Disproportionate Body Lengths Correlate With Idiopathic-Type Curvature in the Curveback Guppy

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Study Design. A comparative allometric study of body lengths in an animal model for human idiopathic-type scoliosis.

Objective. To compare body length variation among adult curved and noncurved *curveback* female guppies.

Summary of Background Data. Tallness and/or abnormal anthropometric parameters have been correlated to idiopathic-type scoliosis (IS) in numerous studies. Heritable curvature in *curveback* has demonstrated morphologic and developmental similarities to human IS. Because control of body length in the guppy is heritable and variable, we investigated whether length might also be correlated to curvature in the *curveback* population.

Methods. Component body lengths were measured from digital photographs for 321 (246 curved and 75 noncurved) females. Sources of experimental variation were omitted by only measuring 2-dimensional curves in mature females all from the same pedigree, and raised under controlled conditions of diet and environment. Body length was divided into 2 component parts (precaudal and caudal). Body lengths were tested statistically for correlation to curvature and curve magnitude.

Results. Although absolute length does not correlate to curvature, this survey of length in the *curveback* model reveals 2 important similarities to anthropometric studies of IS: that there are disproportionate body lengths among females with curvature, and the suggestion of an underlying growth abnormality among curved individuals.

Conclusion. In order to better characterize the relationship between growth, length disproportion, and curvature in the guppy, further studies are warranted. However, this inquiry further supports the usefulness of curveback as a model for understanding the basic biology of idiopathic-type scoliosis and encourages study of growth-related factors.

Key words: idiopathic-type scoliosis, length disproportion, growth, spinal curvature, animal model. **Spine 2010; 35:511–516**

In both humans and guppies, idiopathic-type spinal curvature is a multifactorial deformity that exhibits extensive phenotypic variation. This variation is likely a consequence of interactions among genetic, physiologic, and environmental factors. Success at identifying the etiology of human idiopathic-type scoliosis (IS) has been limited due to trait complexity and the lack of animal models with a noninduced phenotype similar to humans.[1] Preliminary studies in otherwise healthy guppies have revealed that heritable curvature in *curveback* has remarkable morphologic and developmental similarities to human IS (*e.g.*, no vertebral fusion or breaks, distortion of apical vertebrae, curve onset after birth, variable rates of progression and prognoses for curve magnitude, a female bias for severe curves).[2,3] Here, we explore length variation in the *curveback* population, because numerous studies have associated IS with tallness and/or abnormal length proportions along the craniocaudal axis.[4–21]

We surveyed adult *curveback* females for increased length, because mature females with IS have demonstrated increased craniocaudal length.[4] We show that among curved fish, the caudal portion of the body is disproportionately longer relative to the precaudal body, and this disproportion is positively correlated to curve magnitude. As with anthropometric studies, these results suggest that a growth abnormality may be associated with idiopathic-type spinal curvature.

Materials and Methods

Study Population and Data Collection

Guppies are live-bearing teleost fish; offspring are born with a fully ossified skeleton after ~3 weeks of gestation. Curvature begins after birth and is generally stable by sexual maturity (approximately 1 month past birth).[3] In cases of severe curvature, curves will progress into early adulthood (up to 2 months after birth). Breeding pairs were maintained in 4L plastic aquaria, and offspring were separated into individual 600 mL plastic containers after birth. All fish are kept under standardized conditions (*i.e.*, flakes fed every afternoon, supplemented with brine shrimp nauplii; 25–26 C; pH 7–9; RO water reconstituted to 1600–1800 ppm salinity with aquarium salts; 14/10 hour light cycle). Adult females were killed at a minimum of 3 months past birth. Males were not measured because adult coloration obscures the spine. Killed females were photographed on a light table with a digital camera (Toshiba PDR-3310, NYC, US) under 3× magnification.

We measured 246 adult females with curves of varying magnitude, and 75 normal adult females, all from the same pedigree. Curvature is manifest as a sagittal anterior lordosis and posterior kyphosis. A small number of fish exhibit with curvature in the frontal or horizontal planes, and in this study these were omitted. The distribution of curve magnitude is shown in Figure 1, with curve magnitude identified by qualitative scores defined in Gorman *et al*,[3] in which 0 denotes no curvature and 1 to 4 is a scale of increasing magnitude.

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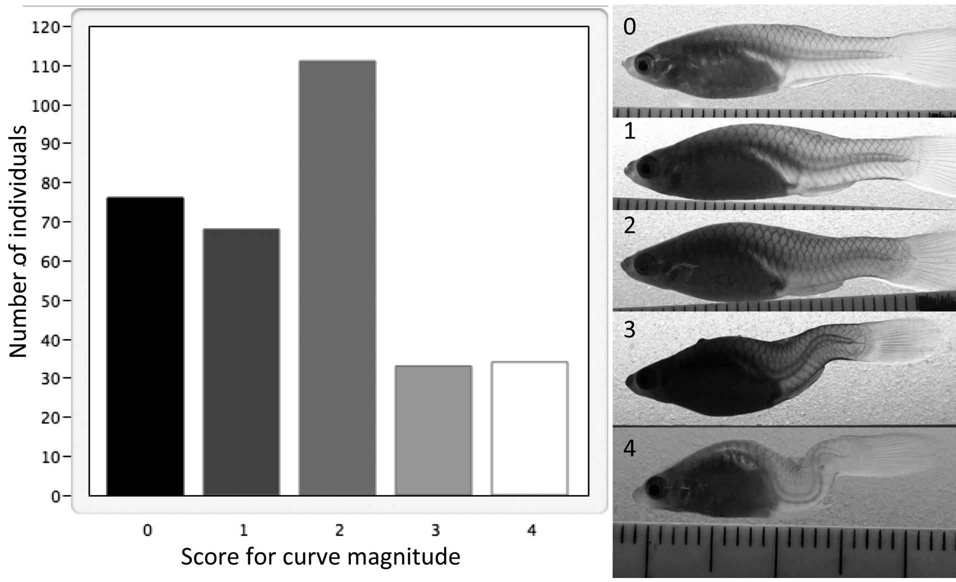

Figure 1. Distribution of curve magnitude within study population. Normal fish have a qualitative score of 0; curved fish were scored as 1 to 4 representing increased curve magnitude. Scale in mm.

To assess whether there are length differences between curved and normal *curveback* females, we divided the body into 2 components along the cranial-caudal axis: the precaudal body and the caudal body (Figure 2). Broadly speaking, these 2 component lengths are comparable to how the trunk and the legs comprise the cranial-caudal lengths measured in anthropometric studies of IS (*e.g.*, chepalocaudal proportions in Nicolopoulos *et al*[13]). In the guppy, the precaudal body contains rib-associated vertebrae. In *curveback,* curvature is manifest in the caudal body, where vertebrae are not associated with ribs. The precaudal length plus the caudal length comprise the "standard length," which is a standard measurement in fish biology.

Measurements

All fish were positioned on their side above a ruler so that there was no variation in orientation. All photographs were taken from above so that the camera looked down on the profile of the fish. To ensure consistency, photographs were taken by the same person throughout the experiment. Photographs were scaled and measured using Image-J (NIH Image).[22] Fish with coronal curvature or axial rotation are not included in the dataset because in photographs, the sagittal profile of the spine would be distorted and measurements would not accurately reflect length.

Body portions were measured as the length of a line drawn between landmark points. Because curvature is located in the caudal portion of the body, we defined the "manifest length" as the length of a line drawn directly between landmark points, and the "true length" as the length of a line traced along the spine within the caudal body. These length measures are similar in concept to the uncorrected and corrected lengths used in anthropometric studies, the difference being that we do not correct for curvature with the formula of Bjure (1968).[23] The true caudal length (line 1 in Figure 3) is the length of a line drawn along the ventral edge of the spine between the top of the cloacal vent to the bottom tip of the caudal peduncle (point A to point B in Figure 3). The manifest caudal length (line 2) is the length of a line drawn directly from points A and B. In a noncurved fish, the manifest length is equal to the true length (*i.e.*,

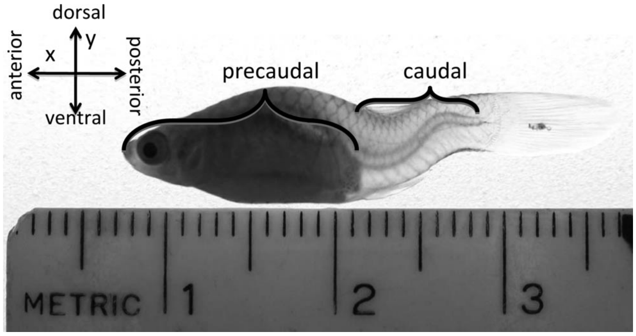

Figure 2. Measured bodies of *curveback* adult females were divided into precaudal and caudal portions: the precaudal portion extends from the tip of the nose to the top of the cloacal vent, and the caudal body portion extends from the top of the cloacal vent to the tip of the caudal peduncle.

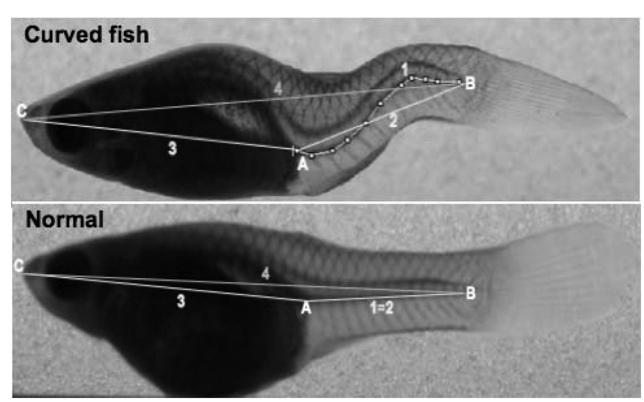

Figure 3. Component measures. Line 1: true caudal length—length of a line drawn along ventral edge of the spine from the top of the cloacal vent (point A) to the tip of the caudal peduncle (point B). Line 2: manifest caudal length—length of a line drawn directly from point A to point B. In a curved fish the length of line 2 is less than the length of line 1. Line 3: precaudal body length—length of a line drawn from the top of the cloacal vent (point A) to the tip of the nose (point C). Line 4: manifest standard length—the length from point C to point B.

the length of line 1 = the length of line 2). The precaudal body length is the length of a line drawn from point A to the anteriormost tip of the nose (point C, line 3 in Figure 3). The manifest standard length (line 4 in Figure 3) is defined as the length of a line drawn from the tip of the nose to the caudal peduncle (point C to point B in Figure 3), and is analogous to the uncorrected standing height in human studies. The lengths of line 1 plus line 3 comprise the true standard length of the fish. In a curved fish the length of line 4 is less than the length of line 1 plus line 3.

The Shapiro-Wilks test was used to test the distributions of length measurements among normal and curved females for normality. Given that most distributions were significantly non-normal ($P < 0.05$), mean values were compared via Wilcoxon tests with α set at 0.05 (χ^2 approximation, 1 *df*). To explore the relationship between length measures, simple linear regression and analysis of covariance (ANCOVA) of log-transformed values were used. As defined by Huxley,[24] the log values of the lengths of 2 body components plotted against each other give a slope of 1 if there is no allometric growth.[24] Statistical analysis was conducted using JMP software for MacOSX, Version 6.0, SAS Institute, INC., Cary, NC.

Results

We first tested whether there are differences between curved and noncurved fish for each length measurement (Table 1 and Figure 3). Wilcoxon pair-wise comparisons show that there is no statistical difference between normal and curved females for mean values of: manifest standard length (line 4); precaudal body (line 3); and manifest caudal length (line 2). However, there is a significant difference between normal and curved females for the true caudal length (line 1) and true standard length (line 1 + line 3).

To test whether the length of the caudal spine is related to curve severity, we tested for allometry between the true and manifest caudal lengths by linear regression on log-transformed values. In curved fish, if there is an allometric relationship between the lengths of lines 1 and 2, then a slope greater than one would show that true caudal length increased faster than manifest caudal length, *i.e.*, that curve severity increased with the length of the caudal spine. Among normal fish, the slope of this line is expected to be one. So, to test for significant differences, we compared the slope for all curved fish to the slope for all normal fish with an ANCOVA. We found that among curved fish, the true caudal length (line 1) increases in proportion with the manifest caudal length (line 2) indicating that a longer caudal spine is not correlated to curve progression (slope for noncurved = 0.99 [R^2 = 1.0]; slope for curved = 0.93 [R^2 = 0.82]; ANCOVA $P = 0.23$).

To investigate the relationship between the length of the caudal spine and the length of the precaudal body, we tested for allometry between the lengths of line 1 and line 3. We found that curved fish have significantly longer caudal spines coupled with shorter precaudal bodies, and that this disproportion is greater among larger females (ANCOVA; $P = 0.0021$) (Figure 4). Our preliminary analysis identified 5 individuals who exhibited extremely small precaudal bodies coupled with long caudal spines. These outliers were omitted from our analysis of the regression shown in Figure 4, but are considered in Figure 5 and in the discussion.

In Figure 4, the regression shows that females with higher curve magnitudes have longer relative caudal spines. Therefore, the relationship between the relative caudal length and curve magnitude was further investigated by comparing mean values for the caudal portion (the length of line1/the length of line 3) for each qualitative category of curve magnitude to the mean caudal portion for normal females. Figure 5 demonstrates that among curved females, as curve magnitude increases, the difference between the mean caudal portion for curved fish compared to normal also increases (Figure 5).

Table 1. Comparison of Length Measurements in Curved *Versus* Noncurved Fish

	Mean Values (SE)		
Length Measures (mm)	Normal	Curved	Significance
Manifest standard length (line 4)	23.2 (0.39)	23.2 (0.20)	NS ($P = 0.82$)
True standard length (lines 1 + 3)	23.2 (0.37)	24.1 (0.20)	$P = 0.05$
Precaudal length (line 3)	14.9 (0.28)	15.1 (0.13)	NS ($P = 0.48$)
Manifest caudal length (line 2)	8.31 (0.13)	8.43 (0.07)	NS ($P = 0.39$)
True caudal length (line 1)	8.35 (0.13)	9.01 (0.07)	$P < 0.0001$*

Nonparametric Wilcoxon pair-wise test used to compare curved and noncurved values (using χ^2 approximation with 1 *df*). Mean value of length measures are given in mm, with standard error (SE) shown for mean values. For all tests, $\alpha = 0.05$.
NS indicates values that are not significant. An asterisk indicates significant values.

Discussion

The present study investigated whether body length might correlate with curvature in the *curveback* guppy model. Our analysis shows that there is no difference between mature normal and curved females for mean values of manifest (*i.e.*, uncorrected) standard and caudal lengths, or precaudal body length. However, there is a significant difference between normal and curved females for true caudal length, although it does not correlate with curve magnitude. Furthermore, there is an allometric relationship between the length of the caudal spine and the length of the precaudal body such that adult curved females demonstrate a disproportionately longer caudal spine relative to the precaudal body length, and this is more pronounced among larger fish and those with greater curve magnitudes.

That a longer caudal spine correlates to curvature generally, but not curve magnitude, suggests that it is not a risk factor for severe curvature. However, disproportionate lengths could be indicative of an underlying growth abnormality that is a risk factor associated with severe idiopathic-type curvature. This is suggested by: (1) disproportion increases with curve magnitude, (2) dis-

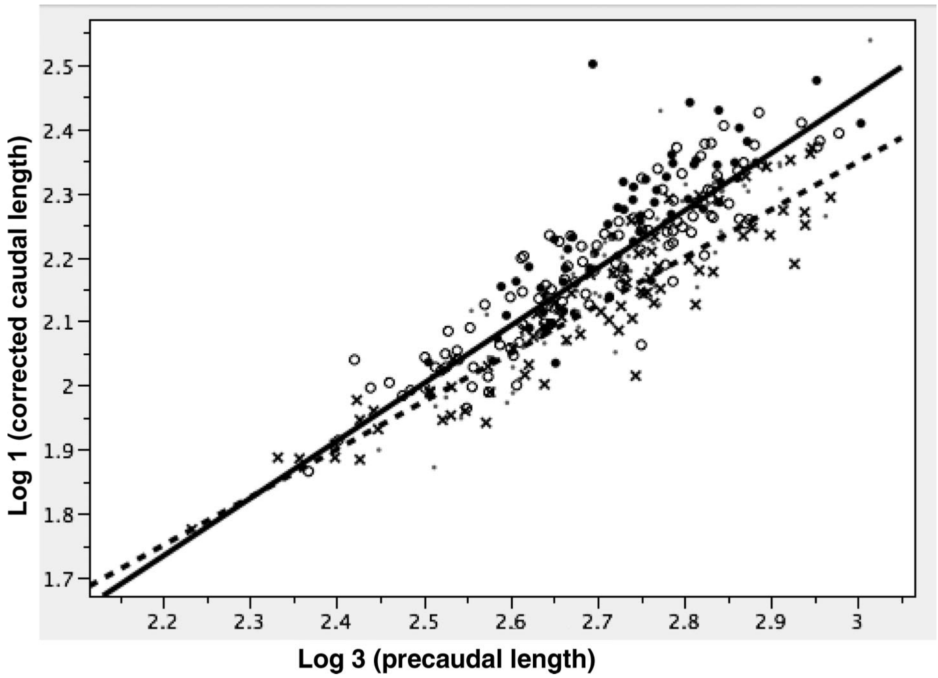

Figure 4. Allometry between precaudal and caudal lengths. There is a significant difference between curved (solid line) *versus* noncurved (dotted line) females for relationship of true caudal length (log of line1) and precaudal length (log of line 3): (slope for noncurved = 0.75 [R^2 = 0.87]; slope for curved = 0.9 [R^2 = 0.79]; ANCOVA; P = 0.0021). Curved individuals are denoted as circles: large solid circles = higher magnitudes (scores 3 & 4), open circles = moderate magnitudes (score 2), small dots = low magnitudes (score 1); normal individuals denoted by a black "x."

proportion is more pronounced among larger fish, and (3) the apparent growth abnormality present in the outliers in Figure 5. Our analysis of relative caudal length shows 5 fish of high curve magnitude that have extreme disproportion (outliers in Figure 5, curve score 4), such that the true caudal length is 75% to 100% of the value of the precaudal length (compared to ~57% in noncurved). On inspection, these females have abnormally small precaudal bodies that suggest an exaggerated growth abnormality (Figure 6). If there is a genetic basis for uncoordinated growth that is linked to curvature, then within the inbred *curveback* pedigree we expect to observe extreme cases of disproportion as we select for higher magnitudes of curvature. Although this appears to be the case, further analysis of inheritance for body length in the *curveback* pedigree is necessary.

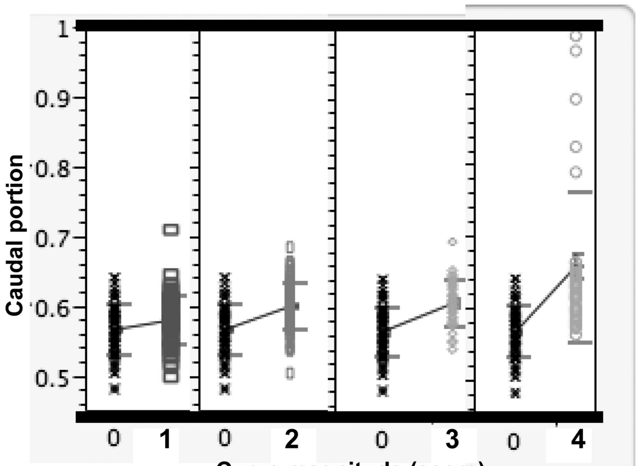

Figure 5. Caudal portion increases with curve magnitude. Wilcoxon pair-wise comparisons of mean caudal portion (line 1/line 3). Each panel shows the caudal portion values for all individuals within a category for curve magnitude (qualitative scores shown in Figure 1), compared to caudal portion values for noncurved fish (mean values connected by line). As curve magnitude increases, the difference between caudal portion and normal increases. Bars of one standard deviation are shown. Note outliers for curve magnitude 4 individuals.

Body Lengths, Growth, and Human Idiopathic-Type Curvature

Many human studies have correlated disproportionate lengths along the craniocaudal axis with IS.[4,10,11,13,17–20] However, inconsistencies between studies in sample sizes and methodology (*e.g.*, at what age a cohort is measured and whether to calculate pelvic height as a component of stature outside of sitting and standing heights) have led to different conclusions regarding which specific body component (trunk or leg length) is relevant, and exactly how the disproportion relates to growth, age, and maturity (*e.g.*, whether pubertal status is considered as a ma-

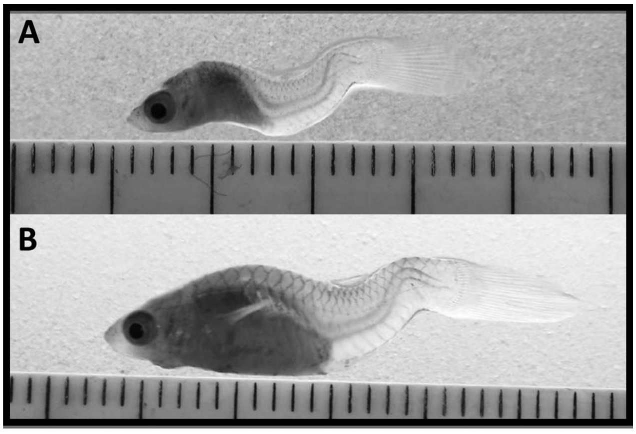

Figure 6. Apparent growth abnormality. **A**, One of the outliers in Figure 5. All outliers have a similar morphology. **B**, Age-matched female showing typical curveback phenotype. Scale shown in mm.

turity marker in addition to age). Further potential for inconsistencies between IS studies is introduced by the fact that methods of correction for loss of height due to curvature use 2-dimensional measurements of a 3-dimensional deformity.[23,25] This means that as the magnitude of the deformity increases, the power to detect an accurate relationship with height/length decreases.

With human IS, the length of the spine has been positively correlated with curve magnitude. However, the methodology behind this correlation needs verification because the commonly used Bjure formula introduces a possible spurious positive correlation between corrected height and curve magnitude (*e.g.*, Cheung *et al*[4]) due to the fact that Cobb angle is used to correct height measurements ($\text{Log}y = 0.011x - 0.177$; where y is the loss of trunk height caused by curvature and x is the Cobb angle of the primary curve).[23] Thus both the dependent and independent variables include curve magnitude.

Despite their inconsistencies, all anthropometric studies address the likelihood that disproportionate lengths in girls with IS are a consequence of abnormal growth patterns. Furthermore, studies that measure females during growth suggest that in cases of IS, abnormal prepubescent stature proportions (trunk or leg lengths) might be related to androgenic affects.[4,13,26] Endocrine factors such as growth hormone activity,[27–31] estradiol levels,[32,33] or testosterone levels[28,33] have been considered as possible risk factors for curve progression in IS. Given that the androgenic and somatotrophic axes are the major hormonal systems regulating postnatal linear growth and have principal roles in regulating skeletal growth and bone mass,[26] it is possible that androgenic and/or somatotrophic pathway interactions are involved in disproportionate cranial-caudal lengths associated with idiopathic-type curvature. For example, associations have been found between allelic variants of the estrogen receptor gene (*PvuII*), growth hormone receptor gene polymorphisms, and adult female height.[34–36] Without an animal model however, hypotheses regarding the effects of these hormones have been difficult to test.

■ Conclusion

As a model, the *curveback* guppy can be used to control for sources of experimental inconsistency, and thus give insight into the relationship between variables such as body lengths, curvature, and curve magnitude. In the present study, possible sources of experimental variation were eliminated by limiting our sample to mature females with 2-dimensional curvature, all from the same pedigree, and grown under controlled conditions of diet and environment. However, by restricting our dataset to mature females, we could not detect important growth-related variation that might be related to body length disproportion and/or curvature. For example, in their comprehensive anthropometric study, Cheung *et al*[4] demonstrated that females with IS were shorter and leaner than maturity-matched controls at their prepubertal growth spurt, but then had significantly greater corrected heights, sitting heights, and segmental lengths (arm span and leg length) after puberty. We therefore suggest further study into the relationship between body lengths and curvature throughout *curveback* growth. Furthermore, using *curveback* families that are inbred for either high or low curve penetrance and expression, it is feasible to experimentally manipulate androgens and/or growth hormone during growth to determine the extent to which these influence length proportions.

Our survey of body lengths in 321 *curveback* females reveals 2 important similarities to anthropometric studies of IS: that there are disproportionate cranial-caudal lengths among females with curvature, and the suggestion of an underlying growth abnormality among curved individuals. These similarities are interesting considering that androgenic and somatotrophic axes are the major hormonal systems regulating postnatal linear growth in all vertebrates, and that recent inquiries reveal extensive conservation of endocrine regulation between humans and teleosts.[37,38] Therefore, this inquiry further supports the usefulness of *curveback* as a model for the basic biology of idiopathic-type scoliosis and encourages study of growth-related factors.

■ Key Points

- As with human IS studies, in the *curveback* model, curved females have disproportionate cranial-caudal body lengths compared to non-curved females.
- Disproportionate body length in *curveback* is positively correlated to curve magnitude.
- As with anthropometric studies, our work with *curveback* suggests that a growth abnormality may be associated with idiopathic-type spinal curvature.

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