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Identification of the relationships between sagittal plane and coronal plane curvature in guppy models

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ABSTRACT

Objective: To explore the relationship between sagittal plane and coronal plane curvatures in guppies by investigating the curvature angles of sagittal and sagittal-coronal guppies.
Methods: After mating between 1000 spinal curvature guppies, 124 guppies (3-month old) were screened from progenies for the present study. Photos of all fishes were taken and the sagittal and coronal angles were calculated via angle measure tool of Photoshop 12.0 software. All data were analyzed by SPSS 11.0.
Results: In sagittal and sagittal-coronal curvature guppies, there was a significant linear correlation between sagittal angles and coronal angles. In 48 sagittal-coronal curvatures, their sagittal angles were above 40°, meanwhile, in 76 sagittal guppies, their sagittal angles were mostly below 40°.
Conclusions: These findings indicated that the occurrence of coronal curvature might be later than sagittal curvature and could be influenced by other factors. Sagittal angles 40° might be involved in the onset of coronal curvature.

1. Introduction

The global prevalence of Idiopathic Scoliosis (IS) among children was 0.5–10% and Scheuermann kyphosis was accounted for 4–10%.[1,2] However, pathogenesis of these idiopathic-type spinal curvatures, no matter genetic factors or the biological processes, were not clearly understood.[3] The complex characteristic of idiopathic-type spinal curvatures has considerably restricted the development of the relevant research work.[3] For example, phenotypic variability was coincided with growth and a genetic-developmental animal model was historic lacking. In addition, there were anatomical differences of spinal curvature between teleost and mammal, corresponding to different biomechanical.[4]

The Guppy (*Poecilia reticulate*), a popular tropical ornamental fish with transparent body, was originated from Venezuela and belonged to the Medaka genus in Cyprinodontidae family.[5] Guppy has been widely employed as a model for the investigation of the morphology, developmental biology, genetics or toxicology since 1920's.[6] Previous literature has documented that guppy was a unique animal model naturally forming the scoliosis without artificial intervention. For example, the guppy curvature lineage has been verified the physiological and developmental similarities to the human Idiopathic Scoliosis syndrome and Scheuermann kyphosis,[3,7] indicating its potential application in clinical researches.

Spinal curvature among teleost could be caused by a various factors, including nutrition, environment and genetics.[7] An arbitrary 3D spinal deformity could be described by a combination of three spatial planes, i.e., the coronal (frontal), sagittal (lateral) and axial (transverse) plane.[8] The curvature phenotype was primarily a sagittal plane deformity in teleost, however, the most human spinal curvatures were in the coronal plane.[6] The spinal curvature in the coronal (frontal) plane was primarily displayed as scoliosis in coronal cross-sections, while the evaluation of spinal curvature in the sagittal (lateral) plane referred to inclination of individual vertebrae or several adjacent vertebrae.[8]

It has been proposed that the curvature deformity in the sagittal plane might reflect the different muscle and ligament organization on the spine.[6] If primary factors caused an imbalance on the sagittal plane of the vertebral column, it was predisposed to collapse onto the coronal plane.[7] Although many guppies with curveback syndrome showed curve in sagittal plane, several emerged the curve in

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coronal plane just as idiopathic scoliosis.[9] Previous study of *hunchback* guppies demonstrated that the curve phenotype associated with complex inheritance showed a greater propensity for coronal deviation, and later onset (around 50 days past birth), than curves that were apparent at birth.[10] In order to clearly understand the occurrence of sagittal plane and coronal plane curvatures, it was necessary to explore the relationship between sagittal plane and coronal plane curvatures.

In the present study, by mating between 1000 spinal curvature guppies, the curvature angles of sagittal plane and coronal plane curvatures from offspring were recorded and analyzed. The linear correlation between sagittal angles and coronal angles was calculated, in the purpose of exploring their relationship. However, further investigations were needed to confirm our hypothesis.

2. Materials and methods

2.1. Sampling

A guppy lineage with a high incidence of spinal curvature (approximately 10% morbidity) was established from laboratory spinal curvature guppies via artificial mating. A total of 1000 spinal curvature guppies mated with each other. Breeding pairs were cultivated separately in 4 L plastic aquaria, and offspring were born after 3 weeks of gestation and moved into individual 600 mL plastic containers keeping under standardized conditions described by Gorman et al.,[5] which were in compliance with Guide for the Care and Use of Laboratory Animals. Total 124 guppies were screened as the samples for the present study, aging 3-month.

2.2. Curvature evaluation

Curvature of fish was scored after birth and then recorded once a month until 3 months old. All fish were positioned on one side above a ruler and the orientation was confirmed. Given adult coloration obscured the spine, males were not measured and females were photographed on a light table with a digital camera (Nikon D700, Japan) under 2-, 3- and 4-× magnification. Briefly, all progenies were placed into a dish (20 mL) and temporarily anesthetized by using the MS-222 anesthetics (international commodity Fingudi, Matacaine). Anteroposterior and lateral photos of vertebral column were taken after the successful anesthesia. The degree of spinal curvature were scored from the side (for sagittal curvature) and above (for coronal curvature) based on the scores defined by Gorman et al.[5] Because curvature was located in the caudal portion of the body, our analysis was limited to the vertebrae therein. The body length of guppies was ranged from 2 to 3 cm. To maintain consistency and precision in scores, the same person evaluated all fish. Subsequently, the curvature angles in the sagittal plane and coronal plane were measured via angle measure tool of Photoshop 12.0 software.

2.3. Statistical analysis

All the data were represented as average ± standard deviation (SD) and analyzed by SPSS 11.0 software. The linear regression and Pearson correlation coefficient (R value) were established by comparing of the curvature angles between sagittal and coronal plane. P value < 0.05 was chose as cut-off criterion.

3. Results

After mating between 1000 spinal curvature guppies, total 124 spinal curvatures guppies were enrolled in the present study and their spinal curvatures were measured. Firstly, the body length of each fish was tested to assess the influence of sample differences (Table 1). *T*-test showed that there was no statistical difference of body length between spinal plane and coronal plane curvatures.

Among the 124 guppies with sagittal plane curvature (average angle: 59°, SD: 15°, Table 1), 76 guppies showed only sagittal plane curvature, meanwhile, other 48 guppies showed both sagittal and coronal plane curvature (average: 59°, SD: 19°; Table 1). The 76 guppies showed sagittal angle without coronal curvature, suggesting that other factors could affect the formation of the coronal curvature (Fig. 1). In 48 guppies with sagittal-coronal plane curvature, Kolmogorov–Smirnov test displayed no significant differences of distribution (Table 2), and Pearson correlation analysis indicated significant correlation between sagittal and coronal angles (R: 0.393, $P < 0.05$; Table 3). Linear regression analysis demonstrated that there was a linear correlation between curvature angles in sagittal-coronal plane curvature. The equation was $y = 0.399x - 6.214$ (y = sagittal angles, x = coronal angles, −6.214 was constant, 0.399 was regression coefficient).

In the 48 sagittal-coronal curvature guppies, it was interesting to find that sagittal angles were all above 40° (Fig. 2). On the other hand, in the 76 sagittal curvature guppies, their sagittal angels were mostly below 40°.

4. Discussion

Previously, the pathogenesis studies about spinal curvature have been focused on genetic factors,[11] molecular biology anomalies (melatonin reduction[12] and calmodulin (CaM) abnormity[13]). Many studies suggested the significant genetic contribution in idiopathic scoliosis. More females showed idiopathic scoliosis than males.[14] More than one single gene might be involved into the inconstant penetrance and expressivity of scoliosis.[15] These findings required further investigation in order to explore the genetic heterogeneity of this disease.[16] Among expressive variation in families, environmental factors might also influence the spinal curve values.[17] For the molecular biology anomalies, it had been suggested a possible preventive effect of melatonin supplements on curve progression, especially in patients with Cobb angles under 35°.[18] The finding of melatonin signaling pathways dysfunction might lead to the development and validation of a clinical blood test for idiopathic scoliosis.[19] Other molecule included calmodulin and growth hormones.[20] Despite advanced research studies, the primary etiology of scoliosis remains unknown.

To thoroughly understand the pathogenesis of spinal curvature, several animal model of scoliosis have been established, including rats,[21] rabbits[22] and chickens,[23] and the outcomes were controversy. Unfortunately, scoliosis progression has not been explained clearly. In human, De Sèze, M. et al.[24] had proposed four main pathogenetic mechanisms, i.e. asymmetric bone growth dysregulation, susceptibility of bones to deformation, abnormal passive spinal system maintenance and disturbed active spinal system maintenance. Gorman et al.[25] have proposed that some guppies possessed the similar curvature pattern as the human idiopathic scoliosis. Briefly, the female guppy were inclined to severe curvature and the male were mild curvature; curvature angle was

Table 1
Statistic analysis of curvatures in 124 guppies.

Catalog	Curvature	
	Coronal plane	Sagittal plane
Number	48	124
Body length (cm)	2.03 ± 0.61	2.21 ± 0.85
Angle (°)	8.95 ± 19.49	59.46 ± 14.51