Age-related Degeneration of the Intervertebral Disc in the African Killifish N. furzeri

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Introduction: The African Killifish Nothobranchius furzeri is the shortest-lived vertebrate that can be cultured in captivity, with age-spans ranging from 3-9 months [1]. As an emerging model for rapid senescence, the N. furzeri exhibits age-related degenerative changes such as lesions and neoplasias in the liver, kidney, heart, and gonads [1]. Furthermore, the longevity of N. furzeri is responsive to environmental stimuli [2] and dietary restriction [3]. State-of-the-art genetic tools and approaches have been developed to directly manipulate the N. furzeri genome, making it further suitable to investigate the molecular level changes that occur with aging [4]. Yet among the age-related changes that occur in the N. furzeri, the musculoskeletal phenotype has been uncharacterized. In particular, we hypothesized that the N. furzeri may be susceptible to degenerative changes in the intervertebral disc.

Methods: The N. Furzeri strain ZMZ1005, which reaches sexual maturation within 4 weeks, was used in this study. The ZMZ1005 survival curve drops at 15 weeks of age with no or few natural deaths before week 15 [4]. The N. furzeri were housed at 25°C in a central filtration recirculating system with a 12 hr light/dark cycle as previously described and fed twice a day with freeze-dried bloodworms until they achieve the desired age for the investigation. 7 fish were collected at ages of 6, 8, 12, 22, 23, 25, and 26 weeks old. As with other fish species, the N. furzeri do not have true intervertebral discs, but rather spinal intervertebral joints (IVJ). Thus the analyses are conducted on the IVJ. X-ray microtomography was used to analyze the IVJ using metrics analogous to clinical assessment of IVD degeneration. Fish were fixed in 70% ethanol, cast in 2% agarose, and subsequently scanned at 12μm resolution (μCT 40, Scanco Medical AG). Spine curvature was determined using a technique similar to the determination of Cobb angle. In contrast to clinical assessment, which only quantifies the curvature at a specific site the angle for the fish was examined at each vertebral level using the last caudal vertebrae with ribs as a reference. Cobb angle was determined along the sagittal plane, which presented the most pronounced curvature. IVJ spacing was determined for all 12 vertebrae by measuring the distance between vertebrae in triplicate and averaging for each level. To reduce errors in the geometric measurements due to out of plane curvature a 3d Bezier path was traced through the midpoint of the each vertebra body and μCT slices were then rectified about 2 or 3 planes for the Cobb angle and IVJ spacing measurements, respectively. All measurements were performed using Osirix DICOM Viewer (Osirix v.5.5.2, open source).

Results: With natural aging, the N. furzeri exhibit a ventral-dorsal kyphosis that occurs progressively in fish older than 20 weeks old examined (Figure 1A). MicroCT analyses reveal that the cause of this increasing curvature is caused by the narrowing of the intervertebral joint, in some cases resulting a total collapse of this space (Figure 1B). Measurement of the IVJ curvature by Cobb angle analyses reveals a significant decrease that is strongly correlated with increasing age (Figure 1C, p < 0.001; R² = 0.63). Moreover, the IVJ spacing is also significantly reduced with age (Figure 1D, p < 0.001; R² = 0.47).

Discussion: Musculoskeletal disorders are among the most prevalent afflictions in the elderly population and pose a significant public health burden. Hence, musculoskeletal phenotypes should be an important part assessing animal models of aging. Here we observed that N. furzeri exhibits an intervertebral joint phenotype as a part of their rapid aging. As etiology of disc degeneration is multifactorial and may include genetic, metabolic, and mechanical factors [5], the aquatic environment that fish live in reduces mechanical loading on the spine, and may allow future investigations to decouple the genetic and mechanical interactions that contribute to degeneration of the disc. The use of the N. furzeri may be a promising model for understanding the conserved mechanisms of intervertebral disc degenerations in humans.

Significance: The accelerated senescence fish, N. furzeri, exhibits degenerative changes in the intervertebral joint. Given its ease of genetic manipulations and the naturally occurring degenerative changes, the N. furzeri may serve as a potential model organism for age-related intervertebral disc degeneration and allow the investigation of conserved molecular pathways that regulate tissue stability and how they're altered with aging and disease. Ultimately, the N furzeri may provide high throughput screening of potential biological therapeutics for intervertebral disc degeneration.

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Fig 1: The African Killfish N. furzeri exhibits an age-dependent dorsal-ventral kyphosis of the spine (A). MicroCT imaging of the N. furzeri spine reveals that the kyphosis is a result of wedging and a reduction of the spacing – and sometimes total collapse – of the intervertebral joint (IVJ) (B). Quantitative measurements of the spine show a significant (p<0.001) increase in the Cobb angle and a significant reduction of IVJ spacing (p<0.001) that are strongly correlated with increasing age (C, D).

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