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Case Report

Histopathology of a wavy medaka

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Abstract: Wavy medakas are medakas that exhibit spinal curvature characterized by dorsoventrally curved vertebrae. We found a spontaneous wavy medaka in our experimental stock and subjected it to a histopathological examination. Macroscopically, the wavy medaka's spine formed an M shape, and its vertebrae displayed a dorsoventral curvature that started at the third vertebral bone. Microscopically, the vertebral cavities were filled with fibrous tissue, which was similar to that seen in the central parts of the intervertebral discs of a normal medaka. The vertebral joints were composed of vacuolated notochord cells without intervertebral disc formation. These changes were also observed in the caudal region, which exhibited less curvature. In the normal medaka, the intervertebral discs form via the regression of the notochord that plays a key role in the development of vertebrae and disc formation. We concluded that notochordal subinvolution had induced intervertebral disc dysplasia, leading to lordokyphosis, in the wavy medaka. (DOI: 10.1293/tox.2015-0070; J Toxicol Pathol 2016; 29: 115–118)

Key words: intervertebral disc, lordokyphosis, notochord, spinal curvature, wavy medaka

Spinal curvature is occasionally observed in various vertebrate species, such as humans, mice, chickens, and teleost fish, etc[1–3]. In teleost fish, spinal curvature is a model of human spinal disease or a factor of decline in the commercial value of such fish[4, 5]. Many previous reports have described the occurrence of spinal curvature in various species of farmed fish and small laboratory fish, such as the sea bream, catfish, medaka and guppy[5–8]. It is known that in fish spinal curvature can be associated with genetic factors. For instance, when medakas that exhibited spinal curvature were repeatedly crossed, 97.8% of the resultant offspring had curved spines after the third generation[9]. The spinal curvature was characterized by vertebrae that curved dorsoventrally around the body axis, producing a wavy shape. Thus, these fish were called "wavy medaka"[1]. The gross appearance and inheritance pattern of the wavy medaka are similar to those seen in the guppy and swordtail fish with curved spines[1]. As far as we know, no previous reports have described the histopathological findings of wavy medakas, although a guppy with spinal curvature was subjected to a histopathological examination in a previous study. We encountered a spontaneous wavy medaka in the stock of fish that we use for experiments. This article describes the histopathological findings of this wavy medaka.

The wavy medaka examined in this study was a non-chemically treated adult male. We found this wavy medaka at three months old and conducted a macroscopic analysis of it at four months old. Except for its unusual morphological appearance, the fish did not exhibit any abnormal clinical signs. Macroscopically, the vertebrae displayed a dorsoventral curvature that started at the third vertebral bone (Fig. 1). In addition, the spine exhibited three curves, i.e., one lordotic and two kyphotic curves, producing an M-like shape. Therefore, the fish's spinal curvature was diagnosed as "lordokyphosis".

The wavy medaka was euthanized using CO_2 gas and fixed *in toto* in Bouin's fluid overnight before being refixed in neutral buffered formalin, embedded in paraffin, cut into 4 μm thick sagittal sections, and routinely stained with hematoxylin-eosin (HE). As a control, a normal male medaka specimen at three months old was prepared.

The histological structure of the normal adult medaka's vertebrae was as follows. The vertebral cavities of the vertebral bones had an acellular structure (Fig. 2A). As for the vertebral joints, the intervertebral discs were divided into fibrous central tissue and spongy peripheral tissue (Fig. 2B). Furthermore, the spongy tissue consisted of regressing vacuolated notochord cells. The intervertebral ligament was composed of a three-layered structure that included the notochordal sheath, the elastic externa, and the extra elastica (Fig. 2C). The elastic externa of the intervertebral ligament was observed at the surface of the vertebral joint as a homo-

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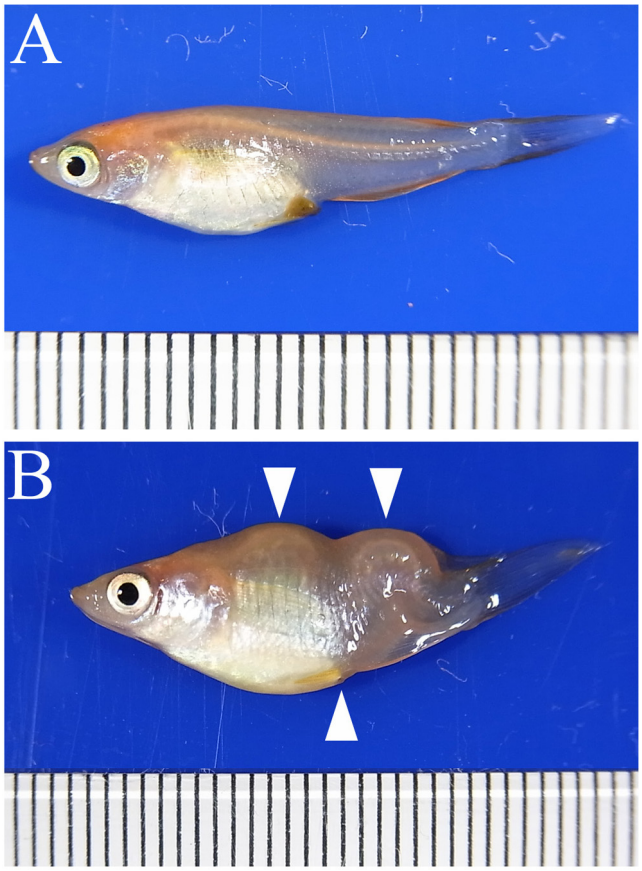

Fig. 1. Gross appearances of the normal and wavy medakas. A. Normal adult medaka. B. Wavy medaka; the anterior, abdominal, and posterior sections of the fish's spine were curved, producing an M shape (arrowhead).

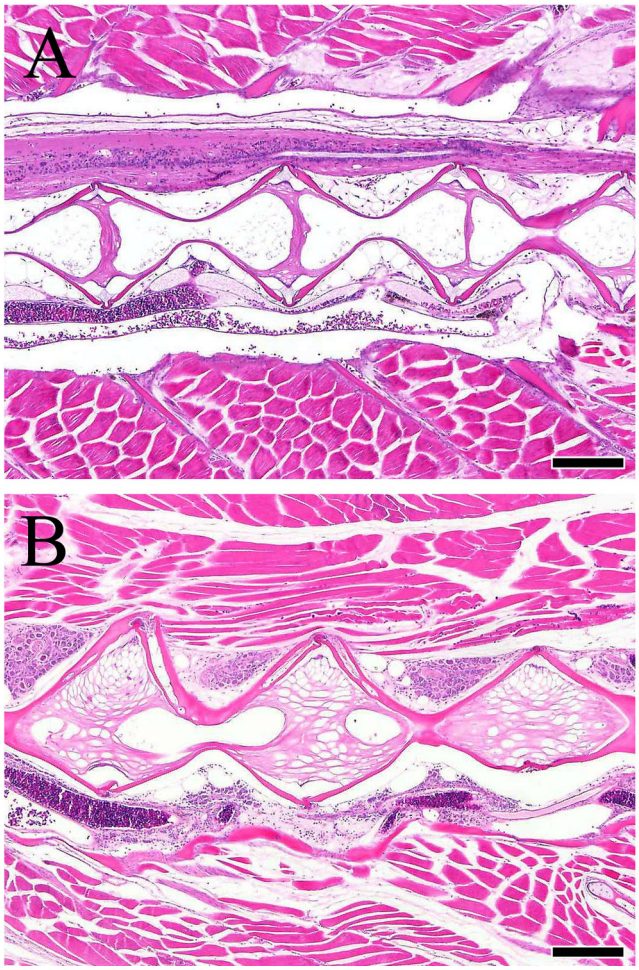

Fig. 3. Histology of the caudal region. A. Caudal vertebral bones of the normal medaka (HE stain, bar = 200 μm); B. Caudal vertebral bones of the wavy medaka; caudal vertebral columns filled with vacuolated notochord cells. Muscle fibers showed a random alignment and were irregular in size (HE stain, bar = 200 μm).

geneous structureless acidophilic tissue with an arch-like shape.

The wavy medaka's vertebrae were curved and deformed without broken bones (Fig. 2D). The wavy medaka's vertebral joints were composed of vacuolated notochord cells, which were similar to the spongy tissue found in the vertebral joints of the normal medaka (Fig. 2E). Therefore, no intervertebral disc formation was seen in the wavy medaka's vertebral joints. The vertebral cavities were filled with fibrous tissue, which was similar to that seen in the central parts of the normal medaka's intervertebral discs. The cortical bone at the edges of its vertebrae had thickened, and these changes were particularly marked on the convex side of each spinal curve (Fig. 2F). In addition, the anterior and posterior vertebral bones of the wavy medaka were fused, and the vestigial intervertebral ligaments remained on the convex side of each spinal curve. There was no hemorrhage, inflammation, or injuries of the spinal cord affected by lordokyphosis, although focal edema was observed in the curved portion of the spinal cord (Fig. 2D). These changes were observed in all vertebrae including the caudal vertebrae, which exhibited less marked curvature than that observed in the abdominal region (Fig. 3). The muscle fibers around these vertebrae demonstrated a random alignment and were irregular in size (Fig. 3).

It is known that spinal curvature can be induced in fishes by hereditary or acquired disorders[4]. In hereditary spinal curvature, the vertebrae usually exhibit multiple curves, the gross appearances of which are simillar among different fish species[5–8]. In guppies, hereditary spinal curvature is already detectable in newly hatched fry, and the degree of curvature gradually increases without causing any bone breakage or spinal injuries during growth[5, 10]. The present wavy medaka had a similar gross appearance to guppies. However, guppies had intervertebral discs that exhibited fibrous tissue in their central regions and spongy tissue in their peripheral regions, although their spongy tissue was compressed and distended due to displacement by the fibrous tissue. As for acquired spinal curvature, a previous fish toxicity study showed organophosphate-induced spinal curvature (producing nonspecific clinical symptoms) in adult medakas and

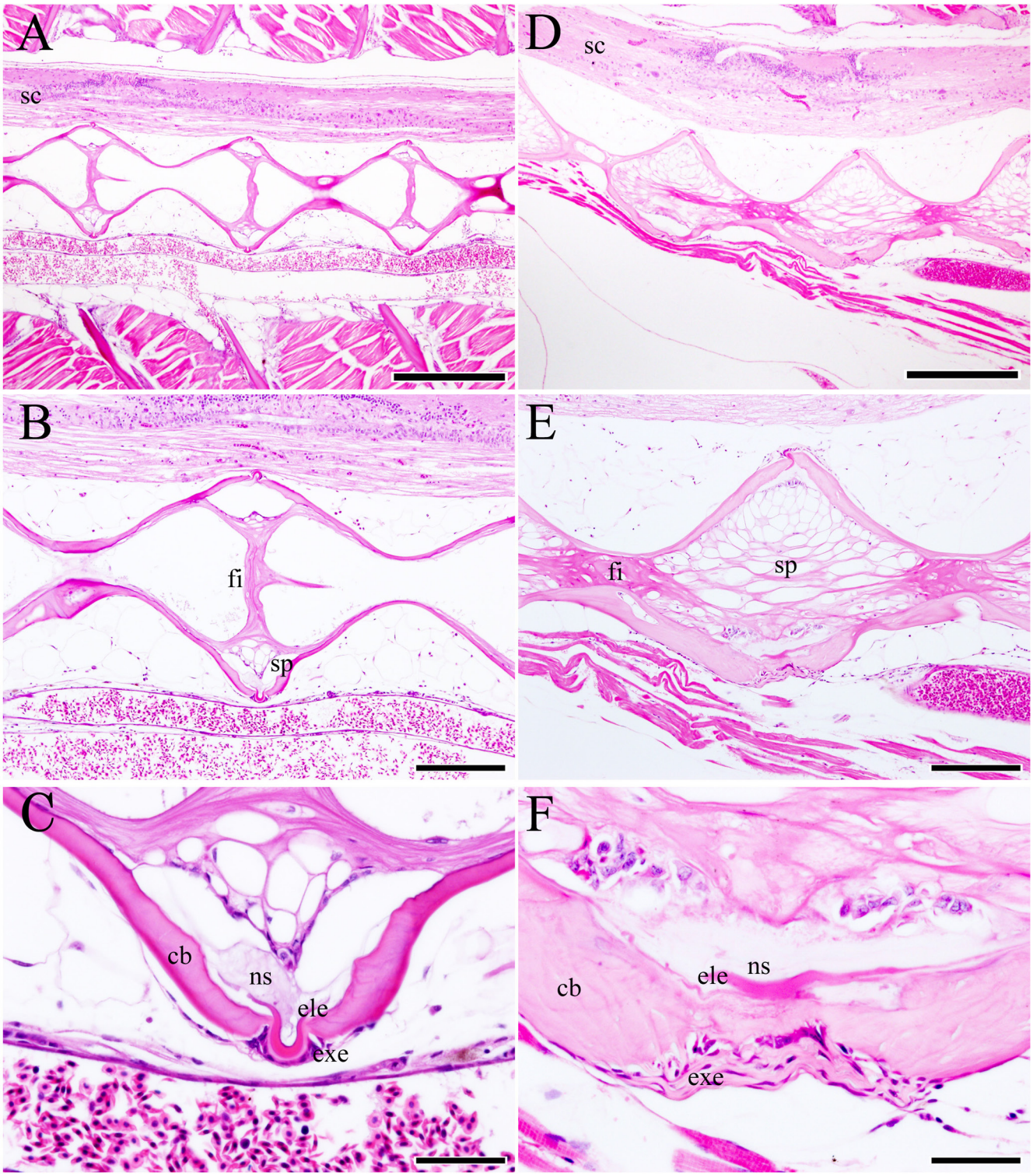

Fig. 2. Histopathology of vertebral bones and intervertebral tissues. A. Vertebral bones of the normal medaka (HE stain, bar = 500 µm). B. Vertebral joints of the normal medaka; fibrous tissue in the central region and spongy tissue in the peripheral region of the intervertebral disc (HE stain, bar = 200 µm). C. Intervertebral ligament of the normal medaka; exterior extra elastica, elastic externa, and notochordal sheath in the intervertebral ligament (HE stain, bar = 50 µm). D. Vertebral bones of the wavy medaka; no abnormalities other than curved vertebral bones and focal edema in the spinal cord (HE stain, bar = 500 µm). E. Vertebral joints of the wavy medaka; vacuolated notochord cells without intervertebral disc formation in vertebral joints. Vertebral cavities were filled with fibrous tissue (HE stain, bar = 200 µm). F. Intervertebral ligament of the wavy medaka; thickened cortical bones and fusion of anterior and posterior vertebral bones (HE stain, bar = 50 µm). cb, cortical bone; exe, exterior extra elastica; ele, elastic externa; fi, fibrous tissue; ns, notochordal sheath; sc, spinal cord; sp, spongy tissue.

black mullets[11]. Each of these fishes displayed a single transverse curve in their spine, resulting from excessive bending caused by convulsions. Therefore, broken vertebrae and hemorrhage were observed in them in the histopathological examinations. In the present case, the vacuolated notochord cells in the vertebral cavity did not regress, so the intervertebral discs did not form. Vacuolated notochord cells remained throughout the whole vertebral cavity, but this did not result in broken bones or spinal injuries. In a normal medaka, intervertebral discs form via regression of the notochord, which consists of vacuolated cells during the embryonic stages of development[12]. In fish, it is known that the notochord plays a key role in the development of vertebrae and in intervertebral disc formation[13, 14]. Moreover, notochord malformation has been found to be associated with vertebral defects[15, 16]. Therefore, we speculated that the spinal curvature seen in the present wavy medaka resulted from genetic notochordal abnormalities, although it was unclear whether this medaka had the spinal curvature at the time of hatching. During development, notochordal subinvolution can induce intervertebral disc dysplasia, leading to M-shaped lordokyphosis.

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