

Cranial cartilages: Players in the evolution of the cranium during evolution of the chordates in general and of the vertebrates in particular

Takayuki Onai^{1,2}  | Toshihiro Aramaki³ | Akira Takai^{4,5} | Kisa Kakiguchi⁶ | Shigenobu Yonemura^{6,7}

¹Department of Anatomy, School of Medical Sciences, University of Fukui, Fukui, Japan

²Life Science Innovation Center, University of Fukui, Fukui, Japan

³Laboratory for Pattern Formation, Graduate School of Frontier Biosciences, Osaka University, Osaka, Japan

⁴Department of Cell Biology, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan

⁵Laboratory for Cell Polarity Regulation, RIKEN Center for Biosystems Dynamics, Research, Osaka, Japan

⁶Laboratory for Ultrastructural Research, RIKEN Center for Biosystems Dynamics, Research, Hyogo, Japan

⁷Department of Cell Biology, Tokushima University Graduate School of Medicine, Tokushima, Japan

Correspondence

Takayuki Onai, Department of Anatomy, School of Medical Sciences, University of Fukui, 23-3, Matsuokashimoaizuki, Eiheiji-cho, Yoshida-gun, Fukui 910-1193, Japan.

Email: tonai@u-fukui.ac.jp

Funding information

Japan Society for the Promotion of Science; University of Fukui

Abstract

The present contribution is chiefly a review, augmented by some new results on amphioxus and lamprey anatomy, that draws on paleontological and developmental data to suggest a scenario for cranial cartilage evolution in the phylum chordata. Consideration is given to the cartilage-related tissues of invertebrate chordates (amphioxus and some fossil groups like vetulicolians) as well as in the two major divisions of the subphylum Vertebrata (namely, agnathans, and gnathostomes). In the invertebrate chordates, which can be considered plausible proxy ancestors of the vertebrates, only a viscerocranium is present, whereas a neurocranium is absent. For this situation, we examine how cartilage-related tissues of this head region prefigure the cellular cartilage types in the vertebrates. We then focus on the vertebrate neurocranium, where cyclostomes evidently lack neural-crest derived trabecular cartilage (although this point needs to be established more firmly). In the more complex gnathostome, several neural-crest derived cartilage types are present: namely, the trabecular cartilages of the prechordal region and the parachordal cartilage the chordal region. In sum, we present an evolutionary framework for cranial cartilage evolution in chordates and suggest aspects of the subject that should profit from additional study.

KEYWORDS

cephalochordates, evolution, head mesoderm, neural crest, neurocranium

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2023 The Authors. *Evolution & Development* published by Wiley Periodicals LLC.

1 | INTRODUCTION

Extant vertebrates include cyclostomes (lampreys and hagfish) and gnathostomes (Janvier, 2015). One of the major features of vertebrates is their highly complex heads consisting of the brain, cranial nerves, skeletal muscles, cranium, and special sensory organs (Gee, 2018). Among the cranial elements, the developmental and evolutionary origins of the cranium has been a major topic in comparative morphology since the 19th century (Huxley, 1858). The cranium is composed of the neurocranium, which encloses the brain and sensory organs, and the viscerocranium, which includes the jaw and pharyngeal skeletons (Romer, 1977). One of the earliest studies on the cranial origin focused on the similarities and differences between the cranium and vertebrae (Goethe, 1790), with the vertebral theory regarding the cranium as a group of vertebrae (Goethe, 1790). The evolution of the jaw on the viscerocranium has been extensively discussed as a serial homolog of pharyngeal arches (de Beer, 1937, reprinted 1971; Huxley, 1874). To understand the formation of the jaw, which is derived from the mandibular arch, key developmental events include interactions between the head mesoderm (premandibular and mandibular mesoderm) and the trigeminal crest, which consists of the supraoptic, infraoptic, and mandibular streams (Kuratani et al., 2013). In the muscular/skeletal system of the jaw, muscles develop from the mandibular arch mesoderm, and skeletons are formed from the mandibular crest. Developmental comparisons between lampreys (jawless vertebrates) and gnathostomes (jawed vertebrates) have shown that the different roles of the neural crest in the premandibular and mandibular regions in these species may provide clues on how the jaw develops (Kuratani et al., 2004, 2013). The difference in the contribution of the neural crest to oral formation is also considered to influence the distance between the nasal sac and hypophysis, and a relationship with the emergence of the trabecular cartilage of the neurocranium in gnathostomes, which is a paired rod-like structure derived from neural crest cells, has been proposed (Kuratani & Ahlberg, 2018). However, whether or not cyclostomes have trabecular cartilage remains controversial.

To understand the early evolution of the neurocranium, it is important to study the ancestral condition of the neural crest cells because they may play a major role in the formation of the neurocranium in early vertebrates. For this purpose, the evolution of neural crest cell types has been studied at the molecular level, which suggests how vertebrate-specific neural crest cell types evolved from neural crest-like cells in chordate ancestors (Cattell et al., 2011; Jandzik et al., 2014). Particularly,

interesting findings have been reported on the similarities between the gene regulatory networks (GRNs) of neural crest cells in vertebrates and mesoderm in chordates such as amphioxus, which is a basal chordate (Jandzik et al., 2014).

In this review, we first summarize the histology of chordate cartilage to understand the similarities and differences between cellular and acellular cartilage as well as their evolutionary relationships. Second, we reconsider how trabecular and parachordal cartilage evolved in early vertebrates.

2 | DIVERSITY OF CARTILAGINOUS SKELETONS IN CHORDATES

Early vertebrates have cartilaginous craniums, and to understand its origins, it is important to define the chordate cartilages before starting a detailed consideration of the evolution of the neurocranium. Cartilage is a special type of fibrous connective tissue that is both elastic and stiff. Similar to other connective tissues, cartilage is composed of cells, called cartilage cells, and intercellular substances, called cartilage matrix (Ito, 2005). In humans, there are three types of cartilage: hyaline, elastic, and fibrocartilage. In hyaline cartilage, which first forms in place of bone during ontogeny, chondrocytes are spherical or oval in shape, and a few chondrocytes are enclosed in the lacuna cartilaginea (a small cavity in the cartilage matrix). Fibrous connective tissue develops at the margins where fibroblasts are well-developed (Ito, 2005). Additionally, in hyaline cartilage, the cartilage matrix consists of fibrils (e.g., type II collagen) and amorphous ground substances (e.g., proteoglycan). Proteoglycans contain mucopolysaccharides, including mucopolysaccharide chondroitin, keratan sulfate, and chondronectin (Ito, 2005). Elastic cartilage is considered to be transformed hyaline cartilage (Ito, 2005). In elastic cartilage, the cells are relatively smaller than those of hyaline cartilage, and there are large amounts of elastic fibrils in the cartilage matrix (Ito, 2005). Meanwhile, fibrocartilage consists of one, two, or three separate cartilage cells, and its matrix contains a large amount of collagen fibers (type I collagen), which makes it relatively soft (Ito, 2005).

Connective tissues include cells such as fibroblasts, fat cells, and pigment cells, while intercellular substances include collagens and elastin for fibrils, proteoglycans (acid mucopolysaccharide and protein complex), and glycoproteins (e.g., fibronectin) (Ito, 2005). Acid mucopolysaccharides (glycosaminoglycans) include hyaluronic acid, heparin, and chondroitin sulfate (Ito, 2005).

The evolution of cartilage in vertebrates has been investigated from the perspective of cartilage in gnathostomes. However, the question is whether ancestral vertebrates have true cartilage (de Beer, 1937, reprinted 1971).

A study of the amphioxus skeletal system revealed cartilage-like structures in adult oral cirri, velar tentacles, endostylar cartilage, gills, and tongue bars (Ruppert, 1997) (Figure 1a–e); these cartilages are classified as cellular or acellular. However, Van Wijhe proposed that oral cirri had true cartilage (Van Wijhe, 1901). According to de Beer, buccal cirri are homologous to oral cartilage in vertebrates (de Beer, 1937, reprinted 1971). The buccal cirri consist of cellular cartilage, and chondrocytes are seen in the skeletal rod, which expresses the fibrillary collagen (*ColA*) gene (Jandzik et al., 2014). Moreover, transmission electron microscopy (TEM) images revealed fibrous collagen sheaths (Ruppert, 1997). However, the amorphous ground substance in cirral skeletons remains unknown.

In amphioxus, velar tentacles, which contain rigid cartilage-like connective tissue (Drach, 1948), were thought to be homologs of gills (Yarrell, 1859).

Additionally, TEM showed that there were multiple heterogeneous cells inside a tentacle (Ruppert, 1997). Meanwhile, the endostyle is a mucus-secreting organ that develops ventrally into the pharynx (Ruppert, 1997). At the base of the endostyle, a paired subendostylar skeletal plate develops to support the endostyle, and the subendostylar skeleton appears to have cartilage-like tissue (Drach, 1948).

Gill skeletons are acellular supporting tissues consisting of gill and tongue bars where skeletal rods are located (Ruppert, 1997). The amorphous ground substance of gill skeletons was suggested to be either chitin (Benham, 1893) or an acid mucopolysaccharide protein complex (Azariah, 1974; Rähr, 1982), of which glycosaminoglycan is a major component based on Alcian blue staining (Rychel et al., 2006). The fibrillar cartilage matrix of gill skeletons has been suggested to be collagenous (Fisher & Franz-Odenaal, 2012; Rychel et al., 2006) or noncollagenous (Wang & Zhu, 2004; Wright et al., 2001). However, Rähr showed that collagen was present around the skeletal rods (Rähr, 1982) (Figure 1c–e). The gill and tongue bar skeletons in amphioxus are not surrounded by branchial muscles as

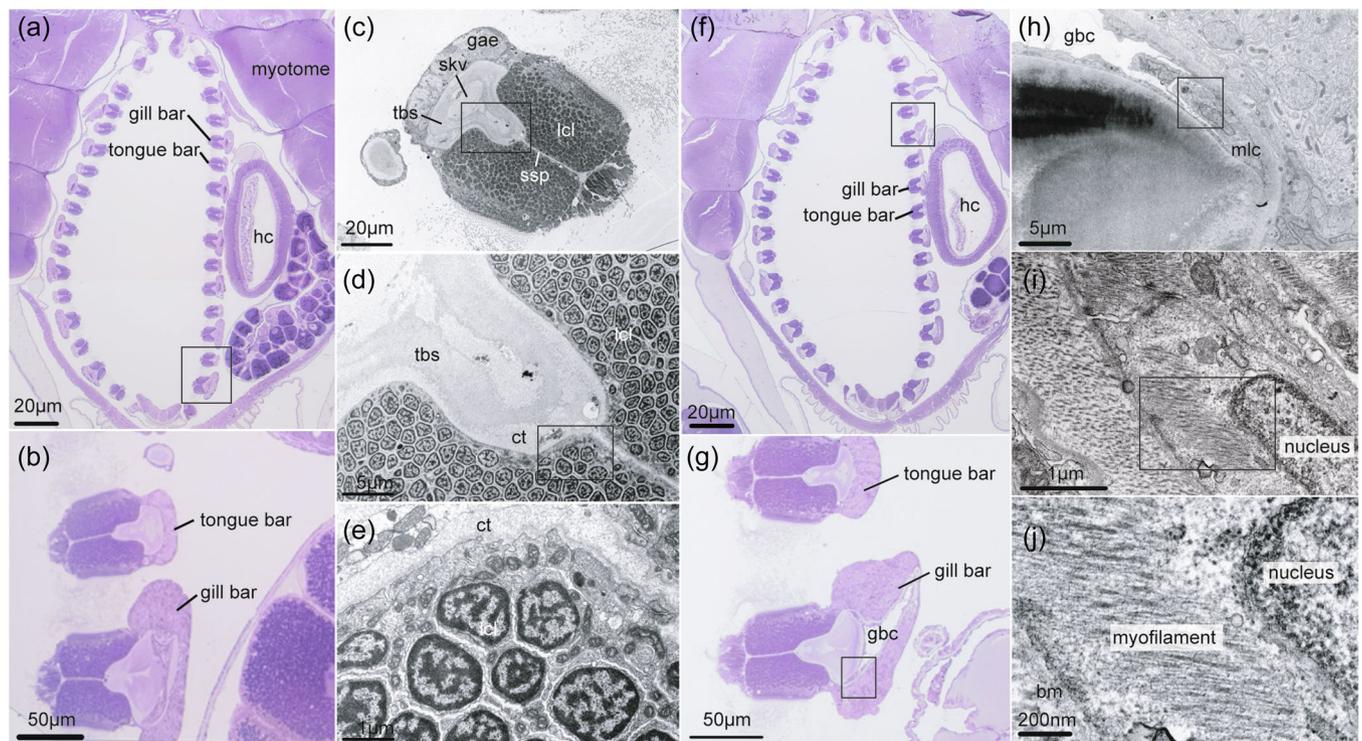


FIGURE 1 Gill skeleton and muscular-like cells in amphioxus. (a, f) The gill skeletons of amphioxus. The black box indicates the magnified area (b). (c) Transmission electron microscope image of a tongue bar in (b). (d) Magnified area as indicated by a black box in (c). (e) Magnified area in D black box. (g) Magnified region in (f) enclosed by a black box. (h) Magnified region is shown as a black box in (g). (i) Magnified area in h (black box). (j) Magnified region as indicated by a black box in (i). ct; connective tissue, gae; glandular atrial epithelium, gbc; gill bar coelom, hc; hepatic cecum, lcl; lateral cells, mlc; muscle like cells, skv; skeletal hemal vessel, ssp; stromal septum, tbs; tongue bar skeletal rod.

in vertebrates, while muscle-like cells in the gill bar coelom were previously reported (Welsch, 1968) (Figure 1f–j). The muscle-like cell faced the basal lamina and probably differentiated from the ventral mesoderm that elongated from the somites at the mid-neurula stages (Figure 1j) (Onai et al., 2017; Yong et al., 2021).

Cyclostomes and gnathostomes split more than 500 million years ago (Kuraku & Kuratani, 2006). Before the split, the presence of at least one whole-genome duplication has been suggested, which is likely to be related to vertebrate-specific morphological evolution (Martik et al., 2019; Ohno, 2013; Simakov et al., 2020). Tendons, which connect muscles to bones, express vertebrate-specific small interstitial leucine-rich repeat proteoglycans (Yoon & Halper, 2005). These genes have been suggested to have evolved through vertebrate-specific genome duplications (Matsushima et al., 2000). The second whole-genome duplication was proposed to have occurred after the split of extant gnathostomes and cyclostomes (Simakov et al., 2020).

The major differences between the skeletal systems of gnathostomes and cyclostomes are (1) absence of bone in cyclostomes and (2) evolution of new proteins in cyclostomes (lamprin, myxinin, and pharymprin) in addition to collagen fibers (Root et al., 2022). Lamprin is an elastin-like fiber expressed in annular, trabecular, and piston cartilage but not in branchial and pericardial cartilages (Fernandes & Eyre, 1999; McBurney & Wright, 1996; Robson et al., 2000). Myxinin is a lamprin-like protein found in hagfish (Wright et al., 1984). Pharymprin is expressed in the third and caudal pharyngeal arch cartilages during lamprey development (Yokoyama et al., 2019). Lampreys also have characteristic cartilages called mucocartilage in the oral and pharyngeal regions that is derived from the mandibular and hyoid arches (Gaskell, 1908; Johnels, 1948). Mucocartilage, which is now considered homogenous for fibrillar collagens (Root et al., 2022), consists of mucopolysaccharides and mesenchyme and is once considered a unique tissue for larval lampreys (Wright & Youson, 1982). A recent study systematically revealed the evolution of the collagen gene family in vertebrates and showed cyclostome-specific gain or loss of genes (Root et al., 2022). Hence, true cartilage in vertebrates can be considered as consisting of type II collagen fibers and chondrocytes. The above evidence of amphioxus and cyclostome cartilages suggests that the basis for true cartilages already present in the common ancestor of chordates and cyclostomes evolved unique repertoires of collagen gene expression patterns in pharyngeal cartilages as well as elastin-like fibers, while extant gnathostomes established the cartilage identity, which consists of type II collagen fibers.

3 | THE PREMANDIBULAR ARCH AND HEAD SEGMENTATION

Trabecular cartilage is a paired rod-like structure located beneath the forebrain. Trabecular cartilage is well conserved in gnathostomes and is a key skeletal element for understanding the basic pattern of the neurocranium (de Beer, 1937, reprinted 1971) (Figure 2a,b). Regarding the origin of trabecular cartilage, Huxley proposed that it evolved from the premandibular arch skeleton (Huxley, 1874) (Figure 2c,d). Meanwhile, de Beer claimed that the premandibular arch mesoderm was located anterior to the mandibular visceral slit in an ancestral amphioxus-like premandibular arch mesoderm (Figure 2e) (de Beer, 1937, reprinted 1971); this hypothesis is deeply related to the head segmentation theory (Goodrich, 1930). According to the head segmentation theory, vertebrate heads are serial homologs of the trunk, which is highly segmented as seen in the vertebrae and spinal nerves (de Beer, 1937, reprinted 1971). Additionally, each segmental unit consists of spinal nerves, somites, and gill slits, and the theory assumes that ancestral vertebrates are amphioxus-like (Goodrich, 1930). From the perspective of head segmentation, there are three pairs of somite homologs, called head cavities, in shark and lamprey embryos, with each cavity situated in the premandibular, mandibular, and hyoid head mesoderm (Goodrich, 1930). Although extant vertebrates do not possess an arch region in the premandibular mesoderm, the premandibular arch hypothesis is expected to be true in ancestors. Evidence for the presence of the premandibular arch is provided by the trigeminal ganglia that separately form V1 and V2–3; the profundus nerve corresponds to the premandibular arch, and the trigeminal nerve corresponds to the mandibular arch as a segmental unit (Goodrich, 1930). For the embryonic origin of the pharyngeal muscles, Noden proposed that from quail-chick chimaeras, branchial muscles are derived from paraxial mesoderm, but not from the lateral plate mesoderm (Noden, 1983). On the branchial arches, Noden considered that branchiomery (segments represented by branchial arches) varies independently from somitomery (segments organized by trunk somites) (Noden, 1983). Further, Northcutt proposed that amphioxus does not have a comparable series of branchiomic muscles (Northcutt, 1996). Current molecular embryology questions Goodrich's model. A developmental comparison between the head cavities and somites in shark embryos highlighted the differences between them (Kuratani & Adachi, 2016), and a secondary comparison between amphioxus and vertebrate mesoderm indicated that the preotic head mesoderm does not express

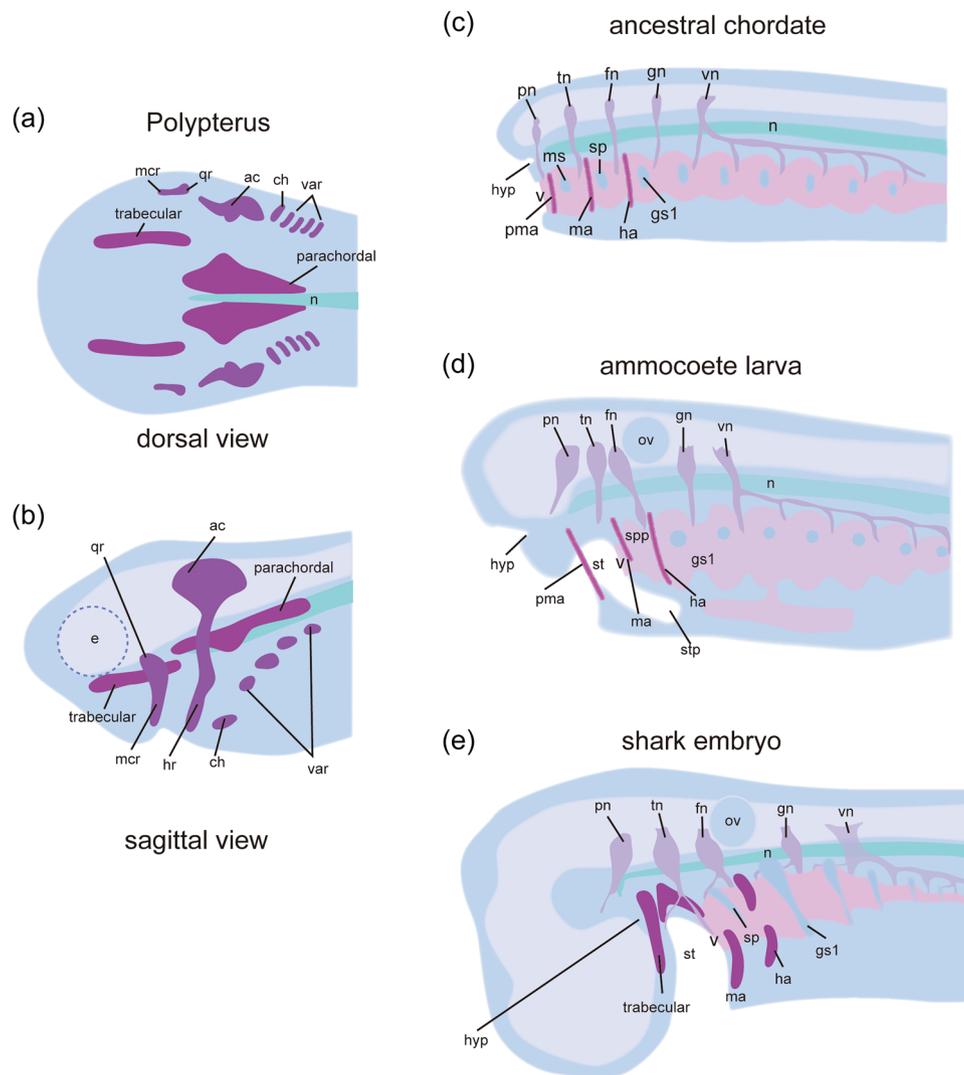


FIGURE 2 Premandibular arch hypothesis and head segmentation. (a, b) The trabecular and parachordal cartilages in polypterus embryos redrawn with modification from de Beer (1937). (a) Dorsal view. (b) Sagittal view. (c) Ancestral chordates having premandibular arch redrawn from De beer (1937). (d) Ammocoete larva of *Petromyzon* redrawn from de Beer (1937). (e) *Scyllium* embryo redrawn from de Beer (1937). ac, auditory capsule; ch, ceratohyal cartilage; e, eye; hyp, hypophysis; fn, facial nerve; gn, glossopharyngeal nerve; gs, gill slit; ha, hyoid arch; hr, rudiment of hyosymplectic cartilage; ma, mandibular arch; mcr, rudiment of Meckel's cartilage; ms, mandibular visceral slit; n, notochord; ov, otic vesicle; pma, premandibular arch; pn, profundus nerve; qr, rudiment of quadrate cartilage; sp, spiracular slit; spp, spiracular pouch; st, stomodaeum; stp, stomodaeal pouches; tn, trigeminal nerve. v, velum; var, rudiment of ceratobranchials.

somitogenesis-related genes (Onai et al., 2015). This evidence deals with the dorsal part of the head mesoderm, which is comparable to trunk somites as both are located dorsally. In the ventrally situated pharyngeal mesoderm, it has been proposed that the head mesoderm is derived from somites based on *tbx1/10* expression in amphioxus somites and *tbx1* expression in the vertebrate pharyngeal mesoderm (Koop et al., 2014).

For evidence of head segmentation in lampreys, the key study is a classic histological study wherein Koltzoff found head somites (premandibular, mandibular, and hyoid somites) during pharyngeal stages (Koltzoff, 1902).

However, our recent study using confocal and TEM microscopes clearly demonstrated the absence of somites in the head mesoderm (Onai et al., 2021). In this paper, we found that there are several somite-rosette-like structures in the mandibular and hyoid head mesoderm in lamprey embryos at pharyngeal stages. However, cell clusters of such pseudo-rosettes were fundamentally different from those in trunk somites. In addition, somitogenesis-related genes were not expressed in those pseudo-rosettes (Onai et al., 2021). A recent re-interpretation of the Cambrian fossil yunnanozoans indicates that the earliest vertebrates are more similar

to cyclostomes than to amphioxus (Tian et al., 2022). Regarding the morphology of stem chordates, recent work suggests that the enigmatic Cambrian fossil called vetulicolians represent stem chordates (Gee, 2018). Vetulicolians share a segmented tadpole shape (McMenamin, 2019). They are diverse and include several groups. Some (e.g., *Bellamyia constricta*) have posterior segments but not anterior, others (e.g., *Dida-zoon haoae*) have segments throughout the body similar to extant amphioxus (McMenamin, 2019). The majority view is that vetulicolians fossils might be represent ancient invertebrate chordates. However, the identity of these enigmatic fossils remains to be settled definitively. There are scientists who think vetulicolians are some sort of arthropod (Butterfield, 2002). And a couple of papers doubt if they really chordates although without suggesting a firm alternative (Briggs et al., 2005; Schubert et al., 2006). There is even one group that thinks vetulicolians are a missing link between amphioxus and tunicates (Syed et al., 2007).

4 | THE EVOLUTIONARY AND DEVELOPMENTAL ORIGIN OF THE TRABECULAR AND PARACHORDAL CARTILAGES IN VERTEBRATES

Trabecular cartilage is derived from the neural crest of gnathostomes (Wada et al., 2005). In zebrafish, transgenic analysis revealed that trabecular and parachordal cartilages develop separately; the former is from the neural crest and the latter is from the mesoderm, which eventually fuse leading to an unclear interface (Kague et al., 2012). In amphibians, lineage trace studies indicate that trabecular cartilage has a neural crest origin (Hanken & Gross, 2005). In lampreys, trabecular cartilage develops as a paired rod-like structure beneath the optic vesicle, similar to gnathostomes (Johnels, 1948). Meanwhile, in a histological study, Damas considered trabecular cartilage to be derived from the neural crest (Damas, 1944). As such, perturbation studies of the neural crest have resulted in conflicting results (Langille & Hall, 1988; Newth, 1956). However, a cell lineage study showed that the trabecular bone originated from the head mesoderm (Kuratani et al., 2004). Therefore, trabecular cartilage in lampreys is a parachordal homolog, with similar results having been reported for hagfish (Oisi et al., 2013). If trabecular cartilage is absent from cyclostomes, it might have emerged in stem gnathostomes as a plate to support the forebrain (Kuratani & Ahlberg, 2018). Therefore, the upper lip of cyclostomes is derived from the premandibular crest, and this cell

population evolved to form trabecular cartilage in jawed vertebrates (Kuratani & Ahlberg, 2018). The proposed transformation fits well with stem gnathostome fossils such as *Romundina*, which revealed a stepwise evolution (e.g., expansion of the distance between the nasal sac and hypophysis and enlarged forebrain) of gnathostome faces (Dupret et al., 2014).

If the parachordal cartilage evolved before the trabecular cartilage, it could be present in jawless vertebrates such as *Metaspriggina*, a Cambrian fossil (Onai et al., 2017). In extant vertebrates, parachordal cartilage differentiates from the presomitic head mesoderm after induction by notochordal signals (McBratney-Owen et al., 2008). If the medial paraxial head mesoderm near the notochord becomes the parachordal cartilage, there are three possible ancestral conditions depending on the contribution of somites: the parachordal cartilage originates from the scleromesothelium of somites or mesenchymal head mesoderm, or expands dorsally from the ventral mesoderm (Aldea et al., 2019; Mansfield et al., 2015; Onai et al., 2015). However, recent findings suggest that contrary to the model proposed by Kuratani et al. (2004), the trabecular cartilage in lampreys may be neural crest-derived and not mesoderm-derived. In stage 26 lamprey embryos, the neural crest cells were recognized above the dorsal neural tube and mandibular arch which are similar to other vertebrates (Trainor, 2013) (Figure 3a–c). If trabecular cartilage is derived from the neural crest in lampreys, it might have been present in early vertebrates. A loss-of-function study of the gnathostome *sox8/9/10* homolog *soxE* (a neural crest marker) in lamprey embryos showed failure of trabecular formation (Lakiza et al., 2011), suggesting that trabecular cartilage may be derived from the neural crest. Histological studies of the lamprey cartilage at stage 27 showed that trabeculae were situated ventral to the eye (Figure 3d,e). Curiously, the prospective trabecular cells were longitudinal and continuous with the neural crest stream from the dorsal neural tube (Figure 3d,e). No medial crest cell population is seen in the cephalic crest, but the situation in lampreys might not be similar based on a cell lineage tracing study (McCauley & Bronner-Fraser, 2003). Collectively, these evidence might be consistent with the neural crest origin of the trabecular in lampreys. If the trabecular cartilage originated from the neural crest already in early vertebrates, their homologs should be located in the lateral neural tube of the last common ancestor of tunicates and vertebrates (Cattell et al., 2011). However, the neural crest-like cells in this pre-vertebrate ancestor did not seem to have been able to differentiate into cartilage cells (Cattell et al., 2011). Molecular comparisons of the pharyngeal mesoderm of amphioxus and arch

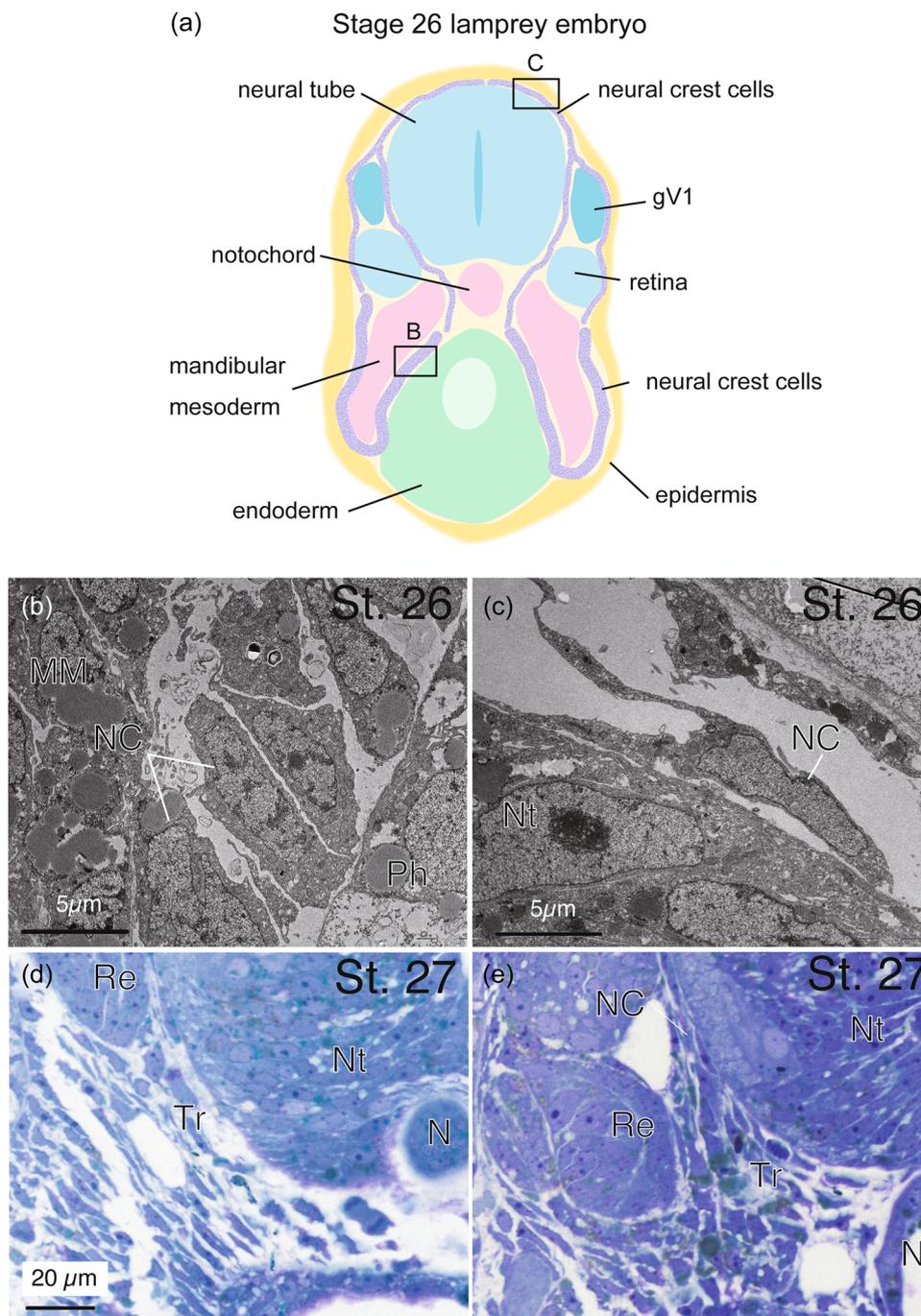


FIGURE 3 Transmission electron microscope (TEM) images of neural crest cells and plastic section images of prospective trabecular cartilages in lamprey embryos (a) A scheme of stage 26 lamprey embryo which was examined in TEM experiments in this study. Black boxes indicate that the regions observed in TEM images (b, c). (b, c) TEM images of mandibular arch crest cells (b) or dorsal neural crest cells (c). (d, e) Plastic section images of prospective trabecular cartilages at stage 27. (d) is rostral to (e). GV1, Ganglion of trigeminal nerve1; MM, mandibular head mesoderm; N, notochord; Nc, neural crest; Nt, neural tube; Re, retina; Tr, trabecular progenitors.

skeletons of vertebrates suggest that cartilage properties in the neural crest evolved by co-option of GRNs in the pharyngeal mesoderm of amphioxus-like ancestors (Cattell et al., 2011; Jandzik et al., 2014). Since amphioxus are more distantly related to vertebrates than

tunicates, the scenario presented here would, however, still be compatible with the neural crest already producing cartilage in the last common ancestor of tunicates and vertebrates. In the initial phase of vertebrate evolution, this animal might have had its

hypophysis derived from the premandibular endomesoderm, which is homologous to the anterior gut diverticulum in amphioxus and consistent with a recent study showing the endodermal origin of hypophysis in zebrafish (Figure 4a) (Fabian et al., 2020). In this animal, the pharyngeal arch skeleton likely formed from the head mesoderm or somites, depending on whether the rostral somites extended into the rostral end (Figure 4a). In the second phase, represented by cyclostomes, the pharyngeal arch skeletons are derived from the mesoderm and neural crest, which recruit cartilage GRNs from the ancestral mesoderm, while the trabecular cartilage is derived from the neural crest (Figure 4b) (Sleight & Gillis, 2020). If so, the evolutionary origin of the

trabecular cartilage is the ancient pharyngeal mesoderm, which, unlike the premandibular arch mesoderm, leads to the conclusion that this skeleton was invented through a stepwise evolution of the neural crest cell types including copying pharyngeal mesoderm GRNs in the chordate ancestor (Figure 4b,c). The neural crest has been considered to be the most important developmental novelty in vertebrate evolution (Northcutt, 1996). From the perspective of neurocranium evolution, the neural crest might have taken skeletogenesis properties from the pharyngeal mesoderm in an ancestral chordate, which eventually might have allowed the pharyngeal mesoderm to generate well-developed branchial muscles for a predatory lifestyle.

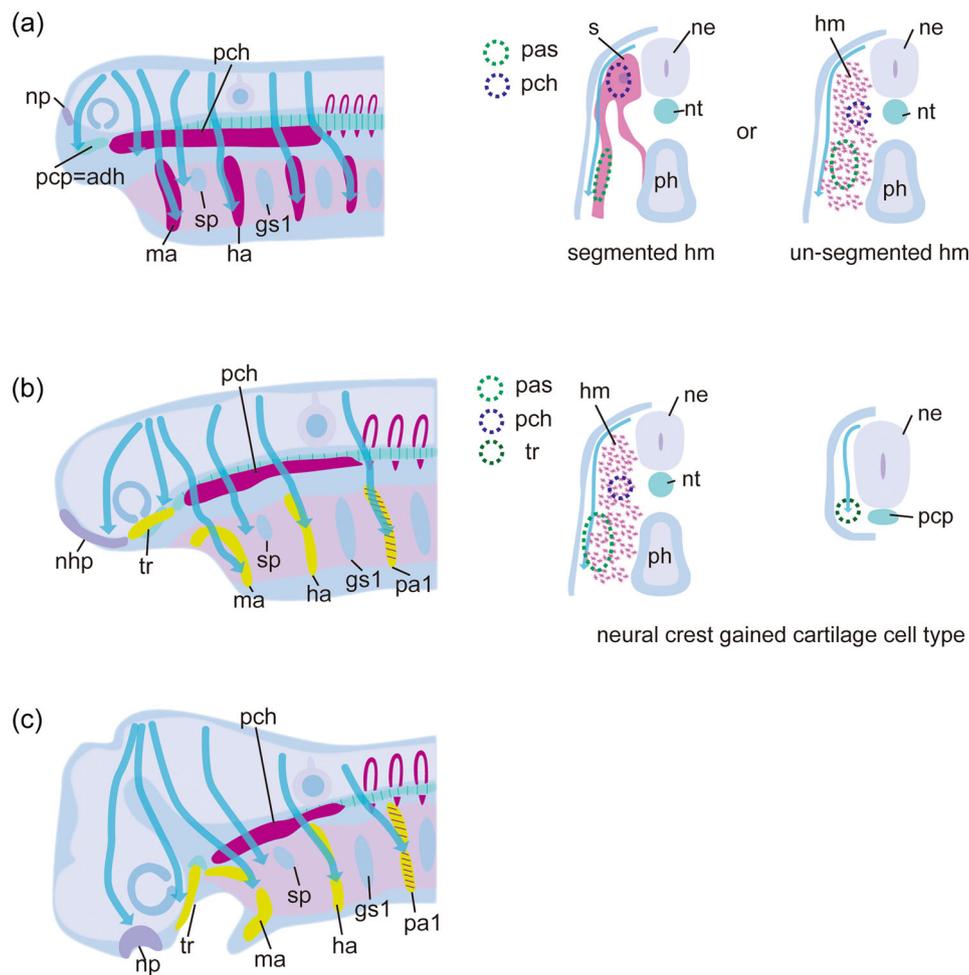


FIGURE 4 Scenario of the evolution of the cranium of vertebrates. (a) Early vertebrates before evolution of the trabecular cartilage. Parachordal cartilage can be present if *Metaspriggina* (Morris & Caron, 2014) is a primitive vertebrate that may have rostral somites forming cartilages. Alternatively, the parachordal cartilages can be derived from the mesenchymal head mesoderm if yunnanozoans are the most basal vertebrates (Tian et al., 2022). In this case, the preotic head mesoderm does not include somites. (b) Jawless vertebrates having trabecular cartilages. (c) Jawed vertebrates. Dark red structure indicates mesodermal cartilage, and yellow structure indicates cartilages from neural crest cells. Light green stream is neural crest migration pathways. adh, adenohypophysis; gs, gill slit; ha, hyoid arch; hm, head mesoderm; ma, mandibular arch; ne, neural tube; nhp, nasohypophysial placode; np, nasal placode; nt, notochord; pas, pharyngeal arch skeleton; pch, parachordal cartilage; pcp, prechordal plate endomesoderm; ph, pharynx; s, somite; sp, spiracle; tr, trabecular cartilage.

5 | CONCLUSIONS

For the early evolution of the vertebrate neurocranium, the developmental origin of trabecular cartilage must be first understood to clarify that trabecular cartilage is a common feature of extant cyclostomes and gnathostomes. However, cell lineage studies of trabecular cartilage in lamprey embryos are insufficient. Therefore, to confirm the theories of neurocranial evolution, future studies should address this problem.

6 | MATERIALS AND METHODS

6.1 | Animal collection

Adult amphioxus were collected from the ocean in Amakusa, Kumamoto, Japan. After collection, the adults were kept in a tank containing artificial seawater. The temperature was maintained at 20°C. Adult lampreys were collected from a river in Hokkaido, Japan. They were placed in a tank that was maintained at 12°C. In vitro fertilization was performed as previously described (Onai et al., 2015). Fertilized eggs were cultured in 10% Steinberg's solution until the stage was fixed.

6.2 | TEM analysis

Samples of adult amphioxus were fixed in 1% paraformaldehyde (PFA) and 1.25% glutaraldehyde (GA) in filtered seawater for 2 h at room temperature, and then for more than 24 h at 4°C. The samples were washed with filtered seawater twice on ice and with 0.1 M cacodylate buffer twice on ice. Lamprey embryos were fixed with 2% PFA and 2.5% GA/PBS (–) and washed with PBS (–). The samples were treated by 1% osmium tetroxide in 0.1 M cacodylate buffer for 2 h in the dark. The samples were then washed thrice with Milli-Q water at room temperature. Block staining was performed by adding 0.5% uranium acetate for 2 h at room temperature. The samples were dehydrated using an EtOH series (50%–100%). Propylene oxide (PO) was added twice for 20 min. PO:resin was added in a 1:1 ratio for 30 min and later in a desiccator overnight. The samples were treated in 100% resin thrice for 2 h at room temperature, evacuated for 30 min, and then placed under vacuum overnight. The samples were then embedded in Polybed 812 for 48 h at 60°C. The sections (90 nm) were double-stained with uranyl acetate and lead citrate and imaged using an electron microscope (JEOL JEM-1010. Japan; Hitachi H-7650, Japan). Regarding identification of the neural crest cells, we applied criteria that the neural

crest arises from the dorsal roof of the neural tube, and the neural crest covers mesodermal core in the mandibular arch. The evidence can be found in (Noden & Francis-West, 2006; Trainor, 2013).

6.3 | Toluidine blue staining

Toluidine blue solution (0.5% toluidine blue O, 1% borax in water) (Waldeck, Germany) was applied to the sectioned samples. The samples were placed on a hot plate for 30 s. They were then washed with Milli-Q water. After the samples were dried at room temperature, they were covered with Entellan (Fuji Film, Japan).

AUTHOR CONTRIBUTIONS

Takayuki Onai designed the study. Takayuki Onai, Kisa Kakiguchi, and Shigenobu Yonemura performed the experiments. Takayuki Onai, Toshihiro Aramaki, and Akira Takai wrote the manuscript and approved the final version.

ACKNOWLEDGMENTS

We thank Dr. N. Adachi of Tokyo Medical Dental University, Japan, for his comments on the manuscript and Mr. Takagi of the University of Fukui for serial sections and TEM analysis of lamprey embryos. We also thank Dr. S. Kuratani for discussions and laboratory space at RIKEN. This work was supported by the University of Fukui Life Science Innovation Center, KAKENHI Grant-in-Aid for Scientific Research (C) Grant Number 19K06787.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Takayuki Onai  <http://orcid.org/0000-0002-5402-3828>

REFERENCES

- Aldea, D., Subirana, L., Keime, C., Meister, L., Maeso, I., Marcellini, S., Gomez-Skarmeta, J. L., Bertrand, S., & Escriva, H. (2019). Genetic regulation of amphioxus somitogenesis informs the evolution of the vertebrate head mesoderm. *Nature Ecology & Evolution*, 3(8), 1233–1240.
- Azariah, J. (1974). Studies on the cephalochordates of the Madras coast. 16. A theory of structural stabilization. *Acta Histochemica*, 50, 62–74.
- de Beer, G. (1937). *The development of the vertebrate skull*. Oxford University Press.

- Benham, W. B. (1893). The structure of the pharyngeal bars of amphioxus. *Quarterly Journal of Microscopical Science*, 35, 97–118.
- Briggs, D. E. G., Lieberman, B. S., Halgedahl, S. L., & Jarrard, R. D. (2005). A new metazoan from the middle Cambrian OF Utah and the nature of the vetulicolia. *Palaeontology*, 48, 681–686.
- Butterfield, N. J. (2002). Leanochoilia guts and the interpretation of three-dimensional structure in Burgess Shale-type fossils. *Paleobiology*, 28, 154–170.
- Cattell, M., Lai, S., Cerny, R., & Medeiros, D. M. (2011). A new mechanistic scenario for the origin and evolution of vertebrate cartilage. *PLoS One*, 6, e22474.
- Damas, H. (1944). Recherches sur le développement de *Lampetra fluviatilis* L. contribution à l'étude de la céphalogenèse des vertébrés. *Archives de Biologie*, 55, 1–248.
- Drach, P. (1948). Embranchement des cephalochordes. In P. P. Grasse (Ed.), *Traite de Zoologie, Tome XI, Echinodermes, Stomochordes, Protochordes* (Vol. XI, pp. 931–1037). Masson.
- Dupret, V., Sanchez, S., Goujet, D., Tafforeau, P., & Ahlberg, P. E. (2014). A primitive placoderm sheds light on the origin of the jawed vertebrate face. *Nature*, 507, 500–503.
- Fabian, P., Tseng, K. C., Smeeton, J., Lancman, J. J., Dong, P. D. S., Cerny, R., & Crump, J. G. (2020). Lineage analysis reveals an endodermal contribution to the vertebrate pituitary. *Science*, 370(6515), 463–467. <https://doi.org/10.1126/science.aba4767>
- Fernandes, R. J., & Eyre, D. R. (1999). The elastin-like protein matrix of lamprey branchial cartilage is cross-linked by lysyl pyridinoline. *Biochemical and Biophysical Research Communications*, 261, 635–640.
- Fisher, S., & Franz-Odenaal, T. (2012). Evolution of the bone gene regulatory network. *Current Opinion in Genetics & Development*, 22, 390–397.
- Gaskell, W. H. (1908). *The origin of vertebrates*. Longmans, Green, and Co.
- Gee, H. (2018). *Across the bridge: Understanding the origin of the vertebrates*. The University of Chicago Press.
- Goethe, J. W. (1790). Das schädelgrüt aus sechs wirbelknochen aufgebaut. *Zur Naturwissenschaft Überhaupt, Besonders zur Morphologie. II 2*.
- Goodrich, E. S. (1930). *Studies on the structure and development of vertebrates*. Macmillan.
- Hanken, J., & Gross, J. B. (2005). Evolution of cranial development and the role of neural crest: Insights from amphibians. *Journal of Anatomy*, 207(5), 437–446. <https://doi.org/10.1111/j.1469-7580.2005.00481.x>
- Huxley, T. H. (1858). The croonian lecture: On the theory of the vertebrate skull. *Proceedings of the Zoological Society of London*, 9, 381–457.
- Huxley, T. H. (1874). On the structure of the skull and of the heart of *Menobranchius lateralis*. *Proceedings of the Zoological Society of London*, 42, 186–204.
- Ito, T. (2005). *Histology revised by Kazuhiro Abe*. Nanzando.
- Jandzik, D., Garnett, A. T., Square, T. A., Cattell, M. V., Yu, J. K., & Medeiros, D. M. (2014). Evolution of the new vertebrate head by co-option of an ancient chordate skeletal tissue. *Nature*, 518, 534–537.
- Janvier, P. (2015). Facts and fancies about early fossil chordates and vertebrates. *Nature*, 520(7548), 483–489. <https://doi.org/10.1038/nature14437>
- Johnels, A. G. (1948). On the development and morphology of the skeleton of the head of *Petromyzon*. *Acta Zoologica*, 29, 139–279.
- Kague, E., Gallagher, M., Burke, S., Parsons, M., Franz-Odenaal, T., & Fisher, S. (2012). Skeletogenic fate of zebrafish cranial and trunk neural crest. *PLoS One*, 7(11), e47394. <https://doi.org/10.1371/journal.pone.0047394>
- Koltzoff, N. K. (1902). Entwicklungsgeschichte des Kopfes von *Petromyzon planeri*; ein Beitrag zur Lehre über Metamerie des Wirbelthierkopfes. *Bulletin de la Societe Imperiale des Naturalistes de Moscou*, 16, 259–589.
- Koop, D., Chen, J., Theodosiou, M., Carvalho, J. E., Alvarez, S., de Lera, A. R., Holland, L. Z., & Schubert, M. (2014). Roles of retinoic acid and *Tbx1/10* in pharyngeal segmentation: Amphioxus and the ancestral chordate condition. *EvoDevo*, 5(1), 36. <https://doi.org/10.1186/2041-9139-5-36>
- Kuraku, S., & Kuratani, S. (2006). Time scale for cyclostome evolution inferred with a phylogenetic diagnosis of hagfish and lamprey cDNA sequences. *Zoological Science*, 23(12), 1053–1064. <https://doi.org/10.2108/zsj.23.1053>
- Kuratani, S., & Adachi, N. (2016). What are head cavities? - A history of studies on vertebrate head segmentation. *Zoological Science*, 33(3), 213–228. <https://doi.org/10.2108/zs150181>
- Kuratani, S., Adachi, N., Wada, N., Oisi, Y., & Sugahara, F. (2013). Developmental and evolutionary significance of the mandibular arch and prechordal/premandibular cranium in vertebrates: Revising the heterotopy scenario of gnathostome jaw evolution. *Journal of Anatomy*, 222(1), 41–55. <https://doi.org/10.1111/j.1469-7580.2012.01505.x>
- Kuratani, S., & Ahlberg, P. E. (2018). Evolution of the vertebrate neurocranium: Problems of the premandibular domain and the origin of the trabecula. *Zoological Letters*, 4, 1. <https://doi.org/10.1186/s40851-017-0083-6>
- Kuratani, S., Murakami, Y., Nobusada, Y., Kusakabe, R., & Hirano, S. (2004). Developmental fate of the mandibular mesoderm in the lamprey, *Lethenteron japonicum*: Comparative morphology and development of the gnathostome jaw with special reference to the nature of the trabecula cranii. *Journal of Experimental Zoology*, 302(5), 458–468. <https://doi.org/10.1002/jez.b.21011>
- Lakiza, O., Miller, S., Bunce, A., Lee, E. M. J., & McCauley, D. W. (2011). SoxE gene duplication and development of the lamprey branchial skeleton: Insights into development and evolution of the neural crest. *Developmental Biology*, 359(1), 149–161. <https://doi.org/10.1016/j.ydbio.2011.08.012>
- Langille, R. M., & Hall, B. K. (1988). Role of the neural crest in development of the cartilaginous cranial and visceral skeleton of the medaka, *Oryzias latipes* (Teleostei). *Anatomy and Embryology*, 177(4), 297–305. <https://doi.org/10.1007/BF00315836>
- Mansfield, J. H., Haller, E., Holland, N. D., & Brent, A. E. (2015). Develop[ment of somites and their derivatives in amphioxus, and implications for evolution of vertebrate sometes. *EvoDevo*, 6, 21.
- Martik, M. L., Gandhi, S., Uy, B. R., Gillis, J. A., Green, S. A., Simoes-Costa, M., & Bronner, M. E. (2019). Evolution of the new head by gradual acquisition of neural crest regulatory circuits. *Nature*, 574(7780), 675–678. <https://doi.org/10.1038/s41586-019-1691-4>

- Matsushima, N., Ohyanagi, T., Tanaka, T., & Kretsinger, R. H. (2000). Super-motifs and evolution of tandem leucine-rich repeats within the small proteoglycans-biglycan, decorin, lumican, fibromodulin, PRELP, keratocan, osteoadherin, epiphykan, and osteoglycin. *Proteins: Structure, Function, and Genetics*, 38(2), 210–225. [https://doi.org/10.1002/\(sici\)1097-0134\(20000201\)38:2<210::aid-prot9>3.0.co;2-1](https://doi.org/10.1002/(sici)1097-0134(20000201)38:2<210::aid-prot9>3.0.co;2-1)
- McBratney-Owen, B., Iseki, S., Bamforth, S. D., Olsen, B. R., & Morriss-Kay, G. M. (2008). Development and tissue origins of the mammalian cranial base. *Developmental Biology*, 322(1), 121–132. <https://doi.org/10.1016/j.ydbio.2008.07.016>
- McBurney, K. M., & Wright, G. M. (1996). Chondrogenesis of a non-collagen-based cartilage in the sea lamprey, *Petromyzon marinus*. *Canadian Journal of Zoology*, 74, 2118–2130.
- McCauley, D. W., & Bronner-Fraser, M. (2003). Neural crest contributions to the lamprey head. *Development*, 130(11), 2317–2327. <https://doi.org/10.1242/dev.00451>
- McMenamin, M. A. S. (2019). Cambrian chordates and vetulicolians. *Geosciences*, 9(8), 354. <https://doi.org/10.3390/geosciences9080354>
- Morris, S. C., & Caron, J. B. (2014). A primitive fish from the Cambrian of North America. *Nature*, 512, 419–422.
- Newth, D. R. (1956). On the neural crest of the lamprey embryo. *Development*, 4, 358–375.
- Noden, D. M. (1983). The embryonic origins of avian cephalic and cervical muscles and associated connective tissues. *American Journal of Anatomy*, 168, 257–276.
- Noden, D. M., & Francis-West, P. (2006). The differentiation and morphogenesis of craniofacial muscles. *Developmental Dynamics*, 235(5), 1194–1218. <https://doi.org/10.1002/dvdy.20697>
- Northcutt, R. G. (1996). The origin of craniates: Neural crest, neurogenic placodes, and homeobox genes. *Israel J. Zool.*, 42(Suppl), 273–313.
- Ohno, S. (2013). *Evolution by gene duplication*. Springer Science and Business Media.
- Oisi, Y., Ota, K. G., Fujimoto, S., & Kuratani, S. (2013). Development of the chondrocranium in hagfishes, with special reference to the early evolution of vertebrates. *Zoological Science*, 30(11), 944–961. <https://doi.org/10.2108/zsj.30.944>
- Onai, T., Adachi, N., & Kuratani, S. (2017). Metamerism in cephalochordates and the problem of the vertebrate head. *The International Journal of Developmental Biology*, 61(10–12), 621–632. <https://doi.org/10.1387/ijdb.170121to>
- Onai, T., Aramaki, T., Inomata, H., Hirai, T., & Kuratani, S. (2015). Ancestral mesodermal reorganization and evolution of the vertebrate head. *Zoological Letters*, 1, 29. <https://doi.org/10.1186/s40851-015-0030-3>
- Onai, T., Sugahara, F., & Adachi, N. (2021). Evolution of the vertebrate head is revealed by the rosette mesoderm. *PREPRINT (Version 1): Research Square*. <https://doi.org/10.21203/rs.3.rs-403612/v1>
- Robson, P., Wright, G. M., Youson, J. H., & Keeley, F. W. (2000). The structure and organization of lamprin genes: Multiple-copy genes with alternative splicing and convergent evolution with insect structural proteins. *Molecular Biology and Evolution*, 17, 1739–1752.
- Romer, A. S. (1977). *The vertebrate body* (5th ed.). Saunders.
- Root, Z. D., Allen, C., Gould, C., Brewer, M., Jandzik, D., & Medeiros, D. M. (2022). A comprehensive analysis of fibrillar collagens in lamprey suggests a conserved role in vertebrate musculoskeletal evolution. *Frontiers in Cell and Developmental Biology*, 10, 809979. <https://doi.org/10.3389/fcell.2022.809979>
- Ruppert, E. E. (1997). Cephalochordata (Acrania). In F. W. Harrison, & E. E. Ruppert (Eds.), *Microscopic anatomy of invertebrates, Vol. 15: Hemichordata, chaetognatha, and the invertebrate chordates* (Vol. 15, pp. 349–504). Wiley.
- Rychel, A. L., Smith, S. E., Shimamoto, H. T., & Swalla, B. J. (2006). Evolution and development of the chordates: Collagen and pharyngeal cartilage. *Molecular Biology and Evolution*, 23, 541–549.
- Rähr, H. (1982). Ultrastructure of gill bars of *Branchiostoma lanceolatum* with special reference to gill skeleton and blood vessels (Cephalochordata). *Zoomorphology*, 99, 167–180.
- Schubert, M., Escriva, H., Xavier-Neto, J., & Laudet, V. (2006). Amphioxus and tunicates as evolutionary model systems. *Trends in Ecology & Evolution*, 21(5), 269–277. <https://doi.org/10.1016/j.tree.2006.01.009>
- Simakov, O., Marlétaz, F., Yue, J. X., O'Connell, B., Jenkins, J., Brandt, A., Calef, R., Tung, C. H., Huang, T. K., Schmutz, J., Satoh, N., Yu, J. K., Putnam, N. H., Green, R. E., & Rokhsar, D. S. (2020). Deeply conserved synteny resolves early events in vertebrate evolution. *Nature Ecology & Evolution*, 4(6), 820–830. <https://doi.org/10.1038/s41559-020-1156-z>
- Sleight, V. A., & Gillis, J. A. (2020). Embryonic origin and serial homology of gill arches and paired fins in the skate, *Leucoraja erinacea*. *Elife*, 9, e60635. <https://doi.org/10.7554/eLife.60635>
- Syed, T., Gudo, M., & Gutmann, M. (2007). Die neue gro beta phylogenie des tierreiches: Dilemma oder fortschritt? *Denisia*, 20, 295–312.
- Tian, Q., Zhao, F., Zeng, H., Zhu, M., & Jiang, B. (2022). Ultrastructure reveals ancestral vertebrate pharyngeal skeleton in yunnanozoans. *Science*, 377(6602), 218–222. <https://doi.org/10.1126/science.abm2708>
- Trainor, P. (2013). *Neural crest cells: Evolution, development and disease*. Academic Press.
- Wada, N., Javidan, Y., Nelson, S., Carney, T. J., Kelsh, R. N., & Schilling, T. F. (2005). Hedgehog signaling is required for cranial neural crest morphogenesis and chondrogenesis at the midline in the zebrafish skull. *Development*, 132(17), 3977–3988. <https://doi.org/10.1242/dev.01943>
- Wang, Y., & Zhu, J. T. (2004). Studies on the gill skeleton in the lancelet, *Branchiostoma belcheri* tsingtauense tchang et koo by transmission electron microscope. *Marine Fisheries Research*, 25, 12–18.
- Welsch, U. (1968). The fine structure of the pharynx, cyrtopodocytes and digestive caecum of amphioxus (*Branchiostoma lanceolatum*). *Symposia of the Zoological Society of London*, 36, 17–41.
- Van Wijhe, J. W. (1901). Beitrage zur Anatomie der Kopfreigion des Amphioxus lanceolatus. *Petrus Camper*, 1, 109–194.
- Wright, G. M., Keeley, F. W., & Robson, P. (2001). The unusual cartilaginous tissues of jawless craniates, cephalochordates and invertebrates. *Cell and Tissue Research*, 304, 165–174.
- Wright, G. M., Keeley, F. W., Youson, J. H., & Babineau, D. L. (1984). Cartilage in the Atlantic hagfish, *Myxine glutinosa*.

- American Journal of Anatomy*, 169(4), 407–424. <https://doi.org/10.1002/aja.1001690404>
- Wright, G. M., & Youson, J. H. (1982). Ultrastructure of mucocartilage in the larval anadromous sea lamprey, *Petromyzon marinus* L. *American Journal of Anatomy*, 165(1), 39–51. <https://doi.org/10.1002/aja.1001650105>
- Yarrell, W. (1859). *A history of british fishes*. John Van Voorst.
- Yokoyama, H., Morino, Y., & Wada, H. (2019). Identification of a unique lamprey gene with tandemly repeated sequences and pharyngeal chondrocyte-specific expression. *Gene*, 701, 9–14. <https://doi.org/10.1016/j.gene.2019.02.094>
- Yong, L. W., Lu, T. M., Tung, C. H., Chiou, R. J., Li, K. L., & Yu, J. K. (2021). Somite compartments in amphioxus and its implications on the evolution of the vertebrate skeletal tissues. *Frontiers in Cell and Developmental Biology*, 9, 607057. <https://doi.org/10.3389/fcell.2021.607057>
- Yoon, J. H., & Halper, J. (2005). Tendon proteoglycans: Biochemistry and function. *Journal of Musculoskeletal & Neuronal Interactions*, 5(1), 22–34. <https://www.ncbi.nlm.nih.gov/pubmed/15788868>

How to cite this article: Onai, T., Aramaki, T., Takai, A., Kakiguchi, K., & Yonemura, S. (2023). Cranial cartilages: Players in the evolution of the cranium during evolution of the chordates in general and of the vertebrates in particular. *Evolution & Development*, 25, 197–208. <https://doi.org/10.1111/ede.12433>