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The Mutant Guppy Syndrome *Curveback* as a Model for Human Heritable Spinal Curvature

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Study Design. This study investigated the morphology, pathogenesis, and inheritance of idiopathic-like spinal curvature in the guppy syndrome, *curveback*.

Objective. To determine whether *curveback* could be applied as a model for the primary factors that contribute to heritable spinal curvature in humans, specifically, the etiopathogenesis of human familial idiopathic scoliosis.

Summary of Background Data. Although a genetic basis is accepted, phenotypic complexity and the lack of an animal model with noninduced curvature have made identification of idiopathic scoliosis etiology difficult. It is well established that humans and fish share many genes with similar tissue and temporal expression characteristics, and comparisons between human and fish genomes have proven to be valuable for understanding the genetics of diseases affecting humans.

Methods. The *curveback* lineage of guppies was constructed from a single curved male crossed to a normal female. Offspring (103) from the original cross were scored from birth until death for the presence and magnitude of spinal curvature. Genetic architecture was investigated through selective inbreeding, analysis of the distribution of curve magnitude in the mature population, and assessment of curve dynamics during development. Computed tomography assessed vertebral detail.

Results. Computed tomography reveals that vertebral breakage or fusion is not associated with the *curveback* syndrome. Inbreeding demonstrates a strong genetic influence on *curveback*, and the distribution of curve magnitude among adult fish suggests polygenic inheritance. There is a female bias for curves of high magnitude and curves that resolve before maturity. There is developmental variability for the age of curve onset, curve progression, and final curve magnitude.

Conclusions. Observed parallels between the *curveback* syndrome and human idiopathic scoliosis suggest that the guppy model is an unexploited resource for the identification of primary etiological factors involved in curvature. As models for biomedical research, teleosts offer great potential regarding spinal stability and deformity.

Key words: guppy, spine, idiopathic, curvature, development, genetic, model, scoliosis. **Spine 2007;32:735–741**

Although the diagnosis, cause, and treatment of human familial idiopathic scoliosis have been the focus of a great deal of research, little progress has been made in identifying the etiology of idiopathic scoliosis. This is largely due to the extreme phenotypic variability of idiopathic scoliosis, and the lack of an appropriate developmental and genetic animal model. Multiple hypotheses such as differences in growth patterns, connective tissue abnormalities, asymmetries in the central nervous system, distribution of melatonin and calmodulin, hormonal variation, spinal slenderness, diet, and posture have all been suggested as causes for idiopathic scoliosis. Although it is well accepted that a genetic basis exists for idiopathic scoliosis, the proportion of phenotypic variation due to genetics and the mode of inheritance of these underlying factors are still a matter of debate.[1–10] Without a tractable model organism for research, navigation through phenotypic complexity to primary causative factors will continue to be difficult. The authors present data on the morphology, development, and inheritance of the guppy mutant *curveback*, demonstrating its potential as a tool for understanding genetic and environmental causes of idiopathic scoliosis.

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Materials and Methods

The guppy, *Poecilia reticulata*, is a small, live-bearing fish, native to the streams of northeast South America, and is a popular aquarium fish. The guppy has been a model organism for ecology, evolution, and genetic research since the 1920s, and was used in this study to investigate the morphology and pathogenesis of the mutant syndrome *curveback*.

Scoring Fish. The guppy spine is visible without magnification. All fish were scored from the side and above while in a glass view tank 4" long × 2" wide × 3" high. The *curveback* phenotype manifests as a primary sagittal lordosis of variable magnitude with some individuals exhibiting posterior kyphosis and/or coronal deviation (Figure 1). Since all affected individuals exhibit sagittal curvature, the degree of lordosis as a standard measure of curvature was used.

Qualitative. The magnitude of lordosis observed in the *curveback* population was categorized into 5- general classes: type 0 (normal); and curve types 1–4, with 4 being extreme curvature (Figure 2). There is also a type of curvature that develops shortly after birth but resolves before maturity; this was scored as a sixth qualitative category (type 5).

Quantitative. Lordosis was measured in adult fish that had been anesthetized with ice water (7 second exposure) and then photographed with a digital camera (Toshiba PDR-3310; New York, NY) under 3× magnification on a standard light table. Using Image-J (NIH Image),[11] a body midline was drawn on digital images between the tip of the caudal peduncle and the center of the occipital orbit (Figure 3). A perpendicular line was then drawn from the midline to the apex of the lordotic curve

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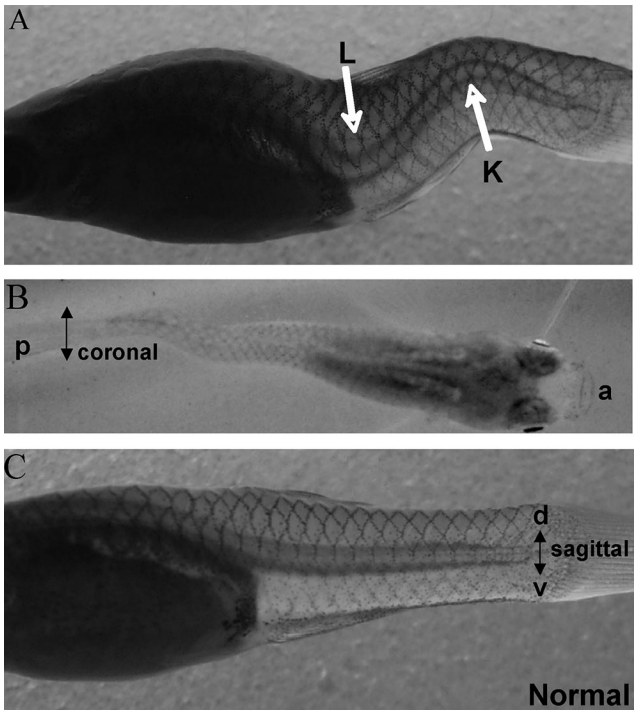

Figure 1. The *curveback* phenotype is a primary anterior lordosis (L) and a secondary posterior kyphosis (K) occurring on the sagittal plane of the animal (**A**). Some individuals demonstrate coronal deviation (anterior axis: a; posterior axis: p) (**B**). Normal fish with sagittal plane and dorsal/ventral axes is shown (**C**). Digital photos of anesthetized adult females (**A** and **C**) taken on a standard light table under 3× magnification. Photo of female (**B**) is taken on an unanesthetized adult under 3× magnification.

("A" in Figure 3). Anterior curve ratio was defined as the ratio of the length of the perpendicular line (magnitude of lordosis) relative to the length of the fish (caudal peduncle to occipital orbit). This measure is similar to that used by Cheung *et al*[12] to measure lateral deviation of apical vertebra. The distribution of the anterior curve ratio in the population was analyzed using the Kolmogorov-Smirnov test.

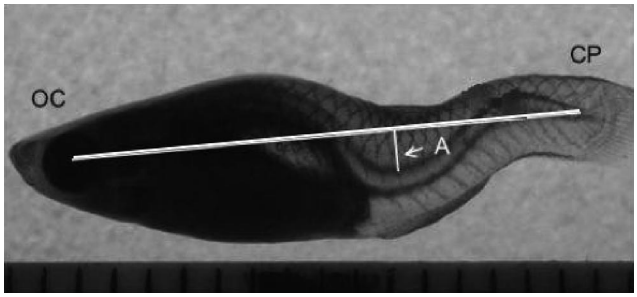

Figure 3. Anterior curve ratio. Quantitative measurement of the magnitude of lordosis (A) is relative to the length of the fish (occipital orbit [OC] to caudal peduncle [CP]).

Skeletal Imaging. Although the guppy spine is visible without magnification, individual vertebrae cannot be discerned. Vertebral detail was observed on a representative normal and a type 4 curved fish by computed tomography (CT) scans at the University of Texas High-Resolution radiograph Computed Tomography Facility,[13] as described by Ketcham and Carlson.[14] Each 3-dimensional (3-D) scan consists of 350 slices 0.4-mm thick, which would allow detection of malformed, fused, or cracked vertebrae.

Establishment and Maintenance of *Curveback* Lineage. The *curveback* lineage originated from a curved male crossed to a normal female, followed by full-sib mating. Both founders were from a population collected in Cumaná, Venezuela,[15] which had been maintained as a laboratory stock for 4 years. To test for recessive genetic components of the syndrome, 2 sib-mate pairs (A and B) were made from F_1 progeny. To test for recessive components in the mother's genotype, an F_1 male was backcrossed to his mother.

Based on the qualitative scores described in Figure 2, we made the following crosses from the F_2 progeny from sib-mate pair A: i, curve type 3 male to normal female; ii, type 4 female; iii, type 4 male to type 4 female; and iv, type 1 male to normal female. From sib-mate pair B: i, type 2 male to type 4 female; and ii, normal male crossed to normal female.

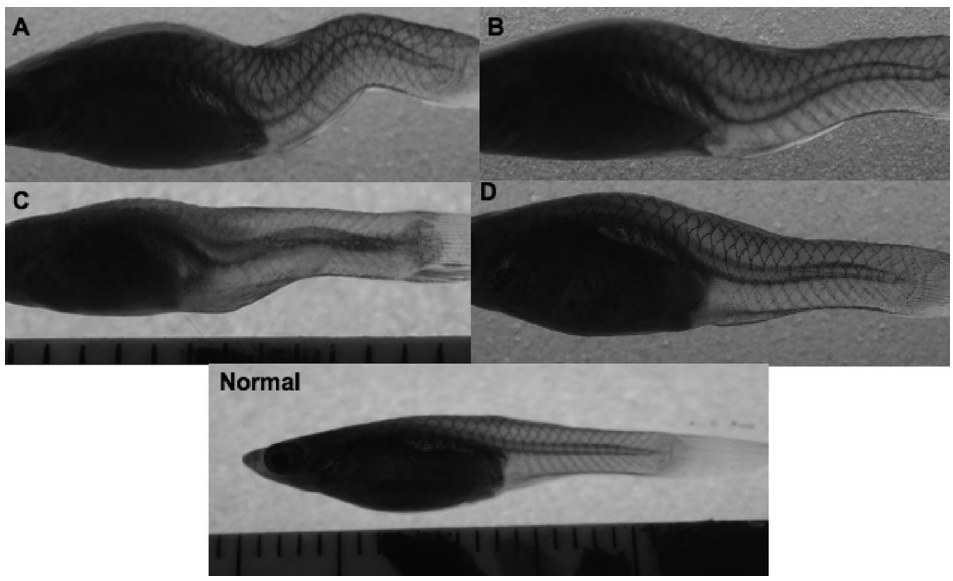

Figure 2. Observed curve types. Shown are 4 general classes of curvature used to score qualitatively lordosis. Progressed curve, referred to as type 4 (**A**). Moderate curve (type 3) (**B**). Slight curve (type 2) (**C**). Nearly curved (type 1) (**D**). All digital photos taken on a standard light table using anesthetized adult fish.

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Selection based on curve magnitude was carried to the fifth filial generation in order to create families enriched with genes for observed curve magnitudes, detect possible genetic linkage for different curve magnitudes, reveal recessive genes, and determine consistency in inheritance of curvature (*i.e.*, amount of variation due to genetic background or modifier genes).

Development of Curvature. One hundred and three fish were qualitatively scored every 2–4 days from birth until sexual maturity, and then at least once per month as adults until death. Sexual maturity occurs between 8 and 10 weeks past birth, as is indicated by development of the male gonopodium and darkening of the female gonoporal region (guppies are livebearers; females give birth to live offspring at about 3–4 weeks postinsemination). Skeletal maturity is estimated as the time when the guppy reaches adult morphologic proportions.

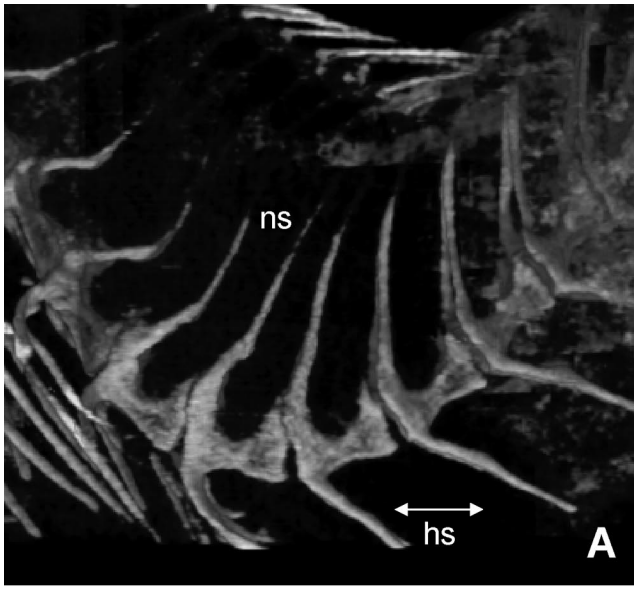

Figure 4. Vertebral detail shows no fusion or breaks. CT of apical vertebrae for curved (**A**) and normal (**B**) guppy. Scan consists of 350 0.4-mm slices. Both guppies are of the *curveback* lineage. hs, indicates hemal spines; ns, neural spines.

■ Results

Curveback Morphology

Of 25 adult fish with lordosis, 8 also had secondary kyphosis. Six of the 8 were of curve type 3 or 4. Of the 17 adults with only lordosis, 4 had curve types 3 or 4. Visual inspection of 5 (1 male, 4 female) fish (out of 103) exhibiting pronounced lordosis, kyphosis, and coronal curvature demonstrate that vertebral rotation can be part of the *curveback* syndrome.

CT scans reveal that vertebral anomalies, breakage, or fusion is not associated with curvature (Figure 4). The 3-D roll-scans allowed for examination of 18 complete precaudal and caudal vertebrae in a normal and severely curved fish; data sets of scans are available for view at http://www.digimorph.org/index.phtml. The scans show that vertebrae involved in curvature are wedge shaped, with the vertebrae at the apex of curvature being most severely warped. Although rotation of the vertebral column is evident in scans of the curved fish, a method to quantify such a character has not yet been optimized.

Curveback Development

Curvature develops after birth and generally does not progress after skeletal maturity. Of the 103 fish scored from birth past sexual maturity, 61% (n = 63) demonstrated curvature during development. Of these, 79% (n = 50) were born with normal spines and developed curvature within the first 2 weeks past birth. The remaining 21% of the fish (n = 13) were born with curved spines that resolved to normal or nearly normal before maturity. Whether curvature resolves does not depend on the magnitude of curvature during development. There is a female bias for curves of high magnitude (15% of scored females have severe curvature compared to 4% of scored males [n = 103]) and for resolving curves (18.5% female *vs.* 8% male [n = 103]), and a male bias for slight curvature (type 2) (Figure 5).

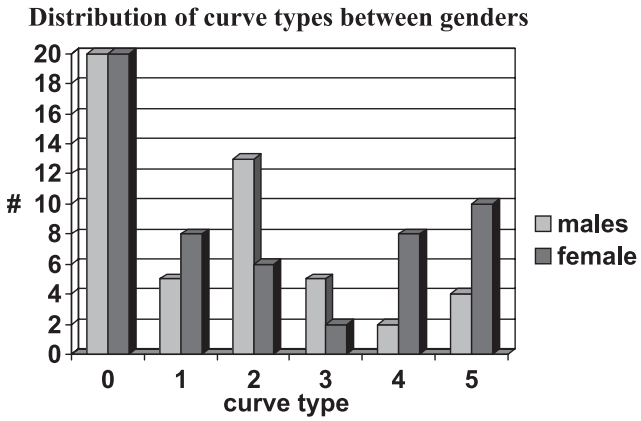

Figure 5. Gender distribution for qualitative scores. Fish were scored from birth to 100 days after birth. Total number (#) of fish scored was 103; number of females was 54, and number of males was 49. Curve types are defined in Figure 2. Normal fish have a curve type "0." Resolving curvature (type 5) is defined as a curve that has decreased by an order of 1 curve type or more.

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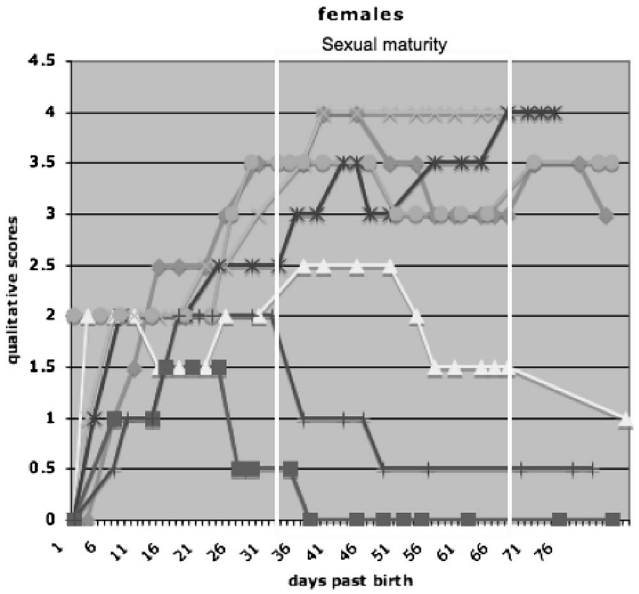

Figure 6. *Curveback* pathogenesis, Curve progression during development is periodic, oscillating between periods of increase or decrease in magnitude and relative stasis. The range for the age of sexual maturity is indicated between the white lines. Male curve progression follows the same general trend as females.

All curves begin small in magnitude, but the initial magnitude as well as the age of onset are variable both within and between families. Fish born on the same day were scored together, which enabled detection of individual differences for onset and progression. The curves of greater amplitude begin within 4 days past birth, while curves that initiate after 7 days tend not to progress past type 2. Progression of curvature is periodic, oscillating between periods of increase or decrease and relative stasis (Figure 6). The general pattern for progression of curvature during growth is the same regardless of whether the fish is born normal or with curvature.

Curveback Inheritance

Our early crosses indicate that recessive genes contribute to *curveback*, and analysis of the distribution of anterior curve ratios among adult fish is not statistically different from normal ($P > 0.1$). F_1 offspring from the original cross were scored only as adults but showed no curvature, suggesting that the gene controlling *curveback* is autosomal recessive. The proportion of affected *curveback* individuals in the lineage increased in further generations with inbreeding, showing a strong genetic influence on the phenotype. Fifty percent of the F_2 offspring demonstrated some degree of curvature during development, regardless of whether they appeared normal as adults. In the F_3 generation, 58% of the offspring demonstrated curvature during development.

Since the *curveback* population originated from a single curved male crossed to a noncurved female and all of the F_1 adult progeny (n = 7) appeared normal, our assumption was that the male was homozygous recessive for *curveback* and that the female was homozygous for wild-type genes. We expected that if the mother had any recessive genes for curvature, we would see curved progeny from a cross to her presumably heterozygous son. Three broods totaling 12 offspring had no curved fish, suggesting that genes for curvature are recessive to normal and that the mother is wild-type. The male was subsequently crossed to a related F_2 noncurved female (from F_1 sib-mate B) and had 12/17 curved progeny, suggesting that the male is heterozygous. Sib-mate A yielded 26% curved F_2 offspring, and sib-mate B yielded 12% curved offspring.

Because the F_1 and early F_2 crosses were conducted before the developmental analysis, animals were scored as adults only for the presence or absence of curvature. We had not detected the subtle "type 1" curve and were unaware of resolving curves until the seventh brood of our F_2 generation. When we examine curve scores throughout development and not just at maturity, inheritance appears more complex. From the F_2 and successive inbred crosses, we see that there is no simple pattern of inheritance for curve magnitude, the time of curve onset, and whether curvature resolves or progresses. If *curveback* were inherited as a Mendelian recessive trait, then we would expect all curved offspring from cross ii and iii of sib-mate A, which is not the case (Figure 7).

■ Discussion

With regard to complex developmental disorders in humans, multiple models are critical for the investigation and manipulation of etiological factors. An experimental model with a strong genetic component will have significant value as a part of scoliosis research. It is well established that humans and fish share many genes with similar tissue and temporal expression characteristics,[16–21] making fish a valuable asset for understanding the genetics of diseases affecting humans.[22–26] Comparisons between human and fish genomes have proven to be a powerful tool for identifying deoxyribonucleic acid sequences that have significant functional activity in all (DNA) vertebrates indentifying genes.[27] Indeed, teleosts show great potential in general as models for biomedical research regarding spinal stability and deformity.[28–31] Parallels between the *curveback* syndrome and idiopathic scoliosis (Table 1) show its potential as a tractable model organism for discovering genetic and developmental factors that influence heritable spinal curvature in vertebrates. The guppy has been an important laboratory organism for genetic analysis since the 1920s,[32] with over 400 papers published on the guppy in the last 10 years alone. A short generation time, the availability of genomic resources, and ease of care and manipulation make the guppy a tractable organism for vertebrate research.

Curveback Morphology

CT scans reveal that the primary lordosis is not associated with vertebral fusion or breakage. Observed distortion of apical vertebrae coincides with typical morphology for human scoliosis where curve vertebrae become warped with progression.[12] Further skeletal analysis us-

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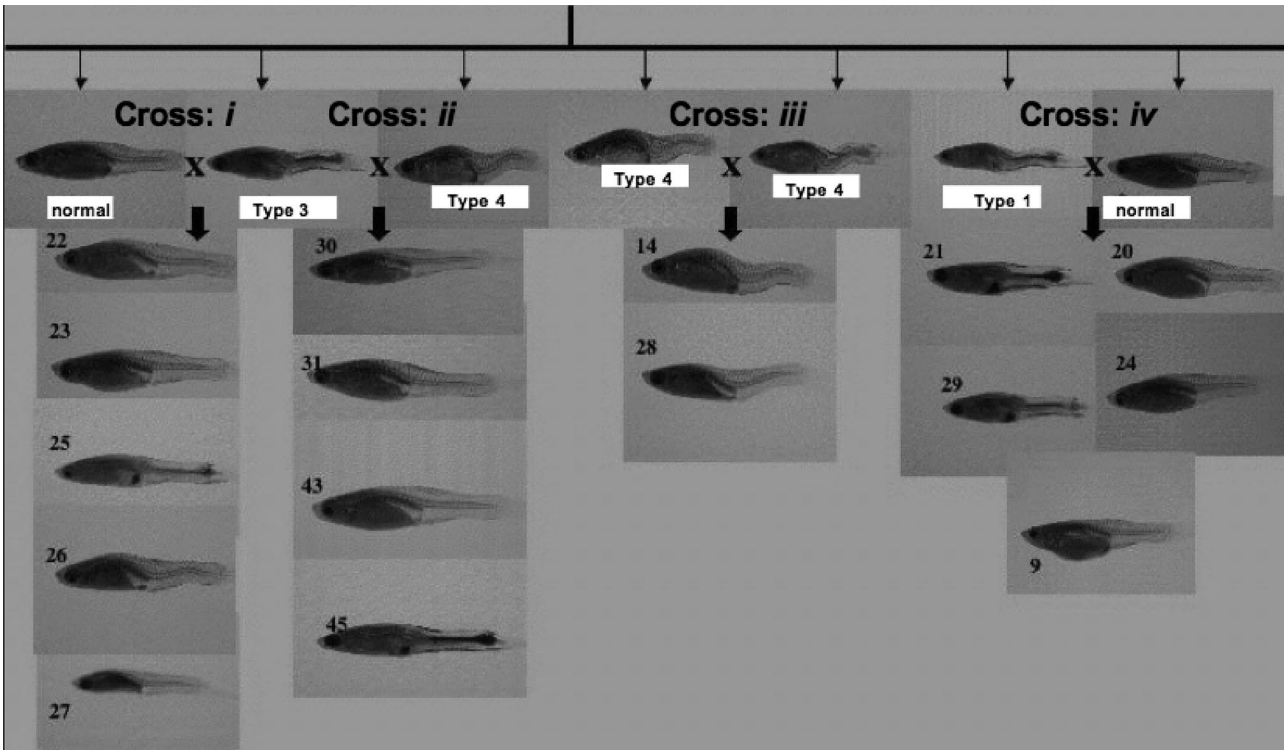

Figure 7. Complex inheritance for curve magnitude. Shown are offspring of sib-mate A (F_3 progeny of the original cross). If inheritance were simple recessive, then crosses ii and iii should have a high number of curved offspring, and cross i no more than 50%. In cross iii, fish No. 28 resolved to normal, and subsequent offspring were normal, and from cross i, fish No. 22 and 26 progressed to type 4 at maturity.

ing CT, and clearing and staining on immature fish will elucidate the timing of structural changes involved in curvature. From such an analysis, we can understand whether variables such as vertebral length, width, or morphologic asymmetry are common correlates to curvature and/or progression.

Biomechanical differences between human and fish may help to delineate secondary from primary phenotypic components. Pinealectomy in hamsters[33] and monkeys[34] did not induce curvature. Pinealectomized rats do not demonstrate spinal curvature unless they are made bipedal.[35] Consequently, scoliosis is thought to be an effect of bipedalism. What is not clear is whether the primary factors that predispose an animal to curvature are present but constrained by quadrupedal biomechanics, as the study with the rat suggests, or if the primary dysfunction is exclusive to humans, perhaps because the scoliosis-causing mutations are specific to the human lineage. As all animals used in orthopedic modeling thus far are quadrupedal, the latter assumption has been favored. Pinealectomy in the guppy and salmon induces spinal curvature with a physiologic response the same as in chickens.[36–38] Furthermore, the teleost spine is not weight bearing due to buoyancy provided by the swim bladder combined with the density of water.[38] Therefore, fish present the opportunity to investigate the factors that influence curvature without the constraints of quadrupedal or bipedal biomechanics. Factors that influence human curvature such as gravity, load, rib association, and lumbar tethering can be omitted from the equation of possible influences for curve progression, thereby illuminating important primary correlates.

Table 1. Parallels Between *Curveback* and Human Idiopathic Scoliosis Phenotypes

	1) No vertebral fusion or breaking
Developmental parallels	
	2) Born normal, curve develops after birth
	3) Curvature does not hinder fitness of individuals
	4) Curve does not progress after skeletal maturity
	5) Curve magnitude increases with age
	6) Incidence of curve that resolves before maturity
	7) Onset time for curvature is variable
	8) Variability among individuals for rate and propensity of curve progression
	9) Female bias for most severe curve
	10) Angle growth is in spurts instead of linear progression
Genetic parallels	
	11) Complex inheritance
	12) Major gene effect but with additional multigenic modifiers
	13) Distribution of curve magnitude in population is continuous

For example, it is hypothesized that the 3-D nature of human idiopathic scoliosis is a consequence of secondary biomechanical modifications to a primary lordosis in the midsagittal plane.[8,39–42] According to Millner and Dickson,[41] although idiopathic scoliosis is a 3-D deformity, the problem is more one of front-back asymmetry as opposed to right-left. This idea has been supported by an observed loss of coupling in the longitudinal growth between the anterior column and the posterior column[43,44] but still remains a matter of debate. The *curveback*

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guppy allows for the investigation of genes that maintain sagittal balance.

Curveback Development

Shared developmental trends listed in Table 1 demonstrate the possibility of conserved biochemical or physiologic processes that may be influencing curve pathogenesis for humans and guppies. Hormones administered to aquarium water can manipulate the rate of sexual maturity and/or growth in order to test for correlation with curvature,[45] and such correlations may help to identify candidate genes.

Incidence of spinal curvature in other animal models is either congenital to other conditions,[46] or lacks correlates to human growth or development (*i.e.*, the curvature occurs in the animal after sexual maturity).[47,48] In order to evaluate the pathogenesis of curvature in the context of development, animal experiments in the past have artificially induced scoliosis. Methods such as tethering, intercostal nerve resection, electrostimulation, irradiation, pinealectomy, magnet implantation, or direct injury to the epiphysial plate have induced curvature in animals such as the rabbit, chicken, goat, dog, rat, and monkey.[49] However, because the curves are artificially induced, there is controversy over whether conclusions made from these experiments relate to primary or secondary influences for curvature.

Curveback Inheritance

The mode of inheritance for idiopathic scoliosis in humans is still a matter of debate. Multifactorial inheritance,[2,50] autosomal dominant,[1,51–53] and X-linked inheritance have all been proposed.[54,55] Uncertainty is thought to arise in part from a common phenotypic threshold for curvature being set at a 10° Cobb's angle, which would obscure minimal yet heritable components of the deformity. Human studies that have targeted candidate genes have not successfully correlated a locus or marker with the idiopathic scoliosis phenotype.[56–58] Complex inheritance and phenotypic variability resemble that of human familial scoliosis, suggesting similarities for genetic architecture. In both human and guppy phenotypes, the time of onset, curve magnitude, morphology, and propensity for curve progression vary within a pedigree. In the *curveback* lineage, observed variability for the distribution anterior curve ratios is similar to that described for the magnitude of lateral curvature in human idiopathic scoliosis,[59] providing support for a multigenic basis. The distribution of qualitative curve types (Figure 5) among males and females shows a major gene effect with modifiers, and suggests sex linkage for a portion of the phenotype. The resolving curve type is similar to that described as human infantile idiopathic scoliosis, but it segregates in the *curveback* population with all curve types.

The visibility of the guppy spine *in vivo* makes detection of curve onset and subtleties regarding curve progression easy to score, thereby facilitating more comprehensive pedigree construction. It has been suggested that human adolescent idiopathic scoliosis is present in a latent form in younger children who manifest a minor degree of curvature.[60] This fact may distort the perceived inheritance in the pedigrees used for linkage analysis. Indeed, from linkage studies with human pedigrees, there is no concordance for identified loci.[7,53,55,61]

To date, the underlying genetic cause of human idiopathic scoliosis has not been identified. The analysis of *curveback* guppies will suggest important genes involved in vertebral stability. In addition to a mapping cross, the *curveback* lineage will allow for a thorough investigation of curve inheritance through inbreeding and selection. Inbreeding of offspring from sib-mate A and sib-mate B has continued so that we presently have 15 families of fish enriched with genes that cause curvature. With a large pedigree, we hope to correlate the segregation of curve types among families to genetic polymorphisms in candidate genes.

■ Key Points

- The *curveback* mutant guppy syndrome offers a unique opportunity to explore the genetic basis of idiopathic-like curvature in vertebrates.
- In the *curveback* guppy, factors affecting curve pathogenesis can be investigated without secondary biomechanical constraints caused by bipedalism.
- Biochemical similarities between humans and fish allow for the experimental manipulation of physiologic processes that may be involved in curve pathogenesis.

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