The Laryngoscope

The Developmental Origin of the Auricula Revisited

Christianne C. A. F. M. Veugen, MD; Frederik G. Dikkers, MD, PhD; Bernadette S. de Bakker, MD, PhD

Objectives/Hypothesis: Congenital auricular anomalies are common. Additionally, the auricle plays an important role in the staging of human embryos. However, little is known about the embryological development of the auricle. The most commonly reproduced developmental theory by His (1885) describes six hillocks; three on the first and three on the second pharyngeal arch. The aim of this study was to assess the validity of this theory by modern techniques and to expand the knowledge of the embryological development and morphology of the auricle.

Study Design: 22 human embryos from the Carnegie collection between Carnegie stage 13 and 23 (28–60 days) were selected based on their histological quality.

Methods: Histological sections of the selected embryos were examined. Three-dimensional (3D) reconstructions were prepared. Additionally, literature research was performed.

Results: The hillocks were absent in most stages. Contrary to common knowledge, the auricle is almost entirely innervated by branches of the facial nerve. The branches of the trigeminal nerve only innervate the tragus and the anterior external auditory meatus (EAM). Consequently, this indicates that almost the entire auricle is derived from the second pharyngeal arch, with the exception of the tragus and the anterior EAM.

Conclusions: The 3D reconstructions show the anatomy and development of the auricle to be different from concepts presented in current textbooks. As a consequence, we propose that preauricular sinuses should be classified as first pharyngeal arch anomalies.

Key Words: Embryology, external ear, congenital anomalies, pharyngeal arch anomalies.

Level of Evidence: NA

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INTRODUCTION

Congenital auricular anomalies are common; 50% of the congenital otorhinolaryngologic anomalies affect the ear.[1] Additionally, the auricle plays an important role in the staging of human embryos, as the Carnegie classification system (CCS) relies on the morphology of the auricle from Carnegie stage (CS)16 until CS18 (Table I).[2] However, little is known about the embryological development of the auricle. Most knowledge is based on early scientific reports, some published more than 100 years ago. The most frequently reproduced developmental theory by His (1885) describes six auricular hillocks: three on the first pharyngeal arch (PA1) and three on the second pharyngeal arch (PA2) (Fig. 1).[3] Understanding the embryological development of the auricle is essential to the understanding of different congenital syndromes and the CCS. To study auricular development, detailed three-dimensional (3D) reconstructions of the forming auricle were made, and an extensive literature search was performed. The aims of this study were to assess the validity of the theory by His and to expand our knowledge of the embryological development and morphology of the auricle.

Background

The Carnegie classification system. The CCS is named after the renowned Carnegie collection of embryonic specimens. Fixation and preservation are associated with shrinkage of tissue, making it difficult to accurately age an embryo on size alone. The CCS was introduced as a staging scheme based on morphological characteristics of the embryonic specimen (Table I).[4,5] The CCS relies in part on the morphology of the auricle from CS16 until CS18.

Morphology—hillocks. In 1885, His described six auricular hillocks that give shape to the human auricle: three on PA1 and three on PA2 (Fig. 1). The hillocks of PA1 form the tragus and the helix as far as the auricular tubercle. The hillocks of PA2 form the rest of the auricle including the lobule (Fig. 1A–C).[3] The existence of His' hillocks has been debated by several authors.[6–8] Authors have had difficulties identifying the hillocks and following their transitions.[6,9] In 1922, Streeter argued that the essential histological change

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TABLE I.
Overview of Embryonic Specimens Used to Examine the Development of the Auricle.

CS	Age, d	Origin*	Specimen	Acquisition Year	CRL, mm	Sex	Fixation Medium	Staining	Plane†	Sections No.	Z-res, μm
13	28–32	CC	5541	1927	4.08	—	Formol	Alum cochineal, eosin	T	379	10.76
13		CC	836	1914	4.09	F	Mercuric chlorine	Alum cochineal (i.e., carmine)	T	247	16.55
14	31–35	CC	8314	1945	5.16	—	Formol	Azan	T	639	8.07
14		CC	6502	1931	5.54	—	Could be Souza	Hematoxylin and eosin	T	1107	5.01
15	35–38	CC	721	1913	4.79	—	Zenker's formol	Hematoxylin and eosin	T	552	8.69
15		CC	3512	1921	6.55	—	Formol	Alum cochineal (i.e., carmine)	T	651	10.06
16	37–42	CC	8773	1950	6.74	—	Bouin	Azan	C	628	10.73
16		CC	6517	1931	10.46	—	Corrosive acetic acid	Alum cochineal (i.e., carmine)	T	547	19.13
17	42–44	CC	6521	1933	10.60	—	Corrosive acetic acid	Alum cochineal (i.e., carmine)	T	1059	10.01
17		CC	6520	1932	12.21	—	Corrosive acetic acid	Alum cochineal (i.e., carmine)	T	684	17.86
18	44–48	CC	6524	1933	9.73	—	Corrosive acetic acid	Aluminum cochineal	T	956	10.18
18		CC	4430	1923	15.85	F	Corrosive acetic acid	Alum cochineal (i.e., carmine)	T	419	37.19
19	48–51	CC	2114	1918	12.59	F	Formalin	Aluminum cochineal	T	309	40.75
19		CC	8965	1952	17.72	—	Zenker's formol	Borax carmine–orange G	T	292	60.69
20	51–53	CC	462	1910	15.93	M	Formol	Aluminum cochineal	T	376	42.36
20		AMC	S2025	~1975	19.77	M	Bouin	Hematoxylin–azophloxine	T	648	30.51
21	53–54	CC	7254	1936	17.36	M	Bouin	Hematoxylin and eosin	T	288	60.12
21		CC	4090	1922	19.43	F	Formol	Alum cochineal (i.e., carmine)	T	195	99.62
22	54–58	BC	895	1914	21.22	F	Formol	Aluminum cochineal	T	420	50.52
22		CC	H983	1962	28.00	M	Formalin	Hematoxylin/trichrome/silver	T	408	53.00
23	56–60	CC	950	1914	23.79	M	Formalin	Aluminum cochineal	T	557	42.71
23		CC	9226	1954	30.01	M	Formol	Azan	T	208	144.28

*Origin of the specimen: AMC = Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands. BC = Boyd Collection: Cambridge University, Cambridge, United Kingdom. CC = Carnegie Collection: National Museum of Health and Medicine, US Army, Silver Spring, MD.
†Plane of sectioning.
C = coronal; CRL = crown-rump-length calculated in millimeters; CS = Carnegie stage; F = female; M = male; T = transversal; Z-res = Z-resolution calculated in micrometers.

that sets the formation of the auricle consists of proliferation and condensation of the mesenchyme, and that the auricular hillocks are foci in which the mesenchymal proliferation is temporarily most rapid. Therefore, the hillocks are incidental characteristics rather than fundamental anatomical entities in the development of the auricle.[7] Nevertheless, the hillock model has prevailed in literature with small alterations over the years. The most frequently reproduced version in embryological literature is that the hillocks of PA1 form the tragus and the anterior part of the helix, and those of PA2 form the bulk of the auricle (Fig. 1D).[10]

Morphology—pharyngeal arches. The question regarding what is derived from PA1 and what is of PA2 origin is probably of more significance than the existence of the hillocks. In the literature, the traditionally held view is that the tragus and at least part of the anterior helix (including the root) are derived from PA1.[3,7,8,10] Prior to His' definition, the entire auricle was believed to be a PA2 derivative.[11] Wood-Jones and I-Chuan concluded from their studies of clinical cases that the auricle is a PA2 derivative, and that the contribution of PA1 is represented only by the tragus and the anterior part of the external auditory meatus (EAM) (Fig. 1D).[6]

Morphology—rotation. His suggested that if the anterior helix is originating from PA1, the first arch has to make a dorsal rotation around the EAM (Fig. 1D).[3] Wood-Jones and I-Chuan proposed that if only the tragus and the anterior EAM is of PA1 origin, PA2 has to make a ventral rotation around the EAM (Fig. 1D).[6]

MATERIALS AND METHODS

Embryonic Specimens

Twenty-two human specimens were used to study the development of the auricle (Table I). All embryos included in this study are historical specimens, which, according to available information, have been collected between 1910 and 1975. Most of the embryos are from the Carnegie collection in Silver Spring, Maryland; one specimen is from the Boyd collection, University of Cambridge, Cambridge, United Kingdom, and one specimen is from the collection of the Department of Medical Biology, Section Clinical Anatomy and Embryology, Amsterdam UMC, Amsterdam, the Netherlands. The studied embryonic specimens range in age from 28 to 60 days, corresponding to CS13 to CS23.[4,5] Histological sections of two specimens per stage were analyzed.

After fixation and embedding, all specimens were sectioned and stained with different histological staining methods. Additional specifications are presented in Table I. The embryos were staged using Streeter's original classification of embryos and its modified version by O'Rahilly and Müller.[4,5] Digital images were taken of the exterior of the original embryos and all histologically stained sections, allowing comparison of the 3D reconstructions to the original embryonic specimens.

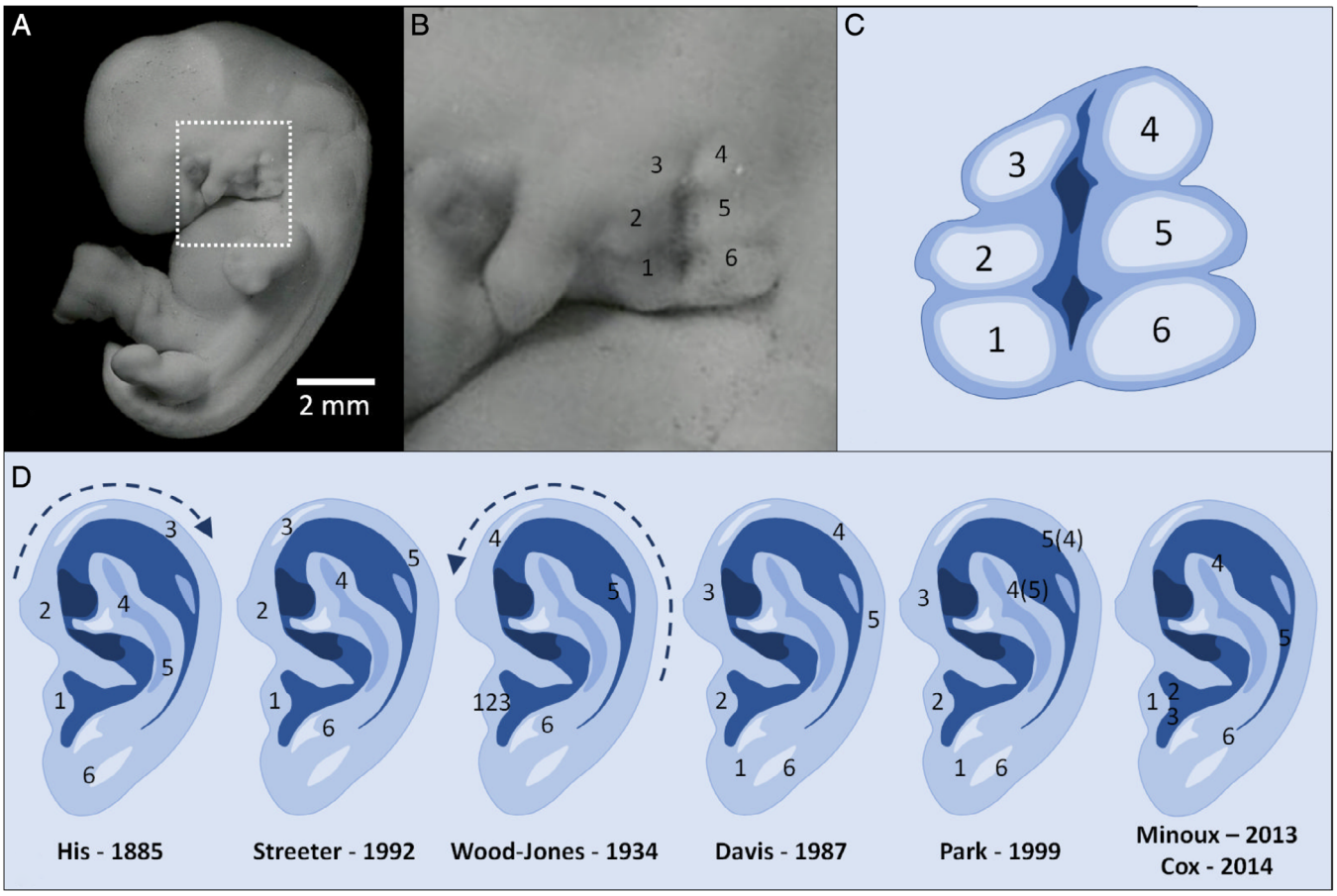

Fig. 1. The proposed theories on the embryological development of the human auricle. (A) Lateral view of the original embryo in Carnegie stage 17 (42–44 days) specimen 6521. (B) Close up of the auricular hillocks as described by His in 1885.[3] Image reproduced with permission of the National Museum of Health and Medicine. (C) Simplified version of the auricular hillocks. Numbers 1 to 3 have been described to be of the first pharyngeal arch origin and 4 to 6 as the second pharyngeal arch derivative. (D) The proposed theories of the embryological development of the human auricle as described by several authors. The fate of the hillocks as described by the authors are indicated by numbers 1 to 6. The first arrow represents the dorsal rotation of the second pharyngeal arch as described by His in 1885.[3] The second arrow represents the ventral rotation of the first pharyngeal arch as described by Wood-Jones and I-Chuan in 1934.[6] [Color figure can be viewed in the online issue, which is available at www.laryngoscope.com.]

3D Reconstructions

Detailed 3D reconstructions of the auricle were made by a trained analyst under supervision of experienced embryologists, by manually segmenting each structure in histologically stained sections, using the software package Amira (version 5.6, http://www.amira.com; Thermo Fisher Scientific, Waltham, MA). The segmented structures were skin surface, auricular cartilages, the PA1 nerve (the trigeminal nerve [nV]), and the PA2 nerve (the facial nerve [nVII]) and their branches. Each structure was bilaterally segmented in each embryo if present in the studied specimen. In the framework of the making of an interactive 3D atlas and quantitative database of human development,[12] adjacent organs and structures were reconstructed as well, permitting reliable positioning of the auricle relative to other structures. Interactive 3D models of two specimens per stage were created. The aligned 3D images were compared with the photographs of the exterior of the original embryos and the alignment was adjusted, if necessary. In each specimen both auricles were represented in 3D, but for the purpose of easy comparison, all figures display the left auricle.

Literature Research

To gain an overview of the available literature concerning the theories on the developmental origin of the auricle, we performed queries in PubMed and reviewed 69 case reports, case series, laboratory research, and literature reviews from 1844 to 2018 including theories about the embryology of the auricle and congenital auricular anomalies and pharyngeal arch anomalies. References of the included papers were reviewed; five additional case reports and literature reviews were included. We reviewed the knowledge about the embryology of the auricle and congenital auricular anomalies that was written in embryological textbooks of the last 150 years.

RESULTS

Morphology—Hillocks

A schematic reproduction based on the 3D reconstructions of the development of the left auricle from CS15 until CS23 is demonstrated in Figure 2. In CS16, the morphology of the 3D reconstruction resembles the hillocks previously described by His (Fig. 1).[3] In the other stages, no hillocks can be identified.

Morphology—Innervation

The PA1 nerve (nV) and its branches are indicated by the purple dotted lines in Figure 2; the PA2 nerve

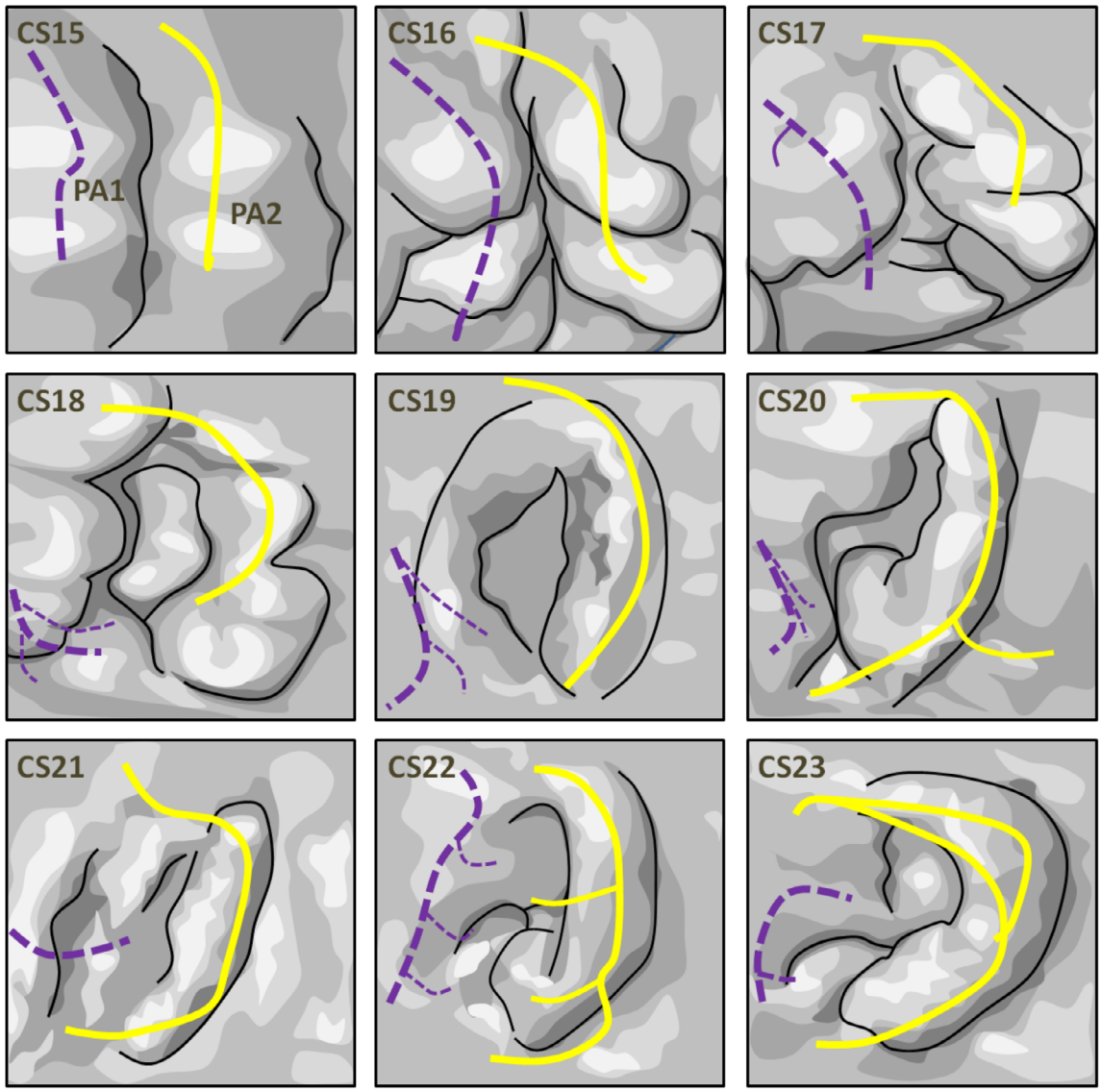

Fig. 2. Schematic reproduction of the development of the auricle based on the three-dimensional reconstructions. The developing auricle is presented in Carnegie stage (CS)15 (35–38 days) to 23 (56–60 days) embryos, displaying the left auricle. The pharyngeal arches are indicated by PA1 (the first pharyngeal arch) and PA2 (the second pharyngeal arch). A schematic reproduction of the trigeminal nerve and its branches is indicated by the purple dotted lines. The facial nerve and its branches are indicated by the yellow continuous lines. [Color figure can be viewed in the online issue, which is available at www.laryngoscope.com.]

(nVII) and its branches are indicated by the yellow continuous lines. In Figure 3, a close up of the 3D reconstruction of the developing nerves is shown. In CS16 the trigeminal branches innervate the entire anterior part of the auricle (Fig. 3A). Later, in CS22 (Fig. 3B) and CS23 (Fig. 3C), only the tragus and the anterior EAM are left to be innervated by the trigeminal branches. In CS16, the facial branches innervate the entire dorsal part of the auricle (Fig. 3A). In CS22 and CS23, the facial branches innervate almost the entire auricle with exception of the tragus and the anterior EAM (Fig. 3B,C).

Morphology—Pharyngeal Arches

The pharyngeal arches are indicated in CS15 by PA1 and PA2 (Fig. 2). Reviewing the innervation of the auricle and the gradual development of the auricle in time in the reconstruction in Figure 2, it appears that in CS22 and CS23, the entire auricle is of PA2 origin, with exception of the tragus and the anterior EAM.

DISCUSSION

Morphology—Hillocks

The hillock model has dominated major embryological textbooks for many decades. In the present study, poor resemblance was found with this model. Only in CS16 does the morphology of the auricle show similarities to the hillocks previously described by His.[3] In no other CS were the authors able to recognize structures that resemble hillocks. This is in accordance to what was

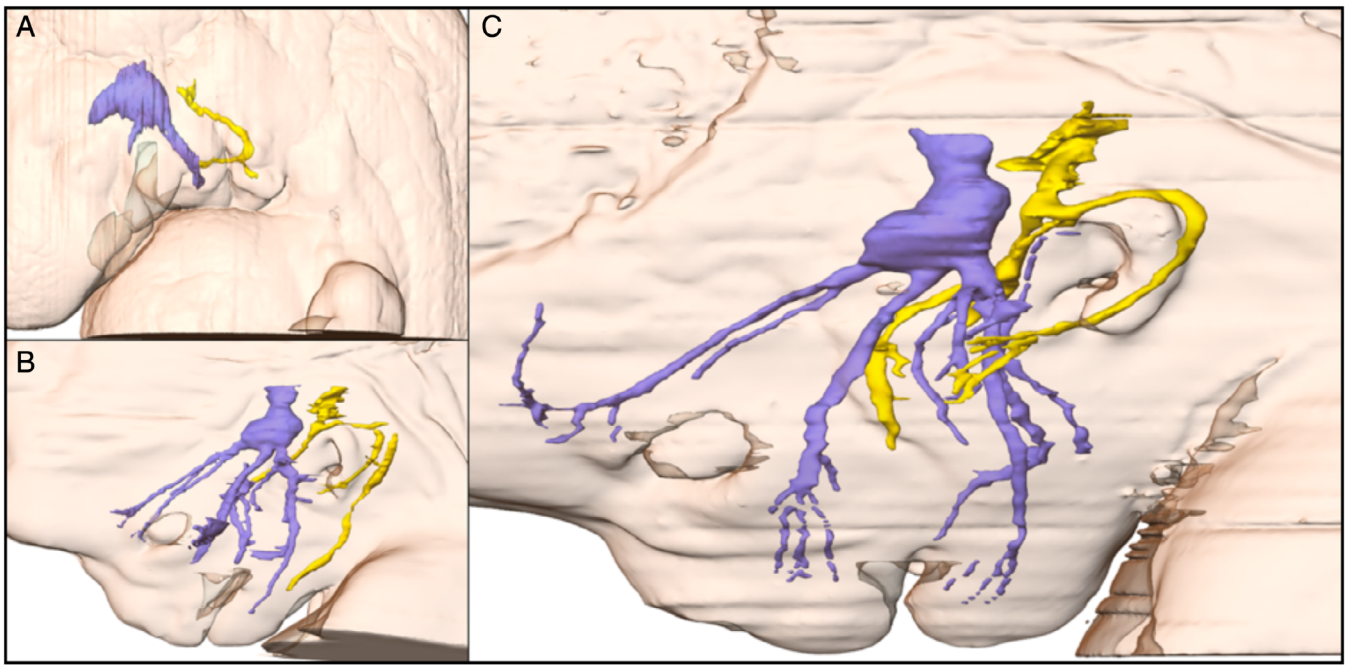

Fig. 3. Development of the trigeminal nerve and the facial nerve. Close up of a three-dimensional reconstruction of the developing left auricular area. The trigeminal nerve is indicated in darker purple; the facial nerve is indicated in bright yellow. (A) Lateral view of a Carnegie stage 16 embryo (37–42 days). (B) Lateral view of a Carnegie stage 22 embryo (54–58 days). (C) Lateral view of a Carnegie stage 23 embryo (56–60 days). [Color figure can be viewed in the online issue, which is available at www.laryngoscope.com.]

objectified by previous authors.[6–10] It supports the theory that the hillocks are merely foci where proliferation is most rapid, as previously suggested by Streeter and later by Porter and Tan.[7,8]

Morphology—Innervation

In the 3D reconstructions of the PA1 nerve (nV), at CS16 the branches innervate the entire anterior part of the auricle. At CS22 and CS23, the branches only innervate the tragus and the anterior EAM. In the 3D reconstruction of the PA2 nerve (nVII), at CS16, the branches innervate the dorsal part of the auricle. At CS22 and CS23, the branches innervate almost the entire auricle, with the exception of the tragus and the anterior EAM. This shift in innervation during embryonic development is an important indication to the pharyngeal arch origin of the auricle, and strengthens our hypothesis that only the tragus and the anterior EAM are of PA1 origin.

Our findings correspond with the knowledge about motoric innervation of the auricular muscles. The auricle has three ex- and six intrinsic muscles, all of which are innervated by motoric branches of nVII.[13] However, the literature states that the auriculotemporal nerve (originating from the nV) is the predominant sensory nerve supply for the anterior helix, the root, and part of the antihelix and tragus.[14] This does not correspond with our reconstructions of the nV. Wood-Jones and I-Chuan explained that the clinical study of innervation of branches of the nVII could have been mistaken for branches of the nV. This mistake can be made easily, due to the fact that the great interchange of fibers between the nV and nVII anatomically precedes the location where the anterior auricular branches are branching off from the nVII. Additionally, when looking at cases of neuritis of the nV, the auricle is not painful. Also, removal of the trigeminal ganglion or resection of the third division of the nV does not result in an anesthetic auricle.[6] Among clinicians, there is strong agreement that the nV supply is limited to the tragus and the anterior EAM.[6,15]

Morphology—Pharyngeal Arches

The question whether the auricle is largely a PA1 or a PA2 derivative has been debated by several authors. Most authors agree that at least part of the anterior helix is derived from the mandibular branch of the PA1.[3,7,8,10] Wood-Jones and I-Chuan, however, stated that only the tragus and the anterior EAM is of PA1 origin.[6] A recent publication by Minoux et al. studied *Hoxa2* mutations in mice.[15] *Hoxa2* encodes a homeobox transcription factor normally expressed throughout the neural crest-derived mesenchyme of PA2; the PA1 mesenchyme is *Hoxa2* negative. In humans, *Hoxa2* partial loss of function induces bilateral microtia. In mice, *Hoxa2* inactivation at early gestational stages results in EAM duplication and absence of the auricle, whereas late inactivation results in a hypomorphic auricle. This indicates that, in mice, the pinna is entirely contributed by PA2 *Hoxa2* neural crest-derived mesenchyme and is therefore a PA2-derived structure.[10,15] Cox et al. suggested that the auricle is mostly a PA2 derivative, but the imprecise use of the

term *pinna* in the former study includes the tragus and root. They observed that structures orthologous to the tragus are present in the adult mouse ears studied by Minoux et al. and consistently mice with mirror-image duplications (seen in ectopic *Hoxa2* expression in the PA1 neural crest) do not show evidence of a tragus.[15] This is consistent with the conclusion that the tragus is a PA1 derived structure.[10] The innervation patterns found in our 3D reconstructions of CS22 and CS23 support the theory that the auricle, with exception of the tragus and the anterior EAM, is derived from PA2.

A second observation in the study by Minoux et al. was that the mouse EAM is entirely lined by *Hoxa2*-negative PA1 mesenchyme and does not, therefore, develop at the first pharyngeal cleft, as previously assumed. These observations suggest that the EAM is instead derived from a distinct invagination within PA1 tissue.[15] It is possible that small branches of the nV innervating the posterior EAM were too small for detailed reproduction in our 3D reconstructions.

Morphology—Rotation

Seen in time, in the 3D reconstructions, the embryological development of the auricle from CS15 until CS23 can be understood as the dorsal part of the auricle—PA2 derivative—rotating ventrally around the EAM. As a result, PA2 will form almost the entire external ear (Fig. 2). This is similar to Wood-Jones and I-Chuan's description (Fig. 1).[6]

Clinical Cases—Preauricular Sinuses and Appendages

A preauricular sinus presents as a small pit, usually located at the anterior margin of the ascending limb of the helix.[16–18] A preauricular appendage presents as a small appendix and has a variety of anatomic locations caused by the line of migration of the auricle during embryonic life.[19] According to His, preauricular sinuses can be explained by incomplete or defective fusion of two hillocks. Preauricular appendages are explained to be accessory hillocks.[3] Wood-Jones and I-Chuan state the localization of preauricular sinuses and appendages form an obtuse-angled V-shaped line in front of the tragus that coincides with the line of the first pharyngeal cleft (Fig. 4).[6] Not previously mentioned by Wood-Jones and I-Chuan is the first branchial (or pharyngeal) cleft anomaly (FBCA).[20–22] A FBCA can present as a cyst, sinus, or fistula. The external component of an FBCA is located anterior to the auricle or in the submandibular region and follows the V-shaped line previously described by Wood-Jones and I-Chuan.[6,20,22] This could implicate that preauricular sinuses should be classified as PA1 anomalies. However, as preauricular appendages can appear farther away from the auricle, the authors are not convinced that their location always coincides with the V-shaped line as earlier proposed by Wood-Jones and I-Chuan.[10,15,23] It has been suggested by other authors that preauricular appendages are of PA2 origin. In mice, conditional ectopic *Hoxa2* expression in the PA1 neural crest is sufficient to induce a mirror-image duplication of the pinna and a loss of the EAM. In addition to the mirror image, they also

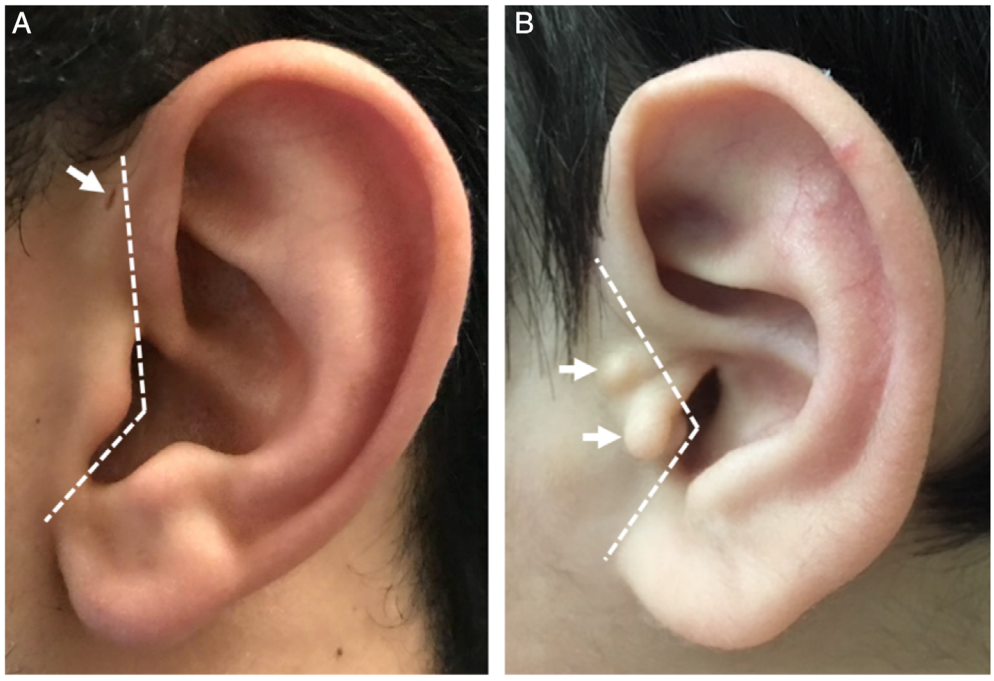

Fig. 4. Clinical cases of anomalies in the development of the auricle. (A) A clinical case of a preauricular sinus. The white arrow points at the ostium. (B) A clinical case of preauricular appendages. The white arrows indicate the two appendages. The dashed lines represent the most frequent localizations of preauricular sinus and preauricular appendages as described by Wood-Jones et al.[6] Pictures were taken with permission of the patients involved. [Color figure can be viewed in the online issue, which is available at www.laryngoscope.com.]

display small ectopic appendages that resemble preauricular appendages. However, no genes that were found in these appendages where specifiers of PA2 identity.[10,15]

Clinical Cases—Interrupted Development of PA1

Wood-Jones et al. describe two cases of absence of the mandibular process of PA1. Failure of development expresses itself over a wide range of malformations, from trivial degrees of micrognathia to complete agnathia. The auricle can be deformed in some of these cases but is never absent. The typical condition seen in cases of agnathia is for the entire auricle being developed behind the EAM; the tragus, however, is entirely unrepresented.[6]

Agnathia-otocephaly, a rare disorder supposedly secondary to failed mandibulofacial development by mesenchymal cells of PA1, is characterized primarily by mandibular hypoplasia or agnathia, microstomia, aglossia, and ventromedial malposition of the ear.[24,25] Usually, the auricle itself is well developed in cases of agnathia-otocephaly. Although auricular dysmorphisms occur, the auricle is never absent. These clinical cases might implicate that the auricle, with exception of the tragus and the anterior EAM, is not a PA1 derivative.

Clinical Cases—Microtia and Oculo-Auriculo-Vertebral Spectrum

Patients with oculo-auriculo-vertebral spectrum (OAVS), alternately known as craniofacial microsomia or Goldenhar syndrome, frequently exhibit microtia together with facial asymmetry (hemifacial microsomia). Often this is accompanied by cervical vertebral anomalies, although they can also have a wider range of defects.[10] Some author suggest microtia to be the mildest expression of OAVS.[23] Microtia is a broad term that encapsulates a diverse array of abnormal appearances of the auricle and is one of the most common external ear abnormalities.[10,15] Microtia can range from grade I, defined as presence of all the normal ear components and the median longitudinal length more than 2 standard deviation below the mean, to grade III, defined as presence of some auricular structures, but none of these structures conform to recognized ear components.[26] Patients with OAVS can present with grade III microtia, normal facial nerve function, and mandibular hypoplasia. One can argue that the clinical manifestations of OAVS are in contradiction with our research when regarding OAVS as a PA1 syndrome. However, multiple researchers have considered the OAVS as a PA1 and PA2 syndrome.[23,27] The clinical findings in the OAVS are therefore in agreement with recent findings.

Limitations

We were only able to study two embryonic specimens per CS. This carries the suspicion that our findings can be the result of individual differences rather than exemplary for the studied stage. This limitation is reduced by the sequential order of the stages, with each stage being very near to the next and the prior. Together they present a logic and flowing "motion picture" that strongly suggests true development. However, the 3D reconstructions are still snapshots in developmental time; what happens between time points remains unknown.

A second limitation is that the process of fixation and embedding is associated with shrinkage of tissue. It is possible that small tissue swellings, such as the hillocks, may be more prone to shrinkage than other surrounding tissue. This could have affected some embryos more than others, depending on the time and type of fixative. This limitation is reduced by the digital images that were taken of the exterior of the original embryos, allowing comparison of the 3D reconstructions to the original embryonic specimens. However, the original embryos studied by His were intact specimens.

As the 3D reconstructions show the predominant innervation of the auricle, it is possible that small branches of nV and nVII were too small for detailed reproduction in our 3D reconstructions.

CONCLUSION

3D reconstructions of the PA1 nerve and the PA2 nerve show that, with exception of the tragus the anterior EAM, the entire auricle is innervated by the PA2 nerve: the facial nerve. This innervation pattern supports the theory that only the tragus and the anterior EAM are of PA1 origin, and that the bulk of the auricle is a PA2 derivative. Our findings do not correspond with the renowned hillock model by His. As a result, we propose preauricular sinuses should be classified as PA1 anomalies.

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