Is the ultimobranchial body a reality or myth: a study using serial sections of human embryos

By

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Summary: Reported morphologies of the ultimobranchial body had varied between researchers: a cluster of mitotic cells, a duct-like structure and a rosette-like cell mass. To clarify the true morphology, we studied tilted horizontal sections of 20 human embryos (crown-rump length 5–18 mm; 4–6 weeks). The sections displayed a ladder-like arrangement of the second to fourth endodermal pouches and, in 5 early embryos we found the fifth pouch attached to the fifth ectodermal groove near the fourth pharyngeal arch artery. The bilateral fifth pharyngeal pouches protruded anterolaterally to form a U-shaped lumen surrounding the arytenoid swelling. The third to fifth pouches were each characterized by a pedal-shaped inferior end. We identified several types of cell clusters as candidates for the ultimobranchial body, but morphologically most of them were, to various degrees, likely to correspond to the blind end of the lower pouch when cut tangentially. Because of the topographical relation to the common carotid artery, a cyst-like structure with a cell cluster seemed to be the most likely candidate of the ultimobranchial body (a common anlage of the thymus and parathyroid). However, we were not able to deny a possibility that a certain plane cutting the pouch end incidentally provided such a cyst-like structure in sections. At any stage, the ultimobranchial body might not appear as a definite structure that is discriminated from others with routine staining. A concept of the ultimobranchial body might be biased by comparative anatomy that shows the ultimobranchial gland in adult birds and reptiles.

Introduction

During embryonic development, the fourth pharyngeal pouch gives rise to the superior parathyroid gland, the ultimobranchial body and, possibly, part of the thymus. The fifth pharyngeal pouch is believed to be entirely taken up into the fourth pouch to provide the so-called caudal pharyngeal complex1, 2). However, in a limited study using human embryos, Merida-Velasco et al.3) ruled out the presence of this complex, and considered a cell cluster at the end of the fifth pouch to be the ultimobranchial body. Hast 4) stated that parts of the epithelial laminae obliterating the primitive laryngeal cavity originated from the fourth pouch. However, we recently demonstrated no contribution of the lower pouch to the laryngeal cavity5).

There have been marked differences in the reported morphology of the ultimobranchial body (Fig. 1): 1) A cluster of mitotic cells in the cord- or plate-like thyroid anlage in rat embryos6); 2) a duct-like structure lined by tall epithelial cells in mouse embryos7); 3) a cell cluster located between the lower ectodermal groove and endodermal pouch in human embryos3); and 4) a rosette-like cell mass with a small lumen in mouse embryos8). According to Rogers6), the ultimobranchial body initially appears at the inferior end of the “third” pharyngeal pouch, but is then quickly incorporated into the cord-like thyroid anlage. However, he did not demonstrate a tran-
sient phase between these morphologies, and it is noteworthy that he did not discriminate the ultimobranchial body from the so-called lateral thyroid, i.e., a contribution of the pharyngeal ectodermal groove to thyroid development\(^1\). Johansson et al.\(^9\) ensured the endodermal origin of the thyroid C cell in the well-differentiated mouse specimen. Both a duct-like ultimobranchial body\(^7\) and one appearing as a cell cluster between the lower groove and pouch\(^3\) appear difficult to discriminate from usual pharyngeal arch structures. A similar situation exists for the cervical sinus, temporarily appearing as an “extra-embryonic” space, which is believed to differentiate into a pathological cyst or fistula with a definite epithelial lining\(^10\).

Consequently, the aim of the present study was to clarify the fetal topographical anatomy of the lower pharyngeal pouches and changes occurring during early development. For this purpose we used mainly tilted horizontal sections to demonstrate the ladder-like appearance of the pharyngeal arches. The present results would reveal whether or not the ultimobranchial body is a morphological entity shown with routine histological methods without immunohistochemistry.

**Materials and Methods**

The study was performed in accordance with the provisions of the Declaration of Helsinki 1995 (as revised in Edinburgh 2000). We used tilted horizontal...
serial sections (5 micron in thickness) of 20 embryos (crown-rump length (CRL) 5–18 mm; approximately 4–6 weeks): 1) 10 embryos (CRL 5–8 mm; 4–5 weeks) for observations of the ladder-like arrangement of the pharyngeal arch structures and 2) another 10 embryos (CRL 11–18 mm, 5–6 weeks) for observations of developmental changes in the putative ultimobranchial body and cervical sinus. The sections had been stained with hematoxylin and eosin (HE) or azan. All specimens were part of the large collection kept at the Embryology Institute, Universidad Complutense, Madrid, and were products of miscarriages and ectopic pregnancies managed at the Department of Obstetrics at the university. The study protocol was approved by our university ethics committee (No. B08/374). None of these specimens showed any morphological abnormality in the brain, the ganglia of the cranial nerves, the lungs, the liver and the intestinal loop including the pancreas. We had no information on the status of pregnancy or family history.

Results

Observations of embryos at approximately 4–5 weeks (CRL 5–8 mm)

Because of the curvature of the head and neck, the trigeminal ganglion with the cervical spinal cord and its associated dorsal root ganglion existed in the same section (Fig. 2). The second to fourth pharyngeal pouches were arranged in a ladder-like manner in a single section or near sections (Figs. 2–4). These pouches were lined by tall endodermal epithelium. Consistently, the upper end of the “ladder” did not correspond to the first pouch and groove, but to the stomodeum (the future mouth), with or without a connection to Rathke’s pouch (Figs. 2E and 3AB). The first pharyngeal pouch and groove (the future middle ear) was located distantly anterosuperior to the other pharyngeal arch structures (Fig. 2AB). Therefore, for brevity, demonstration of the first pouch is limited to Fig. 2 in the present figures.

In contrast to the first pouch restricted anteriorly, the first ectodermal groove extended far postero-inferiorly to a level near the primitive larynx, and its morphology was maintained until the later stages (Figs. 4–6). Unlike the endodermal pouches, the corresponding ectodermal grooves were more difficult to identify because of the inferiorly extending first groove and the dividing medial ends. Notably, we did not clearly identify the second groove in 4 of the 10 embryos (Fig. 3B), in contrast to the distinct second pouch that was identified as a large hollow of the stomodeum (Figs. 2B and 3A). When the second groove was evident, the succeeding third groove became thin, narrow and/or shallow to provide a cord-like arrangement similar to the thyroid gland anlage (i.e., the so-called lateral thyroid; Fig. 2F). Without the second groove (Fig. 4B), the third-fifth grooves provided a marginal structure just similar to that made by the second-fourth grooves (Fig. 2F). Thus, the cervical sinus was likely to face different combinations of the ectodermal grooves.

The third pharyngeal pouch consistently carried a pedal-shaped, lateral protrusion that was connected to the ectodermal groove (Figs. 2DE and 3B–D). Herein, this “pedal” indicated a shape similar to a stamen of lily flowers. Likewise, the fourth pharyngeal pouch with a pedal-shaped inferolateral protrusion appeared smaller than the third pouch (Figs. 2H and 3CD), but the connection with the ectoderm (i.e., the fourth pharyngeal groove) was difficult to identify in 7 of the 10 embryos. The third and fourth pouches were located immediately in front of the nodosa ganglion of the vagus nerve. The bilateral arytenoid swellings sandwiched the slit-like primitive laryngeal cavity (the pharyngoglottic duct) and, in turn, the swellings were sandwiched by the U-shaped bilateral protrusions of the fourth or fifth pouches (Figs. 2F–H and 4A–D).

The fifth pouch was identified near the fourth pharyngeal artery in 5 of the 10 embryos. However, in 3 of the 5 specimens with an identifiable fifth pouch, we did not find the corresponding ectodermal groove (Fig. 2H, I). In the other 2 specimens, the fifth pouch provided a bilateral small mass of cells at the inferior end (Fig. 4E). As seen in the third and fourth pouches, the fifth pouch also carried a pedal-shaped protrusion at the inferolateral end. This inferior end was located anterior to the larynx (Figs. 2H and 4E), in contrast to the laterally located end of the fourth pouch. In front of these lower pouches and developing larynx, the heart or the bulbus cordis was developing. An extra-embryonic space along the surface ectoderm was evident lateral to the fourth and fifth grooves, i.e., the putative cervical sinus (Figs. 2GH and 4B–D). Therefore, the vagus nerve ran near the surface ectoderm or the cervical sinus.

Observations of embryos at approximately 5–6 weeks (CRL 11–18 mm)

The first ectodermal groove still extended far posteroinferiorly to a level near the primitive larynx (Figs. 5 and 6). In contrast, the second to fifth grooves had completely disappeared. Multiple pharyngeal pouch-like structures (with a slit-like lumen lined by tall epithelium) were still evident lateral to the arytenoid swelling: these were regarded as the third, fourth and/or fifth pouches, but their numbering was often difficult because of the solitary morphology without any adjacent upper pouches (Figs. 5–7). These lower pouch remnants were duct-like and extended anterolaterally to the larynx or along the common carotid artery (Figs. 5C–E and 6B–D). We identified bilateral clusters of large mononuclear cells near the thyroid or pericardium as the primitive thymus in 4 of the 10 specimens (Fig. 7BD). Near the developing thyroid and thymus, we identify a cyst-like structure that is
Fig. 2. Ladder-like arrangement of the pharyngeal pouches in an 8-mm-CRL embryo. Tilted horizontal sections tangential to the pharyngeal arches. Panel A (Panel I) is the most anterosuperior (postero-inferior) side of the figure. Intervals between panels are 0.1 mm (A–B, B–C, C–D, D–E), 0.2 mm (E–F) and 0.1 mm (F–G) and 0.2 mm (G–H, H–I), respectively. A ladder-like arrangement of the stomodeum (ST) and the second and third pharyngeal pouches (PP2, PP3) are evident in panels B–D. The second pouch (PP2) extends inferolaterally to connect with the second groove (panels B and C). The cervical sinus is seen in panels E–G. Note a pedal-like morphology of the inferolateral end of the third-fifth pouches (PP3 in panel E; PP4 in panel H and PP 5 in panel I). Because of the curvature of the head and neck, the trigeminal ganglion (TG) exists with the cervical spinal cord and its associated dorsal root ganglion (SP, DRG) in a same section (panels A and B). The future middle and external ear (the first pharyngeal pouch and groove; PP1, PG1) are seen near the trigeminal ganglion in the anterior side of the other pharyngeal arch structures. However, the first groove extends postero-inferiorly (panels C-H) to the level of the primitive larynx (LX). Arrows in panel F indicate a trabecular arrangement, similar to the thyroid gland anlage (TGA), of the third pharyngeal pouch cells. All panels are prepared at the same magnification (scale bar in panel A, 1 mm). Other abbreviations, see the common abbreviation.
Fig. 3. Ladder-like arrangement of the pharyngeal pouches in a 6-mm-CRL embryo. Tilted horizontal sections tangential to the pharyngeal arches. Panel A (Panel I) is the most anterosuperior (postero-inferior) side of the figure. Intervals between panels are 0.1 mm (3A–3B, 3B–3C, 3C–3D, 3D–4A, 4A–4B, 4B–4C) and 0.2 mm (4C–4D, 4D–4E), respectively. A ladder-like arrangement of the stomodeum (ST) and the second and third pharyngeal pouches (PP2, PP3) are evident in panels B-D. The cervical sinus (CS) is seen in the lateral side of the fourth and fifth grooves (PG4, PG5). The fifth pouch (PP5) provides bilateral cell clusters near the bulbus cordis of the heart (star with PP5 in Fig. 4E and its insert). Rathke’s pouch is not yet developed in this specimen. All panels, except for Fig. 4E insert, are prepared at the same magnification (scale bar in panel A, 1 mm). Other abbreviations, see the common abbreviation.
Fig. 4. Ladder-like arrangement of the pharyngeal pouches in a 6-mm-CRL embryo. Tilted horizontal sections tangential to the pharyngeal arches. Panel A (Panel I) is the most anterosuperior (postero-inferior) side of the figure. Intervals between panels are 0.1 mm (3A–3B, 3B–3C, 3C–3D, 3D–4A, 4A–4B, 4B–4C) and 0.2 mm (4C–4D, 4D–4E), respectively. A ladder-like arrangement of the stomodeum (ST) and the second and third pharyngeal pouches (PP2, PP3) are evident in panels B–D. The cervical sinus (CS) is seen in the lateral side of the fourth and fifth grooves (PG4, PG5). The fifth pouch (PP5) provides bilateral cell clusters near the bulbus cordis of the heart (star with PP5 in Fig. 4E and its insert). Rathke’s pouch is not yet developed in this specimen. All panels, except for Fig. 4E insert, are prepared at the same magnification (scale bar in panel A, 1 mm). Other abbreviations, see the common abbreviation.
Fig. 5. Remnants of the lower pharyngeal pouches in an 11-mm-CRL embryo. Tilted horizontal sections tangential to the pharyngeal arches. Panel A (Panel E) is the most anterosuperior (postero-inferior) side of the figure. Panels F and G display higher magnification views of lower pharyngeal pouch structures shown in panel E. Intervals between panels are 0.2 mm (A–B, B–C), 0.1 mm (C–D) and 0.2 mm (D–E), respectively. A ladder-like arrangement is limited to the first and third ectodermal grooves (PG1, PG3) in panel A. We identify a cyst-like structure that is composed of a cell cluster facing to a small lumen as the most likely candidate of the ultimobranchial body (UBB in panels A, E and G). The cervical sinus is absent and, instead, a thick mesenchymal tissue is present in the lateral side of the vagus nerve course (VN). The fifth pharyngeal pouch is not counted, but the fourth pouch (PP4) is similar to the fifth pouch because of the inferior position along the anteroposterior course from the fourth arch artery (PA4) to the common carotid artery (CCA; panel D). The fourth pouch provides bilateral cell clusters near the pericardial cavity (star with PP4 in panels E and F). Asterisk in panel A indicates blood retention due to unknown reason. Panels A–E are prepared at the same magnification (scale bar in panel A, 1 mm), while panels F and G at the higher magnification (scale bar, 0.1 mm). Other abbreviations, see the common abbreviation.
Fig. 6. Remnants of the lower pharyngeal pouches in a 13-mm-CRL embryo. Tilted horizontal sections tangential to the pharyngeal arches. Panel A (Panel E) is the most anterosuperior (postero-inferior) side of the figure. Intervals between panels are 0.2 mm (A–B, B–C, C–D) and 0.3 mm (D–E), respectively. Panel F displays a higher magnification view (scale bar, 0.1 mm) of an area indicated by a broken circle in panel D. A ladder-like arrangement of the pharyngeal arch is absent. We identify a cyst-like structure that is composed of a cell cluster facing to a small lumen as the most likely candidate of the ultimobranchial body (UBB in panels A–D). Instead of the cervical sinus, a thick mesenchymal tissue is present in the posterolateral side of the vagus nerve course (VN). Upper parts of pharyngeal arches are not shown in this figure. The fourth pouch provides bilateral cell clusters (star with PP4 or PP5 in panel D) All panels, except for panel F, are prepared at the same magnification (scale bar in panel A, 1 mm). Other abbreviations, see the common abbreviation.
Fig. 7. Thick mesenchymal tissue around the thymus, thyroid gland and larynx. Panels A and B (Panels C and D) display a 12-mm-CRL embryo (an 18-mm-CRL embryo). Intervals between panels are 0.4 mm (A–B) and 0.3 mm (C–D), respectively. Instead of the cervical sinus, a thick mesenchymal tissue is present in the posterolateral side of the vagus nerve course (VN). We identify a cyst-like structure that is composed of a cell cluster facing to a small lumen as the most likely candidate of the ultimobranchial body (UBB in panel A and its higher magnification in panel E). A cell cluster in panel C (its higher magnification in panel F) is regarded as a part of the thymus (TH) because it is lateral to the common carotid artery (CCA). Asterisk in panels C and D indicates blood retention in the cranial part of the cardinal vein due to unknown reason. The internal jugular vein (IJV) as well as the sternocleidomastoideus muscle (SCM) becomes evident. Panels A–D (or Panels E and F) are prepared at the same magnification: scale bars, 1 mm in panel A, 0.1 mm in panel E). Other abbreviations, see the common abbreviation.
composed of a cell cluster facing to a small lumen as the most likely candidate of the ultimobranchial body (Figs. 6A–C and 7A). This candidate ultimobranchial body was located in the medial and/or lateral side of the common carotid artery. In all 10 embryos, the cervical sinus was not evident because of a thick mesenchymal layer lateral to the course of the vagus nerve (Figs. 5E, 6B–D and 7A–D). Therefore, the inferior ends of the fourth and fifth pharyngeal pouches became distant from the surface ectoderm.

Overall, we did not find the so-called caudal pharyngeal complex in which the fourth and fifth pouches were joined. Instead, a cell cluster suggesting the ultimobranchial body was often seen (stars in Figs. 4–6). However, these cell clusters were, to various degrees, considered likely to correspond morphologically to the blind-end of a pouch when cut tangentially. Finally, we identify a cyst-like structure with a cell cluster as the most likely candidate of the ultimobranchial body (Figs. 6A–C and 7AE).

Discussion

The present study has provided what may be a limited photographic demonstration of the ladder-like arrangement of the first to fifth pharyngeal arches in human embryos. Consistent with the description of Keibel and Mall, these five pharyngeal pouches reached the ectoderm. The fifth pouch bore a pedal-shaped inferior end, being a morphologically reduced version of the inferolateral protrusion of the third and fourth pouches. The fact that the fifth pouch was not usually seen in the present 5-week specimens suggested a rapid change in its morphology. Because regression of the second to fourth pouches coincided with the development of the fifth pouch, the demonstration of a full set of the pharyngeal pouches became difficult or impossible. However, we did not consider the fifth pouch to be a rudimentary structure. The close location to the final larynx was shown in another paper.

We found several forms of the ultimobranchial body, but most of them bore a morphological resemblance to the blind end of the pedal-like lower pouch if cut tangentially. Finally, for the most likely candidate of the ultimobranchial body, we postulated a cyst-like structure that is composed of a cell cluster facing to a small lumen. This structure was similar to that shown by Manley and Capecchi (Fig. 1) although, according to them, it is positive for calcitonin. Actually, the cyst-like structure was likely to be located both in the medial and lateral side of the common carotid artery. The medial one seemed to develop into the parathyroid, while the lateral one into the thymus. However, we were not able to deny a possibility that a certain plane cutting the blind end of the pedal-like lower pouch incidentally provided such a structure in sections. If so, at any stage, the ultimobranchial body might not appear as a definite structure that is discriminated from others with routine staining. The ultimobranchial body might be an imagination in comparative anatomy that shows us the ultimobranchial gland in adult birds and reptiles.

The ultimobranchial body has been considered the origin of not only thyroid C cells or parafollicular cells but also specific cervical tumors containing nest-like structures. However, even without a concept of the ultimobranchial body, thyroid C cell differentiation can be explained in terms of a connection or interaction between the cord-like thyroid anlage and the ectodermal groove (i.e., the so-called lateral thyroid). Actually, endodermal origin of the thyroid C cell is consistent with an endodermal ultimobranchial body by Fontaine (Fig. 1). The theory proposed by Rogers (Fig. 1) is most likely to provide a basis for the ultimobranchial origin of nest-like tumors in the adult neck, especially in the thyroid. The mitotic cell mass in the cord-like thyroid anlage (Fig. 1) is somewhat similar to the adult pathology. However, the thyroid anlage shows a significant change in morphology from a cord to a cluster of follicles. Moreover, we found no intermediate morphology in which the endodermal tall epithelial cell lining of the fourth and/or fifth pouches (a hypothetical origin of the ultimobranchial body) differentiated or evolved into a cord-like thyroid anlage. Conversely, the rosette-like structure existed with the developing thyroid follicles.

Being similar to the ultimobranchial body, the cervical sinus is also clearly described in several textbooks, but its morphology has not been well demonstrated. The “extra-embryonic” space is believed to differentiate into a pathological cyst or fistula with a definite epithelial lining. In this context, the cyst or fistula seems to be covered by the surface ectoderm, as is the case around the cervical sinus. However, the cervical sinus disappeared at 5 weeks due to mesenchymal proliferation lateral to the course of the vagus nerve. Standring concluded that the third and fourth arches were sunk in a retrohyoid depression, corresponding to the cervical sinus. However, near the lower pouches, we did not find any remnant of a space lined by surface ectoderm. Moreover, the fifth pouch was located far medially to the cervical sinus. Even after the cervical sinus had been lost due to caudal growth of the hyoid arches and their fusion with the cardiac elevation, the fifth pouch appeared to maintain its lumen immediately lateral to the arytenoid swelling. The present observations as well as our previous studies using mid-term fetuses suggest that the cervical sinus is unlikely to provide a cyst-like remnant. Overall, we conclude that both the ultimobranchial body and the cervical sinus have been overemphasized as being central to the origins of adult pathologies.
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Figure legend

In all figures, the left-hand side of the figure corresponds to the left side of the body according to morphology for figures of the heart and liver.

Common abbreviation for figures:

AO, aorta;
AS, arytenoid swelling;
BA, brachiocephalic artery;
BC, bulbous cordis (outflow tract of the heart);
CCA, common carotid artery;
CS, cervical sinus;
CV, cranial part of the cardinal vein;
DRG, dorsal root ganglion;
ES, esophagus;
HB, hyoid body;
HBE, hypobranchial eminence;
HGM, hyoglossus muscle;
HGN, hypoglossal nerve;
IJV, internal jugular vein;
LN, lingual nerve;
LX, primitive larynx;
NG, nodosa ganglion of the vagus nerve;
PA4, fourth pharyngeal arch artery;
PC, pericardial cavity;
PG2, PG3, PG4 or PG5, second, third, fourth or fifth pharyngeal ectodermal groove;
PP2, PP3, PP4 or PP5, second, third, fourth or fifth pharyngeal pouch;
PTh, parathyroid;
PX, primitive pharynx;
RC, inferior segment of Reichert’s cartilage;
RP, Rathke’s pouch;
SA, subclavian artery;
SCM, sternocleidomastoides muscle;
SGL, sympathetic ganglion;
SP, spinal cord;
ST, stomeodeum;
TC, thyroid cartilage;
TG, trigeminal nerve ganglion;
TGA, thyroid gland anlage; VC, vertebral column;
TH, thymus anlage; VN, vagus nerve;
TR, trachea;