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Auricular cartilage configuration: a histological study using late-stage human fetuses and adult cadavers
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Running head: Auricular cartilage configuration
Abstract

The auricular cartilage is considered to develop from a funnel-like arrangement of six embryonic hillocks. However, there is little information as to when and how the initial cartilage plate differentiates into the major three hollows or caves: the concha, the scapha and the triangular fossa. We examined semiserial histological sections from 42 human fetuses as well as from seven cadavers of elderly individuals. Tangential sections from adults suggested that three ring-like cartilages were combined to provide a single auricular cartilage and that the external auditory meatus was attached to the lowest ring or concha. All of the fetuses studied carried the three major hollows delineated by skin folds. These skin folds often contained a cartilage loop as a core in place of a thickening or tubercle. Conversely, some of the skin folds corresponded to a highly wavy cartilage plate without looping. According to whether the cartilage loop was present or absent in horizontal sections from 35 fetuses, we classified the cartilage morphology into four patterns, the most frequent of which was absence of the triangular fossa loop (27 fetuses), followed by absence of the scapha loop (11 fetuses). Each pattern was evenly distributed among small and large fetuses. This suggested that some form of cartilage correction or reconstruction was likely to occur after birth, especially at the triangular fossa and/or scapha. Infants appear to show significant region-specific variation in the postnatal growth of the auricular cartilages, especially at the triangular fossa and/or scapha.

Key words: auricular cartilage, scapha, triangular fossa, concha, external auditory meatus

Introduction

Six hillocks from the first and second pharyngeal arches (Streeter, 1922; Wood-Jones and Wen, 1933; Siegert et al., 1994; Karmody and Annino, 1995; Park and Roh, 1999; Porter and Tan, 2005) differentiate into each part of the auricle: hillock-1 into the tragus, hillock-2 into the crus helix, hillock-3 into the ascending helix, hillocks-4 and 5 into the helix, scapha and antihelix, and hillock-6 into the helix and antitragus (Monta and Quintanilla-Dieck, 2017; Honkura et al., 2020). Fusion of the
hillocks is followed by drastic changes in the topographical relationship between parts of the auricle as a result of differential growth, i.e., a considerable difference in growth rate between parts (Kagurasho et al., 2012). However, Kagurasho et al. (2012) seemed not to describe when and how the differential growth occurs to make a specific structure such as the tragus-antitragus complex of the auricle: not the corresponding cartilages but an early-developed muscle (antitragicus) may contribute to deepen the intertragic groove or incisura (Honkura et al., 2020). Because the auricular cartilage is composed of a single plate until midterm (Honkura et al., 2020), we needed to demonstrate when and how the plate provides the folds, thickenings and/or tubercles that delineate the major three hollows or caves: the triangular fossa, the scapha and the concha.

Using histological sections from mid-term and near-term human fetuses, our group has recently demonstrated that 1) the scapha and antihelix develop secondarily through looping of the cartilage near term; 2) extrinsic muscle insertions quite different from those in the adult suggest a postnatal change in the topographical relationships between cartilage and muscle (Honkura et al., 2020). However, because that study focused on muscle insertions and because no tangential sections were examined, we were unable to obtain a comprehensive understanding of when and how the cartilage plate provides the scapha, the concha and the triangular fossa. Moreover, even in adults, histological studies have provided only very limited understanding of auricular cartilage configurations. Therefore, it appears that the starting point of any subsequent study should be a comparison between the adult and late-stage fetus. Therefore our aim in the present study was to clarify the topographical anatomy and variations of the growing auricular cartilage. In addition, the present tangential sections may also provide new evidences of the auricular muscle development.

Materials and Methods

The study was performed in accordance with the provisions of the Declaration of Helsinki 1995 (as revised in Edinburgh 2000). We examined paraffin-embedded histological sections from 42 late-stage fetuses (approximate gestational age 24-40 weeks; crown-rump length
(CRL) 190-330 mm) and seven cadavers of elderly individuals.

The specimens from the seven cadavers (2 males and 5 females; aged 76-97 years at death) were both the left and right auricles from each individual. The cause of death had been ischemic heart failure or intracranial bleeding. These cadavers had been donated to Tokyo Dental College for research and education on human anatomy, and their use for research had been approved by the university ethics committee. The cadavers had been fixed by arterial perfusion of 10% v/v formalin solution and stored in 50% v/v ethanol solution for more than 3 months. From each cadaver, we prepared tissue blocks including the whole auricle. The specimens were decalcified by incubating them at room temperature in Plank-Rychlo solution (AlCl$_2$/6H$_2$O, 7.0 w/v%; HCl, 3.6; HCOOH, 4.6) for a week. After routine procedures for paraffin embedding, we prepared almost sagittal sections from one side and horizontal sections from the other side of the bilateral auricles (10 µm thickness at intervals of 200 or 300 µm) and stained with hematoxylin and eosin (HE). This “almost sagittal” plane was tangential to the external surface of the auricle, and therefore some of the sections contained a large area from the top of the helix to the lobule of the ear.

All 42 late-stage fetuses were part of the collection kept at the Department of Anatomy, Akita University, Akita, Japan. These specimens had been donated to the Department by the families concerned between 1975 and 1985 and preserved in 10% (w/w) neutral formalin solution for more than 30 years. The available specimen data were limited to the date of donation and GA, but there was no information on family name, the name of the obstetrician or hospital, and the reason for abortion. The use of these fetuses for research was approved by the ethics committee of Akita University (No. 1428). The left or right auricle was removed from the fetal head taking special care not to detach the lateral part of the external acoustic meatus from the specimen. After routine procedures for paraffin embedding, we prepared semiserial sections (100-µm intervals; 10 µm thick) and stained them with hematoxylin and eosin (HE). The sectional planes were horizontal (35 fetuses) and tangential or almost sagittal (7 fetuses). Among the horizontal sections, those from 12 fetuses overlapped with materials used for our recent study (Honkura et al., 2020). Therefore, for the present study, we newly prepared horizontal
sections from 23 fetuses as well as tangential sections from 7 fetuses.

Most photographs were taken with a Nikon Eclipse 80, whereas photographs at ultra-low magnification (objective lens less than x1) were obtained using a high-grade flat-bed scanner with translucent illumination (Epson scanner GTX970). Measurements of fetal auricles were not conducted, as the data have already been reported by Honkura et al. (2020).

Results
a. Gross observations of the external skin surface of the auricle

In adult specimens, we identified skin folds surrounding the three hollows or caves – the triangular fossa, the scapha, and the concha – from a superior site to an inferior direction (Fig. 1A). The largest fossa, i.e., the concha, was divided into an upper part (cymba conchae) and a lower part (cavum conchae) by the crus helix. The tragus and antitragus sandwiched a deep intertragic notch that opened to the concha. The external auditory meatus (EAM) started from the bottom of the lower concha.

Unexpectedly, the present fetuses consistently carried all of the triangular fossa, scapha, and concha, and had skin folds delineating these major hollows (Fig. 1B). Therefore, the external morphology of the auricle was quite similar to that in adults. In fetuses, however, the triangular fossa was often shallow and nearly flat.

b. Observations of sections from adults: three basic cartilage rings

Adult tangential sections demonstrated three ring-like cartilages corresponding to the scapha, the triangular fossa, and the concha (Fig. 2). Another (incomplete) cartilage ring, corresponding to the lateral end of the EAM, was attached to the inferior part or the concha ring. The scapha ring was slender and irregularly shaped, and it opened widely to the superior end of the concha ring without a clear border demarcated by cartilage (Fig. 2A). The ring of the triangular fossa was separated from the concha ring by the antihelix (Fig. 2C,D). The largest fossa, i.e., the concha, was divided into the upper and lower rings by the crus helix (Fig. 2E).

The crus was continuous with the tragus cartilage that separated
the concha from the EAM. The antitragus was identified as the lateral end of a thickened cartilage between the upper and lower rings of the concha (Fig. 2F). The deepest sections contained two cartilage rings corresponding to the upper and lower parts of the concha in addition to an incomplete cartilage ring of the EAM (Fig. 2E,G). Finally, it is notable that, in horizontal sections (Fig. 2H-J), we did not find any ring or loop rather but a single, highly wavy plate, except for the cartilages surrounding the EAM. Therefore, in adults, the major three hollows were provided by a combination of acute turns or bends of the cartilage plate, and not by a combination of real cartilage rings.

c. Observations of tangential sections from late-stage fetuses

The present tangential sections partially contained subcutaneous tissues, as the auricle was embedded in the latter to various degrees (Figs. 3 and 4). A cartilage loop of the scapha was always evident in superficial sections but did not provide a complete ring in three of the seven specimens (Fig. 4B,C). Irrespective of whether the ring was complete or incomplete, a candidate for the transverse auricular muscle was seen in the scapha cartilage loop (Figs. 3A and 4B) and it appeared to be continuous with the helix major muscle. An irregularly shaped cartilage ring of the concha was evident in deep sections (Figs. 3H,I and 4J,K), being located superior to the EAM, and – unlike the adult morphology – was not divided by the crus helix (Figs. 3E and 4F). Instead, the crus was identified at a site between the concha and the EAM. Therefore, the concha ring in fetal tangential sections was most likely to correspond to the upper part of the adult concha.

The crus was connected to the tragus cartilage medially and to the antitragus laterally (Figs. 3E,F and 4G,H). A wavy cartilage plate, i.e. the helix, was always continuous with the lateral end of the EAM cartilages (Figs. 3G,H and 4E,F). A cartilage loop corresponding to the triangular fossa was found in only one of the tangential sections (Fig. 3D), whereas in the other six specimens there was no cartilage loop superolateral to the concha loop as well as the helix (Fig. 4). Consequently, a thickening or tubercle of the cartilage provided the crus helix and tragus antitragus, whereas a cartilage loop provided the triangular fossa, scapha and concha.
d. Observations of horizontal sections from late-stage fetuses

Figures 5-8 for horizontal sections are arranged in order from smaller (190 mm CRL) to larger (330 mm CRL) specimens, and therefore the arrangement does not connect to each of the loop variations (Table 1). Above the crus helix, the scapha cartilage loop was identified as a continuation of the deep medial bending of the cartilage plate at a position almost midway along the anteroposterior axis (Figs. 5E-G and 6B,C) or at the posterior portion (Fig. 7C,D). Large specimens did not always carry the scapha loop (Fig. 8A). Since the top of the scapha loop was identified as a small cartilage mass (Figs. 5D and 6A), the loop extended superiorly to make a funnel-like protrusion.

The triangular cartilage loop was identified at the top of the cartilage plate in small fetuses (Fig. 5A,B) and, depending on the superior growth of the cartilage plate, it appeared to descend to an anterosuperior portion in larger fetuses (Fig. 7A). Irrespective, the fossa was always located in a skin projection free from the temporal skin. The triangular fossa also appeared to correspond to an acute medial bending of the cartilage plate below the loop (Fig. 7B). The loop of the triangular fossa was often absent (Table 1).

The concha cartilage loop was always found below or at the same supero-inferior level of the crus helix in horizontal sections (Figs. 6F,G, 7G and 8E). Sometimes, double loops were evident: an upper loop at the level of the crus and a lower loop continuous with the intertragic notch (Fig. 6L). Notably, the upper part of the concha in tangential sections (Figs. 3H and 4J) was identified as an anteriorly located highly wavy plate in horizontal sections (Figs. 6D,E, 7F and 8C), representing a critical difference between tangential and horizontal sections. Because the bottom of the concha loop was identified as a cartilage tubercle near the tragus (Figs. 6H and 8F), the loop extended inferiorly to make a small funnel. Near the crus, the spine of the helix was sometimes evident (Fig. 8D), but it received no muscles. Finally, a future intertragic notch was often embedded in the temporal subcutaneous tissue, and not in a skin projection of the auricle.

Discussion
Throughout this study, we noted three major auricular hollows (or caves): the concha, the scapha, and the triangular fossa. Actually, Honkura et al. (2020) noted a cartilage loop corresponding to the scapha separated from a cartilage plate. However, the present observation of combined cartilage rings in adults (Fig. 2) seems to provide a breakthrough for understanding the configuration of the auricular cartilage. When we sectioned these three rings, i.e., the cartilage part surrounding each of the three major hollows, the rings were simply composed of a highly wavy cartilage plate (Fig. 2H-J). In contrast, the cartilage looping, that was typically seen in the developing scapha (Figs. 5G, 6C and 7C), seemed to be the most striking morphology in cross sections of near-term auricles. There were four patterns according to combinations of whether the loop was present or absent at the three major hollows (Table 1).

Because of the absence in adults and because of the variations at near term, the cartilage looping was likely to be a transient morphology even if present. Therefore, we hypothesized two ways to provide the adult morphology (Fig. 9): irrespective of the transient loop, a simple cartilage plate is likely to provide multiple deep bends or folds corresponding to the major hollows. The looping mechanism is able to provide an independent cartilage separated from the initial plate: such a separation might cause abnormal auricles. In contrast, a restricted thickening of the cartilage plate provides the crus helix, tragus and antitragus. People may consider the triangular fossa to be simply the anterior end of the antihelix, based on the external view (Fig. 1A). However, the antihelix was formed as a “limb” of the scapha loop, while the triangular fossa developed independently superior to the scapha. Therefore, the antihelix appears to be a secondary derivative from the helix. Taken together, with or without looping, an initial cartilage plate seemed to build external protrusions corresponding to the major hollows (Fig. 10) although the drawing does not explain the triangular fossa starting development at the top of the cartilage plate.

Any variation in the transient three-loop morphology (Table 1) is unlikely to be a result of differences in developmental stage, as the four patterns were almost evenly distributed among small and large fetuses. Therefore, at birth, a significant region-specific variation appeared to be present in the auricular cartilage especially in and around the triangular fossa and scapha. The present variations in the growing cartilage seemed to suggest a kind of redundancy in pattern formation of the major three hollows or caves of auricle. Otherwise, it suggested a delay of growth in the limited part although we have no information
about the postnatal growth. Using recent radiological techniques such as ultrasound, further study of the auricles in children is considered necessary to confirm this suggested reconstruction of the cartilage. Nevertheless, since the triangular loop was usually small, we could not rule out the possibility that it was missed in the present semiserial sections cut at an interval of 100 um. This might account for the present small number of fetuses showing a cartilage loop of the triangular fossa.

With regard to muscle insertions, the present study confirmed our previous observations (Honkura et al., 2020), i.e. 1) the superior auricular muscle did not insert to the upper helix, but rather to a site near the crus helix in fetuses; 2) the anterior auricular muscle did not insert to the spine of the helix, but to the anterior end of the cartilage plate. The eminence and ponticulus of the concha in the medial aspect of the auricle are known to be posterior muscle insertions: these structures in the medial aspect was near the crus helix in adult tangential sections (Fig. 2C). The present tangential sections demonstrated that the transverse auricular muscle first appeared within the scapha loop in fetuses. The superior and anterior muscle insertions would likely be corrected through differential growth. Consequently, the classical concept of a single funnel-like cartilage with muscles in the growing auricle might be oversimplified by the adult external morphology.

Acknowledgments
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FIGURE LEGENDS

Figure 1. Right ear auricles of an adult and a late-stage fetus.
Panel A, a 91-year-old man; panel B, a fetus with a CRL of 230 mm (28
weeks). In both specimens, three hollows or caves (concha, scapha and
triangular fossa) are evident. The triangular fossa is located at the upper
end of the antihelix. The concha is divided into upper and lower parts by
the crus helix. Scale bars, 10 mm.

Figure 2. Sagittal and horizontal sections of the auricle from an 85-year-old
man.
Panels A-G, almost sagittal sections tangential to the external aspect of the
auricle; panels H-J, horizontal sections of the contralateral side. Panel A displays
the most lateral or superficial plane in panels A-G. Panel H shows the most
superior plane in panels H-J. In tangential sections, three hollows or caves
(concha, scapha and triangular fossa) can be identified as a ring-like structure,
respectively. A cartilage ring surrounding the scapha is attached to the
superoposterior margin of a large ring corresponding to the concha. The concha
ring is divided into upper and lower parts by the crus helix. The triangular fossa is
separated from the concha ring by the antihelix. In contrast to tangential sections, horizontal sections exhibit a highly wavy cartilage plate without a ring or loop. EAM, external auditory meatus; PAM, posterior auricular muscle; SAM, superior auricular muscle. All panels were prepared at the same magnification (scale bar in panel A, 10 mm).

**Figure 3. Almost sagittal sections tangential to the external aspect of the auricle from a fetus with a CRL of 290 mm (34 weeks).**
Panel A displays the most lateral or superficial plane in the figure, while panel I the most medial or deepest plane. A cartilage loop of the scapha can be seen in the superficial sections ( Panels A and B) and contains a candidate for the transverse auricular muscle (TAM). The triangular fossa (panel D) is formed by acute bending of the cartilage (panel C). The concha loop (panel I) is provided by a highly wavy cartilage plate (panels GH). A skin notch is not evident between the tragus and the antitragus (panel D). ATM, antitragus muscle; EAM, external auditory meatus; HMM, helix major muscle; SAM, superior auricular muscle; SCM, sternocleidomastoideus muscle. Scale bar in panel A, 2 mm.

**Figure 4. Almost sagittal sections tangential to the external aspect of the auricle from a fetus with a CRL of 243 mm (29 weeks).**
Panel A displays the most lateral or superficial plane in the figure, while panel K the most medial or deepest plane. The cartilage loop of the scapha is not closed in superficial sections (Panel C) and contains a candidate for the transverse auricular muscle (TAM). A loop corresponding to the triangular fossa is absent. The concha loop (panel JK) is provided by a highly wavy cartilage plate (panels G-I). A skin notch is not evident between the tragus and the antitragus (panel G). A candidate for the superior auricular muscle (SAM) does not insert to the top of the concha loop (panel I). AAM, anterior auricular muscle; ATM, antitragus muscle; EAM, external auditory meatus; HMM, helix major muscle. Scale bar in panel A, 2 mm.

**Figure 5. Horizontal sections of the auricle from a fetus with a CRL of 190 mm (24 weeks).**
Panel A displays the most superior plane in the figure, while panel N the most inferior plane. A cartilage loop of the triangular fossa is located near the top of the auricle (panel A). The scapha loop is evident superior to the crus helix.
(panels D-G). The concha loop is absent. The tragus and antitragus are located near to each other and connected by a cartilage plate (panels MN). A candidate for the superior auricular muscle (SAM) does not insert to the top of the concha loop (panel I). AAM, anterior auricular muscle; ATM, antitragus muscle; EAM, external auditory meatus; PAM, posterior auricular muscle. Scale bar in panel A, 2 mm.

Figure 6. Horizontal sections of the auricle from a fetus with a CRL of 258 mm (31 weeks).
Panel A displays the most superior plane in the figure, while panel N the most inferior plane. The scapha loop is evident superior to the crus helix. (panels BC), while the concha loop is located in the level of the crus helix (panel FG). A cartilage loop of the triangular fossa is absent. The black star in panel A indicates the top of the scapha loop, while the clear star in panel H indicates the bottom of the concha loop. A candidate for the lower half of the concha loop is evident near the tragus and antitragus and is continuous with the intertragic skin notch (panels KL). AAM, anterior auricular muscle; ATM, antitragus muscle; EAM, external auditory meatus; HMM, helix major muscle; SAM, superior auricular muscle. Scale bar in panel A, 1 mm.

Figure 7. Horizontal sections of the auricle from a fetus with a CRL of 270 mm (32 weeks).
Panel A displays the most superior plane in the figure, while panel G the most inferior plane. The cartilage loop of the triangular fossa is located in the supero-anterior part of the cartilage plate (panel A). The scapha loop is included in the skin projection of the auricle (panels CD). The concha loop is located anterior to the tragus (panel G). The tragus and antitragus are connected by a cartilage plate (panel G). The superior auricular muscle (SAM) inserts to the cartilage plate near the crus, and not to the top of the plate (panel E). ATM, antitragus muscle; EAM, external auditory meatus. Scale bar in panel A, 1 mm.

Figure 8. Horizontal sections of the auricle from a fetus with a CRL of 310 mm (37 weeks).
Panel A displays the most superior plane in the figure, while panel H the most inferior plane. The cartilage loops of the triangular fossa and scapha are absent. The concha loop is located below the crus helix (panel DE). The tragus and
antitragus are connected by a cartilage plate (panel G). The superior auricular muscle (SAM) inserts to the cartilage plate near the crus, and not to the top of the plate (panel B). AAM, anterior auricular muscle; ATM, antitragus muscle; EAM, external auditory meatus; PAM, posterior auricular muscle. Scale bar in panel A, 5 mm.

**Figure 9. Schematic representations showing the hypothetical growth of the auricular cartilage plate.**
A cartilage loop is similar to a deep fold of the cartilage plate in cross sections of near-term auricles. However, even if it is transient, the looping is likely to provide an independent cartilage separated from the mother plate. The present study demonstrated individual variations in cartilage looping for each of the major hollows or caves (Table 1). Therefore, without looping, one or two of the hollows is/are likely to originate from an initial cartilage plate. There seemed to be two ways to build the auricular hollows.

**Figure 10. A diagram showing small cartilage funnels protruding from the auricular cartilage plate.**
Three cartilage protrusions, corresponding to the triangular fossa, scapha and (lower) concha, are formed by a looping of the single cartilage plate, respectively. In association with the elongating tragus, there funnel-like protrusions provide growth of the auricle. However, this model misleads “new” caves (dark portions) in the external view of auricle.
Figure 1. Right ear auricles of an adult and a late-stage fetus.
Figure 2. Sagittal and horizontal sections of the auricle from an 85-year-old man.
Figure 3. Almost sagittal sections tangential to the external aspect of the auricle from a fetus with a CRL of 290 mm (34 weeks).
Figure 4. Almost sagittal sections tangential to the external aspect of the auricle from a fetus with a CRL of 243 mm (29 weeks).
Figure 5. Horizontal sections of the auricle from a fetus with a CRL of 190 mm (24 weeks).
Figure 6. Horizontal sections of the auricle from a fetus with a CRL of 258 mm (31 weeks).
Figure 7. Horizontal sections of the auricle from a fetus with a CRL of 270 mm (32 weeks).
Figure 8. Horizontal sections of the auricle from a fetus with a CRL of 310 mm (37 weeks).
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