Available online at www.sciencedirect.com
ScienceDirect
Comparative Biochemistry and Physiology, Part C 145 (2007) 28–38
CBP
www.elsevier.com/locate/cbpc

Review

Teleosts as models for human vertebral stability and deformity ☆

Kristen F. Gorman *, Felix Breden

Department of Biological Sciences, Simon Fraser University, 8888 University Dr., Burnaby, B.C., Canada V5A 1S6

Received 1 February 2006; received in revised form 12 October 2006; accepted 12 October 2006
Available online 19 October 2006

Abstract

Vertebral development is a dynamic and complicated process, and defects can be caused by a variety of influences. Spinal curvature with no known cause (idiopathic scoliosis) affects 2–3% of the human population. In order to understand the etiology and pathogenesis of complex human skeletal defects such as idiopathic scoliosis, multiple models must be used to study all of the factors affecting vertebral stability and deformity. Although fish and humans have many of the same types of offenses to vertebral integrity, they have been overlooked as a resource for study. The most common morphological deformity reported for fish are those that occur during the development of the spinal system, and as with humans, curvature is a common morphological consequence. Here we review spinal curvature in teleosts and suggest that they are an unexploited resource for understanding the basic elements of vertebral stability, deformity, development and genetics. Fish can be a value to vertebral research because they are tractable, have a diversity of non-induced vertebral deformities, and substantial genomic resources. Current animal models lack non-induced deformities and the experimental tractability necessary for genetic studies. The fact that fish are free of an appendicular skeleton should allow for analysis of basic spinal integrity without the biomechanical constraints observed in quadrupedal and bipedal models. To illustrate the point we review human idiopathic scoliosis and the potential contribution teleosts can make for the identification of causes, risk factors, and treatment options.

Keywords: Development; Genetic model; Guppy; Scoliosis; Spinal deformity; Teleost; Vertebral system; Scoliosis

Contents

1. Introduction

Many factors can compromise the integrity of the vertebral system. Because it is integrated into the body both structurally and functionally, defects of the spinal system have the potential to produce complex phenotypes for which the primary causes are convoluted by secondary phenotypes. Models used so far

☆ This paper is based on a presentation given at the conference: *Aquatic Animal Models of Human Disease* hosted by the University of Georgia in Athens, Georgia, USA, October 30–November 2, 2005.

* Corresponding author. Tel.: +1 604 291 5641; fax: +1 604 291 3496.
E-mail address: kfg@sfu.ca (K.F. Gorman).

1532-0456/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.cbpc.2006.10.004

Table 1
Types of progressive/structural spinal deformities in humans* and reported occurrence in teleosts

Deformity types	Observed in fish?	Circumstances
Idiopathic	Yes	Curvature in aquarium guppy.
Congenital	Yes	Many types: seen in cultured and wild fish.
Neuromuscular	Yes	Laboratory zebrafish
In association with neurofibromatosis	No	Neurofibroma of the Damselfish is a model for NF1 in humans, although there are no reports of spinal curvature.
Traumatic	Yes	In cultured and wild fish.
Due to infection	Yes	*Mycobacterium* (*M. marinum*, *M. chelonei*, *M.fortuitum*)
Due to tumors	Yes	In cultured and wild fish.
Miscellaneous conditions:		
Deformities in adults	Yes	Curvature in older aquarium fish.
Spondylolistheses	?	

*According to the Terminology Committee of the Scoliosis Research Society (1976).

are insufficient to study all of the factors affecting spinal stability and deformity, and thus many of the factors that maintain basic vertebral integrity are unstudied. With regard to complex disorders in humans, multiple models are critical for the investigation and manipulation of etiological factors. The value of teleosts as tools for biomedical research has rapidly become recognized, partly because genomic tools have shown strong human/teleost homology. In this review we discuss the potential of teleost fish as a tool to fill-in some of the gaps in human vertebral research. Defects of the vertebral system in fish and humans have many of the same causes including genetic, physiological (*e.g.* calcium regulation), developmental (*e.g.* fused vertebrae) and infectious (viruses, parasites) (Table 1). In fact, the most common type of deformity seen in fish is vertebral, and most of these occur during development (Brown and Nunez, 1998). Fish systems could be of enormous benefit to vertebral research because they are tractable, exhibit a diverse range of deformities, are free from an appendicular skeleton, and substantial genomic resources have been developed for several species. Here we review spinal curvature in model teleosts and suggest that they are an unexploited resource for understanding the basic elements of vertebral stability, deformity, development and genetics. We illustrate this point by our research into the mutant guppy *curveback* (Fig. 1) as a model for human familial/idiopathic curvature.

2. Spinal curvature in humans

The human spine is normally straight in the coronal plane. At birth there is a slight kyphosis (dorsally directed sagittal curve) from the crown of the head to the buttocks. As a consequence of bipedalism four natural curves develop in the sagittal plane. Control of the head induces cervical lordosis (ventrally directed sagittal curve), and standing causes a lumbar lordosis. Therefore in the normal spine there are cervical and lumbar lordoses and thoracic and sacral kyphoses (Dickson, 2004). There is natural variation among individuals for the magnitude of sagittal curves. Exaggerations of normal curvature are considered abnormalities when they become dysfunctional.

In addition to exaggerations of innate curvature, aberrant spinal curvature can be caused by a variety of influences. Consequently, deformities are classified according to their presumed etiology (Terminology Committee of the Scoliosis Research Society, a glossary of scoliosis terms, 1976). Each etiological category is defined by characteristics that are imposed by the pathophysiology of an underlying condition. Structural curves are those that have the ability to progress during growth (Dickson, 2004). Congenital anomalies (curvature caused by vertebral malformation), idiopathic curvature (curvature with no apparent cause), neuromuscular disorders, neurofibromatosis, connective tissue disorders, and skeletal dysplasia are structural curves specific to the pediatric age group. Although not recognized as an etiological category, (the Scoliosis Research Society) genetics have been identified as the underlying etiology in an increasing number of structural curves. Such is the case for Marfan syndrome, a disorder affecting the connective tissue (Kumar and Guille, 2001; Coucke et al., 2006), and Friedreich's ataxia, a disorder affecting neurological control (Labelle, 2001; Pandolfo, 2006). For curvature such as idiopathic scoliosis and Scheuermann's kyphosis, a genetic basis is widely accepted but there is still no established etiology (Ogilvie et al., 2006; Damborg et al., 2006).

3. Idiopathic scoliosis

Human familial/idiopathic scoliosis represents the largest subgroup of human spinal curvatures. Eighty percent of all

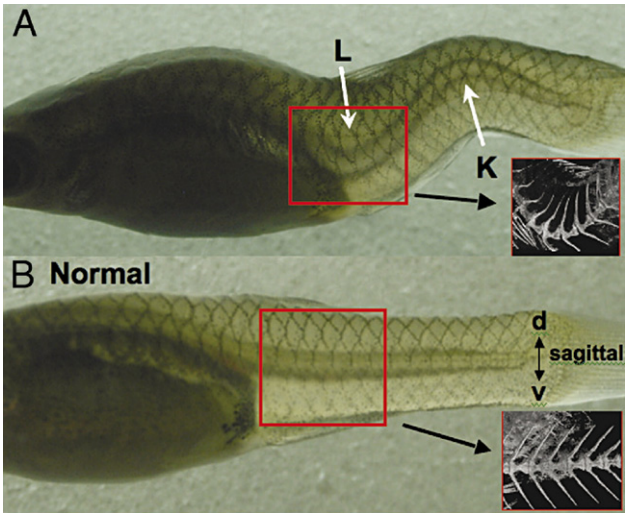

Fig. 1. A: The *curveback* phenotype is a primary anterior lordosis (L) and a secondary posterior kyphosis (K) occurring on the sagittal plane. CT scan shows no vertebral breaks or fusion associated with curvature (some individuals demonstrate coronal deviation—not shown). B: Normal fish with sagittal plane and dorsal/ventral axes shown. Digital photos of anaesthetized adult females taken on a standard light table under 3X magnification, CT scans consist of 350 0.4 mm slices. Images taken at the University of Texas High-Resolution X-ray CT Facility, (datasets of scans available for view at http://www.digimorph.org/index.phtml).

structural curves are idiopathic (IS [MIM 181800]) making IS the most common form of spinal deformity in humans. Generally, its incidence among otherwise healthy school-age children is between 0.15%–10.0% (Lonstein, 1995; Axenovich et al., 1999; Reamy and Slakey, 2001; Asher and Burton, 2006). Since the deformity was first described by Hippocrates, the diagnosis, cause and treatment of IS have been the focus of a great deal of research. However, phenotypic variability, curve pathogenesis that coincides with dynamic growth, and the lack of an animal model with non-induced curvature has made it difficult to identify the etiology. The diagnosis is further complicated by the fact that (without consideration of teleosts) idiopathic-type curvature appears to be exclusive to humans. There is a strong suggestion that IS is not a homogeneous group, but one that is composed of several other subgroups, the exact etiologies of which have not been described.

Generally, it is a characterized structural curvature with no vertebral defects that typically occupies all three planes of the body (the majority of curves exhibiting coronal deviation). The deformity has unique characteristics such as, phenotypic variability for age of curve onset, curve morphology, and rate of curve progression, and a gender bias for girls to develop severe curves. Because of variable ages for curve onset, the deformity is classified on the basis of the child's age when curvature is first identified; for infantile idiopathic scoliosis (IIS) onset is before the age of four years, juvenile idiopathic scoliosis (JIS) from age 4 to 9 years, and adolescent idiopathic scoliosis (AIS) occurs between 10 years and skeletal maturity (James, 1954). Generally, the majority of curves that appear during infancy resolve before sexual maturity is reached, while juvenile onset IS tends to be progressive. Adolescent onset is the most prevalent type of IS. The fact that most cases of IS are diagnosed between 10–14 years may reflect that AIS is present in a latent form in younger children who manifest a minor degree of curvature that develops slowly (Stirling et al., 1996).

Hypotheses for the cause of idiopathic curvature include differences in growth patterns, connective tissue abnormalities, asymmetries in the central nervous system, distribution of melatonin and calmodulin, hormonal variation, ectomorphy/spinal slenderness, diet and posture. Familial clustering (Garland, 1934; Fisher and De George, 1967; Wynne-Davis, 1968; Riseborough and Wynne-Davis, 1973) and concordance among monozygotic twins (Kesling and Reinker, 1997; Andersen et al., 2004) has established a genetic basis as a primary causative factor (Cowell et al., 1972; Beals, 1973; Harrington, 1977), although the mode of inheritance is still a matter of debate. One important outcome of understanding the genetics underlying idiopathic curvature would be earlier detection. There is not an established method for detection of curves in growing children. A recent review of screening methodology showed that the most common method of curve detection was by family or friends. The majority of cases detected by such means presented a curve of such magnitude spinal fusion surgery is necessary (Fazal and Edgar, 2006). Early detection of curvature may mitigate curve progression so that non-operative therapies are more effective.

The deformity is complicated by biomechanical and developmental and possibly genetic variability between individuals (Miller et al., 2006; Ogilvie et al., 2006). Phenotypic components such as curve magnitude and morphology, progression risk, and time of onset can vary within a pedigree. Segregation analysis using human pedigrees has suggested a single gene for the major determinant of IS (Axenovich et al., 1999). However, the study only uses pedigrees with a proband having a reported curve magnitude of at least 15° (Cobb angle; unit of measure for lateral deviation of spine in human IS). Therefore only a portion of the phenotype has been considered in pedigree studies, which is likely to be an effect of detection difficulty for curves of slight magnitude. There is little concordance for identified loci among linkage studies using human pedigrees (Carr et al., 1992; Miller et al., 1996; Bashiardes et al., 2002, 2004; Chan et al., 2002; Inoue et al., 2002; Salehi et al., 2002; Justice et al., 2003; Alden et al., 2006; Ogilvie et al., 2006; Marosy et al., 2006; Miller et al., 2006; Sharipov et al., 2006; Wu et al., 2006). To date, the genetics underlying idiopathic curvature have not been identified. This is most likely a consequence of several factors, including: inconsistent pedigree construction between human studies, an arbitrary consensus threshold for proband curve magnitude that may obscure true heritability, and the lack of a genetic model.

It is hypothesized that the three-dimensional nature of IS is a consequence of secondary biomechanical modifications to a primary lordosis in the mid-sagittal plane (Roaf, 1966; Perdriolle and Vidal, 1987; Millner and Dickson, 1996; Ganey and Ogden, 2001; Burwell, 2003). The human spine must bear the load of the head while tethered at the pelvis, and so theories such as 'column buckling' or the Heuter–Volkmann principle have been used to explain the three-dimensional nature of human curvature (Millner and Dickson, 1996; Schultz et al., 1984; Burwell, 2003; Stokes et al., 2006). If primary factors cause an imbalance on the sagittal plane of the vertebral column, then it is predisposed to collapse onto the coronal plane and in some cases, vertebral rotation is encouraged by the ribs. According to Millner and Dickson (1996), although idiopathic scoliosis is a three-dimensional 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 (Cheng, 2003; Guo et al., 2003).

In order to understand components of the IS deformity phenotype, the spine biomechanics has been extensively modeled in vitro by computer and three-dimensional reconstruction using mammals such as rabbits, goats, and pigs. However, because curve pathogenesis coincides with development, simulation modeling can elucidate only a fraction of the entire deformity. Rates of curve progression and biomechanics are mitigated by individual morphology, relative growth rate, and age of curve initiation (Stokes and Windisch, 2006). Heterogeneity among patients in different studies has further complicated an innately variable morphology (Escalada et al., 2005). Attempts to classify a curve's morphology before skeletal maturity can be complicated by progression of the primary curvature and/or the development of secondary curves (Robinson and McMaster, 1996).

4. Animal models for human idiopathic scoliosis

The suggestion that human biomechanics or physiology can modify primary etiological factors, and the possibility that human IS may consist of many etiological subgroups, implies that more than one type of model will be necessary to address all levels of the complex IS phenotype. Models used to study the pathogenesis of IS curvature rely on induced curvature and are therefore limited (Braun et al., 2006); it is controversial whether conclusions made from such experiments relate to primary or secondary influences for curvature (reviewed in Kawakami et al., 1999). In animals with non-induced curvature the type of scoliosis studied is congenital to other conditions (e.g. hens, Ruble et al., 2002; scoliosis mouse, Adham et al., 2005), or occurs in sexually mature animals (Riggins et al., 1977; Naique et al., 2003), and thus doesn't reflect the human condition. Because heritable, developmental, idiopathic spinal curvature has been exclusive to humans, it has been hypothesized to be a consequence of bipedalism (Burwell et al., 1992; Machida et al., 1999).

Current developmental modeling is further complicated by the fact that most of the animals used thus far are quadrapedal. In these animals, the structure of the spine and the center of gravity are radically different from humans (Mack and Greenberg, 1990; Kawakami et al., 1999; O'Kelly et al., 1999). Despite the fact that the vertebral anatomy of the bird is grossly different from that of non-avian vertebrates (*e.g.*, no intervertebral discs, multiple fused vertebrae), the pinealectomized chicken is the most common animal model of IS. This is largely because pinealectomy induces spinal curvature in most chickens, and because chickens are bipedal. Pinealectomy in hamsters and monkeys does not induce curvature (O'Kelly et al., 1999; Cheung et al., 2005). Pinealectomized rats do not demonstrate spinal curvature unless they are made bipedal (Machida et al., 1999). Likewise, mice made melatonin-deficient without pinealectomy do not show spinal curvature unless they are made bipedal (Machida et al., 2006). Due to conflicting results between human and chicken studies, whether melatonin plays a role in the formation of curvature is also a topic of debate (Bagnall et al., 1996). The forces that act on a bipedal spine are different than those acting on a quadruped. It is not clear whether genes for idiopathic-type curvature are present in terrestrial animals but constrained by quadrupedal biomechanics, as the studies using the bipedal rat suggest, or if the primary etiology is exclusive to humans. As all animals used in orthopaedic modeling thus far are quadrupedal, the latter assumption has been favored.

Fish are ideal models for studying vertebral deformities. The most common deformities among teleosts are those that occur during development of the spinal system, and humans and fish share many etiological factors for non-idiopathic types of curvature (Table 1), demonstrating general homology for spinal deformity. Importantly, pinealectomy in the guppy and salmon induces spinal curvature with a physiological response the same as in chickens (Plugfelder, 1953; Mayer, 2000; Fjelldal et al., 2004), the most common model of IS. Furthermore, the teleost spine is not weight-bearing, due to buoyancy provided by the swim bladder combined with the density of water (Fjelldal et al., 2004). Therefore, fish present the opportunity to investigate factors that influence curvature without the constraints of quadrupedal or bipedal biomechanics. Factors that influence human curvature such as gravity, vertical loading, rib association and lumbar tethering can be omitted from the equation of possible influences for curve progression, thereby illuminating important primary correlates.

5. Spinal curvature in model teleosts

The abundance of spinal deformities among fish suggests their innate power to reveal important variables for basic spinal stability and deformity. To illustrate the point, in Fig. 2 we review 63 studies from over 20 species showing vertebral deformities. Types of deformity commonly seen are lordosis, kyphosis, scoliosis, and broken/cracked vertebrae. Causes can be physical injury, environmental, nutritional, infection, or genetic (Fig. 2). Until recently, the ontogeny of fish spinal curvatures in non-model teleost species has received relatively little attention and most studies have been largely descriptive. Consequently, few deformities have been explored beyond the level of association with particular causative factors (Brown and Nunez, 1998).

Among Euteleosts, the model fish species guppy (*Poecilia reticulata*), medaka (*Oryzias latipes*) and swordtail (*Xiphophorus maculatus*) have been shown to exhibit similar (although not characterized in swordtail) spontaneous spinal curvature mutants (Fig. 3). Curvature in these closely related teleosts is similarly described as a primary dorso-ventral deviation (lordosis and/or kyphosis), with some fish exhibiting coronal curvature and vertebral rotation. Clear and stain of medaka *wavy* and guppy *curveback* mutants shows normal vertebrae, and as with human idiopathic curves, the vertebral centra are warped at the point of greatest curvature (curve apex) in severe curves (Takeuchi, 1960; Fig. 1). Historical characterization of non-induced spinal curvature in the guppy and medaka has been largely descriptive, with particular focus on inheritance, calcium and phosphorous ratios in the vertebrae, and tissue analysis (Harrison, 1941; Rosenthal and Rosenthal, 1950; Rosenthal, 1953, 1954, Rosenthal et al., 1958; Takeuchi, 1960; Marquet and Sobel, 1969). The *fused* medaka mutant and the *stubby* and *palla* guppy mutants are similarly described as fusion of a variable number of vertebrae that causes shortening of the spine (Aida, 1930; Schultz, 1963; Lodi, 1978).

A review of the curve phenotypes in Fig. 3 suggests that multiple genes are associated with curvature, and that some of the genes may be conserved between medaka and guppy. The *wavy* medaka, *hunchback* swordtail, and *curveback* guppy phenotypes have a very similar curve morphology (primary lordosis/kyphosis), inheritance pattern, as well as trait expressivity (variable penetrance for magnitude of lordosis and kyphosis). Conservation of gene function has been confirmed among orders of Euteleosti (Sato et al., 2000), and so it is not unreasonable to assume that the guppy, swordtail, and medaka share genes involved in the spinal curvature phenotype. Both the guppy and medaka genomes have been shown to have

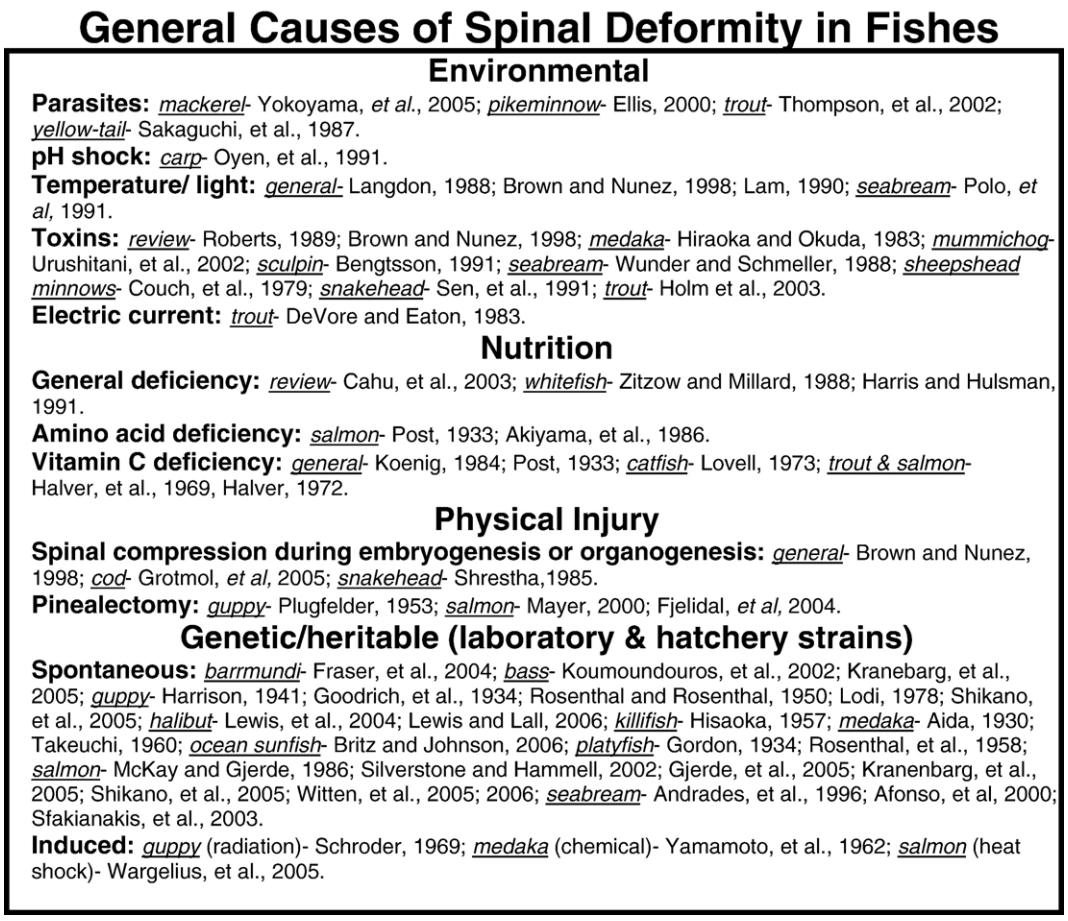

Fig. 2. Spinal deformities in fish can be caused by a variety of influences. Shown here are general categories for insults to vertebral integrity. Because spinal deformities are so prevalent among fishes, this is not an exhaustive list of publications.

strong homology with the swordtail genome (Brummel et al., 2006). The fact that guppy and medaka also exhibit the heritable fused/stubby vertebral mutants with strong phenotypic and genetic similarity, further suggests conserved genes for vertebral development and function among these species. Interestingly, the zebrafish model is more distantly related than the guppy and medaka (*i.e.,* is not a Euteleost: Crollius and Weissenbach, 2005), and has no incidence of heritable, idiopathic-type curvature. This may be a reflection of genetic differences, as zebrafish have different copy-numbers and expression patterns for important developmental genes than medaka and pufferfish (Schartl, 2004; Gajewski et al., 2006; Kurosawa et al., 2006).

Differences in curve morphologies and inheritance patterns between species and/or strains of fish could either indicate modifying genes or modified expression patterns of the same genes involved in the phenotype. With all curve phenotypes, genetic complexity is apparent in curve morphology and the time of curve onset. As seen in Fig. 3, there are single factor recessive and complex inheritance patterns observed in different populations of swordtail and guppy. Analysis by Harrison (1941) of *hunchback* guppies revealed two curve phenotypes with different inheritance patterns, although they segregated in the same pedigree. The curve phenotype associated with complex inheritance demonstrated a greater propensity for coronal deviation, and later onset (around 50 days past birth), than curves that were apparent at birth. Curves present at birth showed Mendelian recessive inheritance and curvature that increased steadily with growth. Studies by Rosenthal (1951) and Goodrich et al. (1934) describe Mendelian recessive inheritance for curvature present at birth in guppies, but mention individuals for whom curvature matures after birth and exhibits a different and more complex inheritance pattern. However, no further analysis was published. Ando et al. (1995) demonstrated that different curve morphologies within strains of guppies are correlated to differential segregation of genetic markers, demonstrating that multiple genes account for some curve phenotypes. Furthermore, there are two deformities involving fused vertebrae in the guppy, one with dominant inheritance, and one recessive. These studies demonstrate the genetic complexity of basic spinal stability, reinforcing the contribution of teleosts to the study of the genetic basis of spinal deformities.

The studies in Fig. 3 indicate that spinal curvature does not hinder fitness in laboratory conditions (*e.g.*, lifespan, feeding, activity). Although not correlated to the propensity for curvature,

Species	mutant	inheritance	analysis	references
Medaka	*fused*	recessive	descriptive	Aida, 1930; Ogawa, 1965
	wavy	recessive + modifiers	larval spine dev., spine ratio, eggs	Aida, 1930; Takeuchi, 1960
Swordtail	*hunchback*	complex	descriptive	Gordon, 1934
	(no name)	recessive/ single factor	spine [Ca/P], males sterile	Rosenthal *et al.*, 1958
Guppy	*palla*	dominant	inheritance	Lodi, 1978
	stubby	recessive	descriptive	Schultz, 1963;
	hunchback	both recessive and complex observed	descriptive, inheritance	Harrison, 1941 Goodrich *et al.*, 1943
	lordosis	recessive/ single factor	bone [Ca/P] metabolism, embryonic, inheritance	Rosenthal and Rosenthal, 1950; Rosenthal, 1951;1953; 1954;1958
	lordosis	recessive/ sex linked (females only)	cellular/histological	Marquet and Sobel, 1969
	curveback	multifactorial	descriptive, development, inheritance	Gorman and Breden
	multiple	not indicated	selection	Ando, *et al.*,1995

Fig. 3. A summary of the research conducted on model teleosts with regard to heritable spinal curvature. For further reading regarding: Medaka fused spinal mutants: Aida, 1930; Ogawa, 1965. Medada wavy spinal mutants: Aida, 1930; Takeuchi, 1960. Swordtail spinal mutants: Gordon, 1934; Rosenthal, et al., 1958 Guppy spinal mutants: Lodi, 1978 (palla); Schultz, 1963 (stubby); Harrison, 1941 and Goodrich, et al., 1943 (hunchback); for lordosis in guppies: Rosenthal and Rosenthal, 1950; Rosenthal, 1951, 1953, 1954, 1958; Marquet and Sobel, 1969; Ando, et al., 1995.

the eggs of the *wavy* medaka mutant strain are "weaker" than normal fish eggs and tend to die during embryonic development (Takeuchi, 1960). There are no differences in the embryonic development of the lordotic and normal guppies (Rosenthal, 1953). Rosenthal's report (1951) of semi-lethalism in the male lordotic guppy is questionable, because he compares the inbred lordotic guppy strain to wild-caught guppies. Our experience in the lab with guppies is that fecundity decreases in general as laboratory strains become more inbred, and thus the lower fecundity may reflect the inbreeding during the maintenance of the lordotic lines.

Chemical analysis in curved fish has focused on calcium and/or potassium levels of vertebral structures and embryonic or larval calcification (Harrison, 1941; Rosenthal and Rosenthal, 1950; Rosenthal, 1953, 1954, 1958; Rosenthal et al., 1958; Takeuchi, 1960). Although calcium and potassium levels during guppy embryogenesis are not different among fish in lineages of lordotic guppies (Rosenthal, 1953), Rosenthal suggests that there is an alteration of calcium metabolism in the vertebrae but not muscle of adult curved fish (1954, 1958). Abnormal calcium metabolism has been suggested as a possible component of human IS (Cheung et al., 2006). Excess crystalline inclusions were found in the endoplasmic reticulum and nuclear envelop of the spinal cord oligodendroglia of curved guppies (Marquet and Sobel, 1969). This is interesting because virus-like particles have been noted in the paraspinal muscle of humans with idiopathic scoliosis (Webb and Gillespie, 1976; Green et al., 1979). It is possible that the crystalline inclusions in guppies are an indication of excess cellular protein. Whether the human particles indicate a similar cellular process or are an indication that general cellular disruption can cause curvature has not been investigated. Morphological analysis in the *wavy* medaka such as spine ratios or vertebral structure was measured. In the *wavy* mutants the length of the vertebral column is the same as in normal medaka, but vertebral spines are shorter on the convex side of curvature (Takeuchi, 1960).

Growing research in fish vertebral ontogeny, a history of genetic and molecular research regarding spinal curvature of model teleosts, and the growing field of comparative genomics can make model teleosts valuable for human vertebral research. Without many of the complications that have been observed in previous models, fish offer an opportunity to explore the basic elements of vertebral stability and deformity. Comparisons between the human genome and that of non-mammal vertebrates such as fish has proven to be a powerful tool for identifying DNA sequences that have significant functional activity in all vertebrates (Boffelli et al., 2004). The fact that humans and fish share many genes with similar tissue and temporal expression characteristics is well established (Aparicio et al., 2002).

6. *Curveback* as a model for human familial/idiopathic scoliosis deformity

An experimental model that has a strong genetic component and does not rely on induced curvature will have significant value for scoliosis research. Discovery of conserved genes in humans for idiopathic-type spinal curvature and testing the effects of suspected risk factors for curve progression may suggest new therapeutic interventions. In human IS, the contribution of factors such as biomechanical force, load, growth rate trajectories, hormone levels, spinal length, vertebral distortion, and sex has been debated. Using the tractable guppy model, we will be able to test each factor as a separate

Table 2
Parallels between curvesback and human idiopathic/familial scoliosis phenotypes

1) No vertibral 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.

hypothesis and determine whether it contributes to the progression of curvature. We have shown how developmental trends in the *curveback* lineage parallel the human condition of familial/idiopathic scoliosis (Gorman et al., in press). Our results show the guppy's potential as a tractable model organism for investigation into genetic and developmental factors that influence spinal curvature.

Spinal curvature in the *curveback* guppy develops after birth, when the skeleton is fully ossified. Guppies are livebearers, and are born about 3 weeks post-fertilization. The primary force/load on the guppy spine is directed in a cranial to caudal (anterior to posterior) direction, principally due to motion through water, so that the orientation of primary curvature is perpendicular to the load (similar to human IS). Very importantly, CT scans and clear and stain analysis of skeletons show that curvature is not caused by vertebral malformation, making *curveback* the first animal model for human IS with non-induced curvature. As in humans, there is a female bias for extreme/progressed curves, despite the fact that there is no apparent gender bias at the time of curve onset. In both the human and *curveback* guppy populations the majority of curves are slight in magnitude, while some progress to a great extent, and there is a class of curves that resolve before the individual reaches skeletal maturity. Variability in the *curveback* guppy population for age of curve onset, propensity to demonstrate progression of curvature, and curve magnitude resembles that described for the human population (Gorman et al., in press). The observed distribution for curve magnitude is continuous in both the *curveback* and human IS populations (Miller et al., 2005; Gorman et al., in press). The pattern of curve progression for guppies is similar to humans in that the angle grows in spurts instead of a linear progression through development (Escalada et al., 2005). These similarities between the guppy *curveback* mutant and human IS are summarized in Table 2.

The guppy has been an important laboratory organism for genetic analysis since the 1920's (Winge, 1927). A growing number of genomic resources make gene identification possible in the near future. Candidate genes identified in teleosts can be screened in human pedigrees for association with IS. Much of the work demonstrating human/teleost homology has been done with medaka, often in systems implicated in the IS phenotype (*e.g.*: *pineal gland*: Alunni et al., 2004; *neuronal development;* Okubo et al., 2006; *somitogenesis*; Gajewski et al., 2006; *bone formation*: Wagner et al., 2003; Renn et al., 2006; *vertebral column formation*: Ohtsuka et al., 2004; Yasutake et al., 2004). Medaka is closely related to the guppy, and several lines of evidence (such as same mode of inheritance, and similar trait morphology and expressivity) suggest that the mutant *wavy* is genetically similar to *curveback* in the guppy. Using two models with similar curve phenotypes will increase the likelihood of identifying genes conserved in human developmental processes that are involved in IS.

7. Conclusion

The growing number of sequenced genomes and overall genomic resources available for fish has made them an important tool for the investigation of human genetics, development and disease (*e.g.* reviews from: Danzmann and Gharbi, 2001; Prince and Pickett, 2002; Wittbrodt et al., 2002; Boffelli et al., 2004; Cossins and Crawford, 2005). Experimental studies of spinal deformities in fish are an unexploited resource capable of answering questions of vertebral development, basic stability, and deformity. The spinal column of fish is free from an apendicular skeleton, and is not weight-bearing, allowing for an opportunity to investigate the factors that maintain the vertebral skeleton without the constraints of quadrupedal or bipedal biomechanics. Complicating factors such as gravity and load can be omitted from the equation of possible influences for curve progression, thereby illuminating important factors for basic spinal stability.

By using model teleosts for understanding conserved features of vertebral stability, research into vertebral deformities will be able to distinguish between primary and secondary etiological factors of deformity phenotypes, something that has been very difficult with the accepted animal models so far. We expect that with model teleosts it will be possible to manipulate suspected etiological correlates such as hormone and mineral (*e.g.* calcium) levels. With inbreeding not only can inheritance be investigated in detail, but also selective breeding will allow for the creation of families enriched for genes contributing to deformities. Mapping crosses, experimental crosses, and transgenics are all possible in model teleosts, making them a valuable tool for the understanding of the development of the vertebral system and the etiology of deformities.

Acknowledgments

We acknowledge the advice and encouragement of S. Tredwell of the Department of Orthopaedics, British Columbia Children's Hospital, and thank Julian Humphries and Tim Rowe at the University of Texas High-Resolution X-ray Computed Tomography Facility, the Natural Sciences and

Engineering Research Council of Canada and the Scoliosis Research Society for the support.

References

Adham, I.M., Gille, M., Gamel, A.J., Reis, A., Dressel, R., Steding, G., Brand-Saberi, B., Engel, W., 2005. The scoliosis (sco) mouse: a new allele of Pax1. Cytogenet. Genome Res. 111 (1), 16–26.

Aida, T., 1930. Further genetical studies of *Aplocheilus latipes*. Genetics 15, 1–16.

Alden, K.J., Marosy, B., Nzegwu, N., Justice, C.M., Wilson, A.F., Miller, N.H., 2006. Idiopathic scoliosis: identification of candidate regions on chromosome 19p13. Spine 31, 1815–1819.

Alunni, A., Blin, M., Deschet, K., Bourrat, F., Vernier, P., Retaux, S., 2004. Cloning and developmental expression patterns of *Dlx2, Lhx7 and Lhx9* in the medaka fish (*Oryzias latipes*). Mech. Dev. 121, 977–983.

Andersen, M., Thomsen, K., Kyvik, K., 2004. Genetic components in adolescent idiopathic scoliosis, a cohort study of twins. Spine J. 4, 55S.

Ando, D., Nakajima, M., Fujio, Y., 1995. Strain difference of vertebral abnormality in the guppy *Poecilia reticulata*. Tohoku J. Agric. Res. 46, 29–34.

Aparicio, S., Chapman, J., Stupka, E., Putnam, N., Chia, J-m., Dehal, P., Christoffels, A., Rash, S., Hoon, S., Smit, A., Gelpke, M.D.S., Roach, J., Oh, T., Ho, I.Y., Wong, M., Detter, C., Verhoef, F., Predki, P., Tay, A., Lucas, S., Richardson, P., Smith, S.F., Clark, M.S., Edwards, Y.J.K., Doggett, N., Zharkikh, A., Tavtigian, S.V., Pruss, D., Barnstead, M., Evans, C., Baden, H., Powell, J., Glusman, G., Rowen, L., Hood, L., Tan, Y.H., Elgar, G., Hawkins, T., Venkatesh, B., Rokhsar, D., Brenner, S., 2002. Whole-genome shotgun assembly and analysis of the *Fugu rubripes*. Science 297, 1301–1310.

Asher, M.A., Burton, D.C., 2006. Adolescent idiopathic scoliosis: natural history and long term treatment effects. Scoliosis 1–10.

Axenovich, T.I., Zaidman, A.M., Zorkoltseva, I.V., Tregubova, I.L., Borodin, P. M., 1999. Segregation analysis of idiopathic scoliosis, demonstration of a major gene effect. Am. J. Med. Genet. 86, 389–394.

Bagnall, K.M., Raso, V.J., Hill, D.L., Moreau, M., Mahood, J.K, Jiang, H., Russell, G., Bering, M., Buzzell, G.R., 1996. Melatonin levels in idiopathic scoliosis. Diurnal and nocturnal serum melatonin levels in girls with adolescent idiopathic scoliosis. Spine 17, 1974–1978.

Bashiardes, S., Veile, R., Wise, C.A., Szappanos, L., Lovett, M., 2002. Positional cloning strategies for idiopathic scoliosis. Stud. Health Technol. Inform. 91, 86–89.

Bashiardes, S., Veile, R., Allen, M., Wise, C.A., Dobbs, M., Morcuende, J.A., Szappanos, L., Herring, J.A., Bowcock, A.M., Lovett, M., 2004. SNTG1, the gene encoding gamma 1-syntrophin, a candidate gene for idiopathic scoliosis. Hum. Genet. 115, 81–89.

Beals, R.K., 1973. Nosologic and genetic aspects of scoliosis. Clin. Orthop. 93, 23–32.

Boffelli, D., Nobrega, M.A., Rubin, E.M., 2004. Comparative genomics at the vertebrate extremes. Nat. Genet. 5, 456–465.

Braun, J.T., Ogilvie, J.W., Akyuz, E., Brodke, D.S., Bachus, K.N., 2006. Experimental scoliosis in an immature goat model: A method that creates idiopathic-type deformity with minimal violation of the spinal elements along the curve. Spine 28 (19), 2198–2203.

Brown, C.L., Nunez, J.M., 1998. Disorders of development. In: Leatherland, J. F., Woo, P.T.K. (Eds.), Fish Diseases and Disorders. . Non-infectious Disorders, vol. 2. CAB International, pp. 1–17.

Brummel, M., Kazianis, S., Davidson, W.S., Breden, F., 2006. Conservation of synteny between guppy and Xiphophorus genomes. Zebrafish 3 (3), 347–357.

Burwell, R.G., 2003. Aetiology of idiopathic scoliosis, current concepts. Pediatr. Rehabil. 6, 137–170.

Burwell, R.G., Cole, A.A., Cook, T.A., Grivas, T.B., Kiel, A.W., Moulton, A., Thirwall, A.S., Upadhyay, S.S., Webb, J.K., Wemyss-Holden, S.A., et al., 1992. Pathogenesis of idiopathic scoliosis. The Nottingham concept. Acta Orthop. Belg. 58 (Suppl 1), 33–58.

Carr, A.J., Ogilive, D.J., Wordsworth, B.P., Priestly, L.M., Smith, R., Sykes, B., 1992. Segregation of structural collagen genes in adolescent idiopathic scoliosis. Clin. Orthop. 274, 305–310.

Chan, V., Fong, G.C.Y., Luk, K.D.K., Yip, B., Lee, M.K., Wong, M.S., Lu, D. D., Chan, T.K., 2002. A genetic locus for adolescent idiopathic scoliosis linked to chromosome 19p13.3. Am. J. Hum. Genet. 71, 401–406.

Cheng, J.C., 2003. Osteopenia. Spine, State Art Rev. 14, 339–348.

Cheung, K.M.c., Wang, T., Poon, A.M.S., Carl, A., Tranmer, B., Hu, Y., Luk, K. D., Leong, J.C., 2005. The effect of pinealectomy on scoliosis development in young nonhuman primates. Spine 30 (18), 2009–2013.

Cheung, C.S., Lee, W.T., Tse, Y.K., Lee, K.M., Guo, X., Qin, L., Cheng, J.C., 2006. Generalized osteopenia in adolescent idiopathic scoliosis-association with abnormal pubertal growth, bone turnover, and calcium intake? Spine 31, 330–338.

Cossins, A.R., Crawford, D.L., 2005. Fish as models for environmental genomics. Nat. Rev., Genet. 6, 324–333.

Coucke, P., Van Acker, P., De Paepe, A., 2006. Mutation analysis of the FBN1 gene in patients with Marfan syndrome. Methods Mol. Med. 126, 81–95.

Cowell, H.R., Hall, J.N., MacEwen, G.D., 1972. Genetic aspects of idiopathic scoliosis, a Nicholas Andry Award Essay, 1970. Clin. Orthop. 86, 121–131.

Crollius, H.R., Weissenbach, J., 2005. Fish genomics and biology. Genome Res. 15, 1675–1682.

Damborg, F., Engell, V., Andersen, M., Kyvik, K.O., Thomsen, K., 2006. Prevalence, concordance, and heritability of Scheuermann kyphosis based on a study of twins. J. Bone Jt. Surg., Am. Vol. 88, 2133–2136.

Danzmann, R.G., Gharbi, K., 2001. Gene mapping in fishes, a means to an end. Genetica 111, 3–23.

Dickson, R.A., 2004. Spinal deformity-basic principles. Curr. Orthop. 18, 411–425.

Escalada, F., Marco, E., Duarte, E., Muniesa, J., Belmonte, R., Tejero, M., Caceres, E., 2005. Growth and curve stabilization in girls with adolescent idiopathic scoliosis. Spine 30 (4), 411–417.

Fazal, M.A., Edgar, M., 2006. Detection of adolescent idiopathic scoliosis. Acta Orthop. Belg. 72, 184–186.

Fisher, R.L., De George, F.V., 1967. Idiopathic scoliosis, an investigation of genetic and environmental factors. J. Bone Jt. Surg. 49, 1005.

Fjelldal, P.G., Grotmol, S., Kryvi, H., Gjerdet, N.R., Taranger, G.L., Hansen, T., Porter, M.J., Totland, G.K., 2004. Pinealectomy induces malformation of the spine and reduces the mechanical strength of the vertebrae in Atlantic salmon, *Salmo salar*. J. Pineal Res. 36, 132–139.

Gajewski, M., Elmasri, H., Girschick, Sieger, D., Winkler, C., 2006. Comparative analysis of *her* genes during fish somitogenesis suggests a mouse/chick-like mode of oscillation in medaka. Dev. Genes Evol. 216, 315–332.

Ganey, T.M., Ogden, J.A., 2001. Development and maturation of the axial skeleton, In: Weinstein, S.L. (Ed.), The Pediatric Spine, Principles and Practice, 2nd ed. Lippincott Williams & Wilkins, Philadelphia, pp. 3–54.

Garland, H.G., 1934. Hereditary scoliosis. Br. Med. J. 1, 328.

Goodrich, H.B., Harrison, R.W., Josephson, Trinkhaus, 1934. Three genes in *Lebistes reticulates*. Genetics 28, 75.

Gordon, M., 1934. Hunchback; a story about a platyfish. Aquarium 162–164 (Nov.).

Gorman, K.F., Tredwell, S.J., Breden, F., in press. The mutant guppy syndrome *curveback* as a model for human heritable spinal curvature. Spine.

Green, R.J.L., Webb, J.N., Maxwell, M.H., 1979. The nature of virus-like particles in the paraxial muscles of idiopathic scoliosis. J. Pathol. 129, 9–12.

Guo, X., Chau, W.W., Chan, Y.L., Cheng, J.C., 2003. Relative anterior spinal overgrowth in adolescent idiopathic scoliosis. Results of disproportionate endochondral-membranous bone growth. J. Bone Jt. Surg. 85B, 1026–1031.

Harrington, P.R., 1977. The etiology of idiopathic scoliosis. Clin. Orthop. 126, 17–25.

Harrison, R.W., 1941. A contribution to the genetics of *Lebistes reticulatus* the nature and inheritance of 'Hunchback'. Thesis, Wesleyan University, Connecticut, USA.

Inoue, M., Minami, S., Nakata, Y., Takaso, M., Otsuka, Y., Kitahara, H., Isobe, K., Kotani, T., Maruta, T., Moriya, H., 2002. Prediction of curve progression in idiopathic scoliosis from gene polymorphic analysis. Stud. Health Technol. Inform. 91, 90–96.

James, J.I.P., 1954. Idiopathic scoliosis. The prognosis, diagnosis, and operative indications related to curve patterns and the age of onset. J. Bone Jt. Surg., Am. Vol. 36-B (1), 36–49.

Justice, C.M., Miller, N.H., Marosy, B., Zhang, J., Wilson, A.F., 2003. Familial idiopathic scoliosis, evidence of an X-linked susceptibility locus. Spine 28, 589–594.

Kawakami, N., Deguchi, M., Kanemura, T., 1999. Animal models of scoliosis. In: An, Y.H., Friedman, R.J. (Eds.), Animal Models in Orthopaedic Research. CRC Press, pp. 549–564.

Kesling, K., Reinker, K.A., 1997. Scoliosis in twins. Spine 22, 2009–2015.

Kumar, S.J., Guille, J.T., 2001. Marfan syndrome, In: Weinstein, S.L. (Ed.), The Pediatric Spine, Principles and Practice, 2nd ed. Lippincott Williams & Wilkins, Philadelphia, pp. 505–516.

Kurosawa, G., Takamatsu, N., Takahashi, M., Sumitomo, M., Sanaka, E., Yamada, K., Nishii, K., Matsuda, M., Asakawa, S., Ishiguro, H., Miura, K., Kurosawa, Y., Shimizu, N., Kohara, Y., Hori, H., 2006. Organization and structure of hox gene loci in medaka genome and comparison with those of pufferfish and zebrafish genomes. Gene 370, 75–82.

Labelle, H., 2001. Spinal deformities in Friedreich's ataxia, In: Weinstein, S.L. (Ed.), The Pediatric Spine, Principles and Practice, 2nd ed. Lippincott Williams & Wilkins, Philadelphia, pp. 809–818.

Lodi, E., 1978. Palla, A hereditary vertebral deformity in the guppy, *Poecilia reticulata* Peters, Pisces, Osteichthyes. Genetica 48, 197–200.

Lonstein, J.E., 1995. Idiopathic scoliosis, In: Lonstein, J.E., Bradfud, D.S., Winter, R.B., Ogilive, J.W. (Eds.), Moe's Textbook of Scoliosis and Other Spinal Deformities, 3rd ed. WB Saunders, Philadelphia, pp. 219–256.

Machida, M., Murai, I., Miyashita, Y., Dubousset, J., Yamada, T., Kimura, J., 1999. Pathogenesis of idiopathic scoliosis-experimental study in rats. Spine 24, 1985–1989.

Machida, M., Dubousset, J., Yamada, T., Kimura, J., Saito, M., Shiraishi, T., Yamagishi, M., 2006. Experimental scoliosis in melatonin-deficient C57BL/6J mice without pinealectomy. J. Pineal Res. 41, 1–7.

Mack, J.D., Greenberg, R.A., 1990. Review of scoliosis research at the University of Michigan. Perspectives 2 (www.curedisease.com).

Marosy, B., Justice, C.M., Nneka, N., et al., 2006. Lack of association between the aggrecan gene and familial idiopathic scoliosis. Spine 31, 1420–1425.

Marquet, E., Sobel, H.J., 1969. Crystalline inclusions in the nuclear envelope and granular endoplasmic reticulum of the fish spinal cord. J. Cell Biol. 41, 774–785.

Mayer, I., 2000. Effect of long-term pinealectomy on growth and precocious maturation in Atlantic salmon, *Salmo salar parr*. Aquat. Living Resour. 13, 139–144.

Miller, N.H., Mims, B., Child, A., Milewicz, D.M., Sponseller, P., Blanton, S.H., 1996. Genetic analysis of structural elastic fiber and collagen genes in familial adolescent idiopathic scoliosis. J. Orthop. Res. 14, 994–999.

Miller, N.H., Justice, C.M., Marosy, B., Doheny, K.F., Pugh, E., Zhang, J., Dietz III, H.C., Wilson, A.F., 2005. Identification of candidate regions for familial idiopathic scoliosis. Spine 30, 1181–1187.

Miller, N.H., Marosy, B., Justice, C.M., Novak, S.M., Tang, E.Y., Boyce, P., Pettengil, J., Doheny, K.F., Pugh, E.W., Wilson, A.F., 2006. Linkage analysis of genetic loci for kyphoscoliosis on chromosomes 5p13, 13q13.3, and 13q32. Am. J. Med. Genet., A 140A, 1059–1068.

Millner, P.A., Dickson, R.A., 1996. Idiopathic scoliosis, biomechanics and biology. Eur. Spine J. 5, 362–373.

Naique, S.B., Porter, R., Cunningham, A.A., Hughes, S.P., Sanghera, B., Amis, A.A., 2003. Scoliosis in an Orangutan. Spine 28, E143–E145.

Ogawa, N.M., 1965. A phylogenetic study of the vertebral fused (f) in the medaka, *Oryzias latipes*. Embryologia 9, 13–33.

Ogilvie, J.W., Braun, J., Argyle, V., Nelson, L., Meade, M., Ward, K., 2006. The search for idiopathic scoliosis genes. Spine 31, 679–681.

Ohtsuka, M., Kikuchi, N., Yokoi, H., Kinoshita, M., Wakamatsu, Y., Ozato, K., Takeda, H., Inoko, H., Kimura, M., 2004. Possible roles of zic1 and zic4, identified within the medaka Double anal fin (Da) locus, in dorsoventral patterning of the trunk-tail region (related to phenotypes of the Da mutant). Mech. Dev. 121, 873–882.

O'Kelly, C., Wang, X., Raso, J., Moreau, M., Mahood, J., Zhao, J., Bagnall, K., 1999. The production of scoliosis after pinealectomy in young chickens, rats, and hamsters. Spine 24, 35–43.

Okubo, K., Sakai, F., Lau, E.L., Yoshizaki, G., Takeuchi, Y., Naruse, K., Aida, K., Nagahama, Y., 2006. Forebrain gonadotropin-releasing hormone neuronal development: insights from transgenic medaka and the relevance to X-linked Kallmann syndrome. Endocrinology 147, 1076–1084.

Pandolfo, M., 2006. Friedreich ataxia: detection of GAA repeat expansions and frataxin point mutations. Methods Mol. Med. 126, 197–216.

Perdriolle, R., Vidal, J., 1987. Morphology of scoliosis, three-dimensional evolution. Orthopaedics 10, 909–915.

Prince, V.E., Pickett, F.B., 2002. Splitting pairs, the diverging fates of duplicated genes. Nature 3, 827–837.

Plugfelder, O., 1953. Wirkung der epiphysektomie auf die postembryonalentwicklung von *Lebistes reticulata* (Peters). Wilhelm Roux' Arch. Entwickl. mech. Org. 146, 115–136.

Reamy, B.V., Slakey, J.B., 2001. Adolescents idiopathic scoliosis, review and current concepts. Am. Fam. Phys. 64, 111–116.

Renn, J., Schaedel, M., Volff, J.-N., et al., 2006. Dynamic expression of *sparc* precedes formation of skeletal elements in the medaka (*Oryyzias latipes*). Gene 372, 208–218.

Riggins, R.S., Abbott, U.K., Ashmore, C.R., Rucker, R.B., McCarrey, J.R., 1977. Scoliosis in chickens. J. Bone Jt. Surg. 59, 1020–1026.

Riseborough, E.J., Wynne-Davis, R., 1973. A genetic survey of idiopathic scoliosis in Boston, Massachusetts. J. Bone Jt. Surg. 55A, 974–982.

Roaf, R., 1966. The basic anatomy of scoliosis. J. Bone Joint Surg. 48B, 786–792.

Robinson, C.M., McMaster, M.J., 1996. Juvenile idiopathic scoliosis. Curve patterns and prognosis in one hundred and nine patients. J. Bone Jt. Surg., Am. Vol. 78, 1140–1148.

Rosenthal, H.L., 1951. Studies on semi-lethalism in the male lordotic guppy *Lebistes reticulates*. J. Heredity 42, 257–259.

Rosenthal, H.L., 1953. Embryonic development of the lordotic and normal guppy, *Lebistes reticulates* (Peters). Biol. Bull. 105, 160–165.

Rosenthal, H.L., 1954. Composition of bone and muscle from the lordotic and normal guppy, *Lebistes reticulates*. Nature 173, 693.

Rosenthal, H.L., 1958. Comparative uptake of calcium-45 and strontium-90 by wild and lordotic guppies (23826). Proc. Soc. Exp. Biol. Med. 97, 624–627.

Rosenthal, H.L., Rosenthal, R.S., 1950. Lordosis, a mutation in the guppy *Lebistes reticulates*. J. Heredity 41, 217–218.

Rosenthal, H.L., Myers, P.R., Brunings, M.K., 1958. Spinal curvature, a mutation in the swordtail, *Xiphophorus*. J. Heredity 49, 238–242.

Ruble, R., Silverman, S., Pisenti, J., Wakenell, P., 2002. Amelia/ectromelia in association with scoliosis in three commercial layer hens, *Gallus gallus* forma domestica. Avian Pathol. 31, 429–433.

Salehi, L.B., Mangino, M., De Serio, S., De Cicco, D., Capon, F., Semprini, S., Pizzuti, A., Novelli, G., Dallapiccola, B., 2002. Assignment of a locus for autosomal dominant idiopathic scoliosis, IS, to human chromosome 17p11. Hum. Genet. 11, 401–404.

Sato, A., Figueroa, F., Murray, B.W., Malaga-Trillo, E., Zaleska-Rutcznska, Z., Sultmann, H., Toyosawa, S., Wedekind, C., Steck, N., Klein, J., 2000. Nonlinkage of major histocompatibility complex class I and classII loci in bony fishes. Immunogenetics 51, 108–116.

Schartl, M., 2004. A comparative view on sex determination in Medaka. Mech. Dev. 121, 639–645.

Schultz, R.J., 1963. Stubby, a hereditary vertebral deformity in the viviparous fish *Poeciliopsis prolifica*. Copeia 2, 325–330.

Schultz, A.B., Sorensen, S.E., Anderson, G.B.J., 1984. Measurements of spine morphology in children, ages 10–16. Spine 9, 70–73.

Sharipov, R.N., Zaidman, A.M., Zorkol'tseva, I.V., Aksenovich, T.I., Dymshits, G.M., 2006. Polymorphism of the aggregan gene in families with idiopathic scoliosis. Mol. Biol. 40, 493–495.

Stirling, A.J., Howel, D., Millner, P.A., Sadiq, S., Sharples, D., Dickson, R.A., 1996. Late-onset idiopathic scoliosis in children six to fourteen years old. A cross-sectional prevalence study. J. Bone Jt. Surg., Am. Vol. 78-A, 1330–1336.

Stokes, I.A.F., Windisch, L., 2006. Vertebral height growth predominates over intervertebral disc height growth in adolescents with scoliosis. Spine 31, 1600–1604.

Stokes, I.A.F., Aronsson, D.D., Dimock, A.N., Cortright, V., Beck, S., 2006. Endochondral growth in plates of three species at two anatomical locations modulated by mechanical compression and tension. J. Orthop. Res. 24, 1327–1334.

Takeuchi, K., 1960. Study of the mutant, wavy, in the medaka, *Oryzias latipes*. Annot. Zool. Jpn. 33, 124–131.
Terminology Committee of the Scoliosis Research Society, 1976. A glossary of scoliosis terms. Spine 1, 57–58.
Wagner, T.U., Renn, J., Riemensperger, T., 2003. The teleosts fish medaka (*Oryzias latipes*) as genetic model to study gravity dependent bone homeostasis *in vivo*. Adv. Space Res. 32, 1459–1465.
Webb, J.N., Gillespie, W.J., 1976. Virus-like particles in paraspinal muscle in scoliosis. Br. Med. J. 2, 912–913.
Winge, O., 1927. The location of eighteen genes in *Lebistes reticulatus*. J. Genet. 18, 1–43.
Wittbrodt, J., Shima, A., Schartl, M., 2002. Medaka—a model organism from the Far East. Nat. Genet. 3, 53–64.
Wu, J., Qiu, Y., Zhang, L., Sun, Q., Qiu, X., He, Y., et al., 2006. Association of estrogen receptor gene polymorphisms with susceptibility to adolescent idiopathic scoliosis. Spine 31, 1131–1136.
Wynne-Davis, R., 1968. Familial, idiopathic scoliosis. A family survey. J. Bone Jt. Surg. 50B, 24–30.
Yasutake, J., Inohaya, K., Kudo, A., 2004. Twist functions in vertebral column formation in medaka, *Oryzias latipes*. Mech. Dev. 121, 883–894.

Further reading

Afonso, J.M., Montero, D., Robaina, L., Astorga, N., Izquierdo, M.S., Gines, R., 2000. Association of a lordosis–scoliosis–kypohsis deformity in gilthead seabream (*Sparus aurata*) with family structure. Fish Physiol. Biochem. 22, 159–163.
Akiyama, T., Murai, T., Mori, K., 1986. Role of tryptophan metabolites in inhibition of spinal deformity of chum salmon fry caused by tryptophan deficiency. Bull. Jpn. Soc. Sci. Fish. 52, 1255–1259.
Bengtsson, A., 1991. Effects of bleached pulp mill effluents on vertebral defects in fourhorn sculpin, *Myoxocephalus quadricornis* L. in the Gulf of Bothnia. Arch. Hydrobiol. 121, 373–384.
Britz, R., Johnson, G.D., 2006. Occipito-vertebral fusion in ocean sunfishes (Teleostei: Tetraodontiformes: Molidae) and its phylogenetic implications. J. Morphol. 266, 74–79.
Cahu, C., Zambonino, J., Takeuchi, T., 2003. Nutritional components affecting skeletal developmental in fish larvae. Aquaculture 227, 245–258.
Couch, J.A., Winstead, J.T., Hansen, D.J., Goodman, L.R., 1979. Vertebral dysplasia in young fish exposed to the herbicide trifluralin. J. Fish Dis. 2, 35–42.
DeVore, P.W., Eaton, J.G., 1983. An investigation of spinal deformity of trout, *Salmo sp*. in the Brule river, Wisconsin. J. Great Lakes Res. 9, 69–73.
Ellis, S.G., 2000. Characterization of skeletal deformities in three species of juvenile fish from the Willamette River Basin. Willamette River Basin Studies, Ecological Health Technical Study. EVS Environment Consultants, Inc., Seattle, WA. 50 pp.
Fraser, M.R., Anderson, T.A., de Nys, R., 2004. Ontogenic development of the spine and spinal deformities in larval barramundi, *Lates calcarifer*. culture. Aquaculture 242, 697–711.
Gjerde, B., Pante, M.J.R., Baeverfjord, G., 2005. Genetic variation for a vertebral deformity in Atlantic salmon, *Salmo salar*. Aquaculture 244, 77–87.
Grotmol, S., Kryvi, H., Totland, G.K., 2005. Deformation of the notochord by pressure from the swim bladder may cause malformation of the vertebral column in cultured Atlantic cod *Gadus morhua* larvae, a case study. Dis. Aquat. Org. 65, 121–128.
Halver, J.E., 1972. The role of ascorbic acid in fish disease and tissue repair. Bull. Jpn. Soc. Sci. Fish. 38, 79–92.
Halver, J.E., Ashlet, L.M., Smith, R.M., 1969. Ascorbic acid requirements of coho salmon and rainbow trout. Trans. Am. Fish. Soc. 98, 762–771.
Harris, K.C., Hulsman, P.F., 1991. Intensive culture of lake whitefish, *Coregonus clupeaformis*, from larvae to yearling size using dry feeds. Aquaculture 96, 255–268.
Hisaoka, K.K., 1957. Lordoscoliosis in the tropical fish *Panchax lineatus*. J. Heredity 48, 251–252.
Hiraoka, Y., Okuda, H., 1983. Characteristics of vertebral abnormalities of medaka as a water pollution indicator. Hiroshima J. Med. Sci. 32, 261–266.
Holm, J., Palace, V.P., Wautier, K., Evans, R.E., Baron, C.L., Podemski, C., Siwik, P., Sterling, G., 2003. An assessment of the development and survival of wild rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*) exposed to elevated selenium in an area of active coal mining. In: Browman, H.I., Skiftesvik, A.B. (Eds.), The Big Fish Bang. Proceedings of the 26th Annual Larval Fish Conference. Inst. of Marine Research, Bergen, Norway, pp. 257–273.
Koenig, J., 1984. Importance of vitamin C in ichthyophysiology and practice of pisciculture. Ichthyophysiol. Acta 8, 41–57.
Koumoundouros, G., Maingot, E., Divanach, P., Kentouri, M., 2002. Kyphosis in reared sea bass (*Dicentrarchus labrax* L.): ontogeny and effects on mortality. Aquaculture 209, 49–58.
Kranenbarg, S., Waarsing, J.H., Muller, M., Weinans, H., van Leeuwen, J.L., 2005. Lodrotic vertebrae in sea bass (*Dicentrarchus labrax* L.) are adapted to increased loads. J. Biomech. 38, 1239–1246.
Lam, T.J., 1990. Technology development in aquaculture, new ideas and approaches. In: Ming, C.L., Ng, P.K.L. (Eds.), Essays in Zoology. National University of Singapore Press, Singapore, pp. 357–370.
Langdon, J.S., 1988. Investigation of fish kills. In: Bryden, D.I. (Ed.), Fish Diseases-Refresher Course for Veterinarians-Proceedings, vol. 106. The University of Sydney, Australia, pp. 167–223.
Lewis, L.M., Lall, S.P., 2006. Development of the axial skeleton and skeletal abnormalities of Atlantic Halibut (*Hippoglossus hippoglossus*) from first feeding through metamorphosis. Aquaculture 257, 124–135.
Lewis, L.M., Lall, S.P., Witten, P.E., 2004. Morphological descriptions of the early stages of spine and vertebral development in hatchery-reared larval and juvenile Atlantic halibut (*Hippoglossus hippoglossus*). Aquaculture 241, 47–59.
Lovell, R.T., 1973. Essentiality of vitamin C in feeds for intensively fed caged channel catfish. J. Nutr. 103, 134–138.
McKay, L., Gjerda, B., 1986. Genetic variation for a spinal deformity in Atlantic Salmon, *Salmo salar*. Aquaculture 52, 263–272.
Oyen, F.G.G., Camps, L.E., Wendelaae-Bonga, S.E., 1991. Effect of acid stress on the embryonic development of the common carp (*Cyprinus hippurus*). Aquat. Toxicol. 19, 1–12.
Polo, A., Yufera, M., Pascual, E., 1991. Effects of temperature on egg and larval development of *Sparus aurata* L. Aquaculture 92, 367–375.
Post, G.W., 1933. Nutrition and nutritional diseases of salmonids. In: Stoskopf, M. (Ed.), Fish Medicine. Saunders, Philadelphia, pp. 343–357.
Roberts, R.J., 1989. Fish Pathology, 2nd edn. Balliere Tindall, London. 467 pp.
Sakaguchi, S., Hara, T., Matsusato, T., Yamagata, Y., Kawai, H., Maeno, Y., 1987. Scoliosis of cultured yellowtail caused by parasitic *Myxobolus buri*. Bull. Natl. Res. Inst. Aquacult. Jpn./Yoshokukenho 12, 79–86.
Schroder, H., 1969. Inheritance of radiation-induced spinal curvature in the guppy *Lebistes reticulates*. Can. J. Genet. Cytol. 11, 937–947.
Sen, G., Behera, M.K., Patel, P.N., 1991. Toxicity of zinc to the fish *Channa punctatus* Bloch. with behavioral, morphological and skeletal abnormalities. Environ. Ecol. 9, 1023–1027.
Sfakianakis, D.G., Koumoundouros, G., Divanach, P., Kentouri, M., 2003. Development of saddleback-like syndrome in reared white seabream *Diplodus sargus* (Linnaeus, 1758). Aquaculture 217, 673–676.
Shikano, T., Ando, D., Taniguchi, T., 2005. Relationships of vertebral deformity with genetic variation and heterosis in the guppy *Poecilia reticulata*. Aquaculture 246, 133–138.
Shrestha, T.K., 1985. Further observations on vertebral deformity in *Heteropneustes fossilis* (Bloch). MATSYA—Bull. Indian Soc. Ichtyol. 11, 82–84.
Silverstone, A.M., Hammell, L., 2002. Spinal deformities in farmed Atlantic salmon. Can. Vet. J. 43, 782–784.
Thompson, K.G., Nehring, R.B., Bowden, D.C., Wygant, T., 2002. Response of rainbow trout *Oncorhynchus mykiss* to exposure to *Myxoboluscerebralis* above and below a point source of infectivity in the upper Colorado River. Dis. Aquat. Org. 49, 171–178.
Urushitani, H., Shimizp, A., Katsu, Y., Iguchi, T., 2002. Early estrogen exposure induces abnormal development of *Fundulus heteroclitus*. J. Exp. Zool. 293, 693–702.
Wargelius, A., Fjelldal, P.G., Hansen, T., 2005. Heat shock during early somitogenesis induces caudal vertebral column defects in Atlantic salmon (*Salmo salar*). Dev. Genes Evol. 215, 350–357.

Witten, P.E., Gil-Martens, L., Hall, B.K., Huysseunne, A., Obach, A., 2005. Compressed vertebrae in Atlantic salmon (*Salmo salar*) evidence for metaplastic chondrogenesis as a skeletogenic response late in ontogeny. Dis. Aquat. Org. 64, 237–246.

Witten, P.e., Obach, A., Huysseune, A., Baeverfjord, G., 2006. Vertebrae fusion in Atlantic salmon (*Salmo salarl*): development, aggravation and pathways of containment. Aquaculture 258, 164–172.

Wunder, W., Schmeller, H.-B., 1988. Deformations of the spinal cord in one-year-old *Salvelinus fontinalis* caused by aluminum in acid water. Fisch. Teichwirt 39, 178–179.

Yamamoto, T., Tomita, H., Matsuda, N., 1962. Hereditary and nonheritable vertebral anchylosis in the medaka, *Oryzias latipes*. Jpn. J. Genet. 38, 36–47.

Yokoyama, H., Freeman, M.A., Itoh, N., Fukuda, Y., 2005. Spinal curvature of cultured Japanese mackerel *Scomber japonicus* associated with a brain myxosporean, *Myxobolus acanthogobii*. Dis. Aquat. Org. 66, 1–7.

Zitsow, R.E., Millard, J.L., 1988. Survival and growth of lake whitefish, *Coregonus clupeaformis*, larvae fed only formulated dry diets. Aquaculture 69, 105–113.