentiation, asymmetrical endochondral bone formation in spine	Infantile-onset scoliosis, average Cobb angle ~ 30 degrees	No	*Dis Model Mech, 2019* [121]
Sox9	Mouse	Genetic	Constitutive knockout (postnatal inactivation), Cre–LoxP	Kyphosis at cervical region	Loss of trabecular bone, stunted growth, dedifferentiation of growth plate chondrocytes, IVD degeneration, other widespread bone and cartilage defects		No	*J Bone Miner Res, 2013* [119]
Adgrg6 conditional mutant	Mouse	Genetic	Conditional knockouts in osteochondroprogenitor cells (Col2a1–Cre); mature osteoblasts (Bglap-Cre); and committed chondrocytes (Aggrecan-enhancer, tetracycline inducible-Cre)	Scoliosis		Approximately 60% penetrant, with onset at P20; Curvature apex at T8-T9; Mean Cobb angle 11–46 degrees	Yes	*eLife, 2021* [144]

Table 3 Summary of non-mammalian genetic models of scoliosis

Genetic mutation(s) or model name	Species	Model type	Genetic type	Scoliotic phenotype	Secondary phenotypes	Additional scoliosis details, if provided	Associated with human studies of IS?	References
"curveback" mutation	Guppy	Genetic	Spontaneous mutation	Lordosis, kyphosis	None noted	Sagittal anterior lordosis, posterior kyphosis. Variable curve magnitude. Female bias for severe curves; no vertebral fusions, breaks	No	*Med Hypotheses,* 2009 [71]
"wavy" mutation	Medaka	Genetic	Spontaneous mutation	Scoliosis	IVD dysplasia		No	*J Toxicol Pathol*, 2016 [145]
ptk7 and other motile cilia genes	Zebrafish	Genetic	Zygotic mutation	Scoliosis	Ependymal cell cilia defects, cerebrospinal fluid flow defects	Late onset, severe curves more prevalent in females	Yes	*Nature Communications*, 2014 [72]
ccdc40, ccdc151, dyx1c1	Zebrafish	Genetic	Constitutive knockout after RNA rescue during embryonic period	Scoliosis	Ependymal cell cilia defects, cerebrospinal fluid flow defects		No	*Science, 2016* [70]
c21orf59 conditional mutant	Zebrafish	Genetic	Conditional (temperature sensitive)	Scoliosis	Ependymal cell cilia defects, cerebrospinal fluid flow defects		No	*Science, 2016* [70]
kif6 mutant	Zebrafish	Genetic	Constitutive knockout	Scoliosis	Reissner fiber defects, central canal cilia defects		Yes	*Dev Dyn*, 2014 [128]
poc5 overexpression	Zebrafish	Genetic	Morpholino knockdown, followed by injection of patient-specific RNAs	Scoliosis	None noted	Mild to severe curvatures in ~ 50% of embryos, curvature at juvenile stage	Yes	*J Clin Invest*, 2015 [124]
lbx1b mosaic expression	Zebrafish	Genetic	Mosaic expression	Scoliosis	Asymmetrical somite arrangement	Scoliosis visible by 21 dpf; progressive scoliosis observed by 55 dpf	Yes	*PLoS Genet, 2016* [125]
mapk7 mosaic mutant	Zebrafish	Genetic	CRISPR–Cas9	Scoliosis	None noted	62% of adult mutant zebrafish displayed scoliosis (ranging from mild to severe)	Yes	*Hum Mutat,* 2017 [146]
sspo LOF mutants	Zebrafish	Genetic	Constitutive indel mutations, CRISPR–Cas9	Scoliosis	Impaired CSF flow, irregular *sspo* deposition in brain ventricles, other	Scoliotic individuals associated with loss of Reissner fiber	No	*CurrBiol*, 2020 (Rose) [131]

Table 3 (continued)

Genetic mutation(s) or model name	Species	Model type	Genetic type	Scoliotic phenotype	Secondary phenotypes	Additional scoliosis details, if provided	Associated with human studies of IS?	References
sspo hypomorphic mutants	Zebrafish	Genetic	Constitutive indel mutations, CRISPR–Cas9, genetic screening	Scoliosis	Reissner fiber disassembly, somite displacement, other	50% curved by 7 dpf, complete penetrance by 20 dpf	No	*Curr Biol*, 2020 (Troutwine) [132]
kif7 hypomorphic mutants	Zebrafish	Genetic	Constitutive indel, CRISPR–Cas9	Scoliosis	Longer central canal cilia	Severe, juvenile-onset curvatures	Yes	*Hum Mutat,* 2020 [136]
ttll11 mutant	Zebrafish	Genetic	Constitutive mutation (insertion), CRISPR–Cas9	Scoliosis, body curvature in embryos	Disorganization of retinal cone cells; Death of curved embryos by 2 weeks past fertilization; higher survival for individuals that developed curvature later	Spinal curvature across two planes with rotation	Yes	*Sci Rep,* 2021 [147]
Cfap298 mutant	Zebrafish	Genetic	Constitutive mutation	Scoliosis		63% penetrant, juvenile onset, more frequent in females, thoracolumbar curvatures	No	*Sci Rep,* 2021 [148]

genes, including the candidate IS gene *mtnr1b* [123], but due to the lack of genetic resources in the guppy the causal mutation(s) were never fully defined.

Recently, zebrafish (*Danio rerio*) have become prominent in scoliosis research, with key advantages and available resources as listed in *Overview of Popular Animal Models*. Initial overexpression experiments in zebrafish of mutations in *POC5* [124] (POC5 Centriolar Protein) and near *LBX1* [125] (Ladybird Homeobox-1) were used to validate human genetic findings.

Zebrafish models have shown that tissue-specific mutations in motile cilia genes, including *ptk7* (Protein Tyrosine Kinase 7), lead to phenotypes that resemble aspects of IS including late-onset spine curvatures without vertebral malformations, and a higher penetrance and severity in female mutant fish [72, 126]. Curvatures were associated with defects in cerebrospinal fluid flow, hypothesized as linked to malfunctioning motile cilia of the brain's ependymal cells [70]. The *ptk7* model sparked additional interest in ependymal cilia defects as related to IS pathology [124, 127], which were also seen in *kif6−/−* (Kinesin Family Member 6) scoliotic zebrafish [128, 129]. Further research pinpointed neuroinflammation as a potential cause of the scoliosis or progression of the scoliosis in *ptk7* mutant fish; interestingly, as treatment of the *ptk7* mutant zebrafish with non-steroidal anti-inflammatory drugs (e.g., aspirin and N-acetyl cysteine) drastically reduced the incidence of spinal curvatures [130].

Recent studies have implicated the Reissner fiber, a threadlike glycoprotein strand running the length of the spinal cord central canal, as essential for proper spine morphogenesis in larval zebrafish [131, 132]. Moreover, these studies show that the loss of motile cilia function and disrupted cerebrospinal fluid flow are tightly correlated with the disassembly of the Reissner fiber. Whether these mechanisms are shared with IS in humans remains controversial, as it is unclear whether humans possess a Reissner fiber past infancy [133, 134]. Regardless, the mechanism of how the Reissner fiber is controlling spine morphogenesis warrants further research. Recent studies implicating the stimulation of the cerebrospinal fluid-contacting neurons (CSF-cNs) by physically interacting with the Reissner fiber are intriguing [135]. This is even more relevant in light of recent work showing that alterations in the expression of urotensin neuropeptides in CSF-cNs can lead to improper control of the body axis and result in scoliotic curvatures in zebrafish. Additional recent work has found that defects in sco-spondin (encoded by *SSPO*), the main protein component of the Reissner fiber, cause scoliosis in zebrafish and is a conserved mechanism, along with neuroinflammation, across different genetic zebrafish models of scoliosis [131].

Current limitations and future directions of IS animal modeling

With the exception of mutant *Adgrg6* mice [120], which also show a pectus excavatum phenotype [120], and potentially *kif7*^{co63} zebrafish [136], which have not yet been fully characterized, there is currently a lack of animal models that show isolated, IS-like spinal curvatures without secondary phenotypes, including hydrocephalus, skeletal malformations, degeneration, or bone quality defects. Additionally, with a few exceptions [71, 72], most animal models of scoliosis have not shown sexual dimorphism. Severe IS presents in females by a 9:1 ratio, which has been hypothesized as being caused by differences in the prepubertal growth spurt [137, 138], muscle mass [137], inflammatory responses [139], and hormones [138, 140, 141].

IS is now largely understood as a complex genetic disease caused by combinations of mutations that alone are mildly deleterious, perhaps in combination with environmental risk factors [142]. Most animal models to date have manipulated single genes or variants to determine the effect of the genomic region in isolation. Future animal modeling research may move towards combinatorial approaches, where 2 + characterized mutations are induced within one model, or genetic models are combined with environmental or epigenetic perturbations also suspected to contribute to IS [143].

Conclusion

IS is a multifactorial condition that has been challenging to model in animals due to complex genetic and unique biomechanical factors believed to cause disease onset. Despite these challenges, genetic animal models have served as crucial tools to understand spinal development and pathology, test scientific hypotheses, and validate potential mutations found in human data. We advocate careful selection of animal models depending on the scientific question, and careful consideration of IS-like curvatures within the context of the animal model being studied. Future research directions may include induction of multiple mutations or environmental effects within one animal model to reflect current understanding of the human condition.

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Declarations

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