

Switching of the Laryngeal Cavity From the Respiratory Diverticulum to the Vestibular Recess: A Study Using Serial Sagittal Sections of Human Embryos and Fetuses

*Masahito Yamamoto, †Yohei Honkura, ‡Jose Francisco Rodríguez-Vázquez, §Gen Murakami, †Yukio Katori, ||Baik Hwan Cho, and *Shin-ichi Abe, *Tokyo, †Sendai, and §Iwamizawa, Japan, and ‡Madrid, Spain, and ||Jeonju, Korea

Summary: A cecum-like protrusion of the pharynx (the laryngeal cecum or vestibular recess [VR]) develops immediately anterior to the laryngeal part of the respiratory diverticulum. An expansion of the VR has been well described, whereas the fate of the diverticulum is still obscure, although its pharyngeal opening corresponds to the glottis. We observed sagittal sections of 10 embryos (five specimens at 5–6 weeks and another five at 7–8 weeks) and eight fetuses at 25–30 weeks. At 5–6 weeks, a lumen of the laryngeal part of the respiratory diverticulum appeared, and subsequently, the VR opened into the epithelial lamina. Because of this discrete separation, it seemed unlikely that the pharyngeal pouches contributed to the laryngeal epithelium. At 6–7 weeks, the VR exhibited a high boot-shaped lumen with canalization to the diverticular lumen at the level of the cricoid cartilage. Thus, in a midline area between the bilateral arytenoid cartilages, double laryngeal lumina were evident, separated by the thick midline epithelial lamina. At 25–30 weeks, the inferior part of the VR lumen had become enlarged because of the destruction of the epithelial lamina along the arytenoid and corniculate cartilages. In contrast, candidates for the initial diverticular lumen remained as epithelial slits in the anterosuperior side of the transverse arytenoid muscle. Therefore, the final anterior and lateral laryngeal walls seemed to originate from the VR with canalization, in contrast to the part of the posterior wall derived from the initial diverticular wall.

Key Words: Respiratory diverticulum–Laryngeal cecum–Vestibular recess–Laryngeal ventricle–Pharyngeal pouch–Human embryo.

INTRODUCTION

In the embryonic larynx, the laryngeal cecum,¹ sometimes referred to as the vestibular recess (VR),² is a distinct structure protruding inferiorly from the anterior pharyngeal wall near the fourth pharyngeal pouch. It develops later than the respiratory diverticulum. Keibel and Mall³ published a series of diagrams showing sequential changes in this structure without any accompanying explanation (Figure 1): these diagrams suggested that the laryngeal cecum may temporally open to the trachea to provide the so-called vestibulotracheal canal. Thus, the term “VR” seems to be suitable for the laryngeal cecum. During development of the larynx, two openings of the future laryngeal cavity to the pharynx are present: one is for the VR and the other corresponds to the laryngeal part of the respiratory diverticulum, or the so-called pharyngotracheal duct (initial phase in Figure 2). Because the transverse arytenoid muscle develops at the posterior margin, the pharyngeal opening of the diverticulum corresponds to the future glottis.¹ The VR and the laryngeal part of the diverticulum are tightly

and extensively connected by a thick epithelial lamina until 7 weeks, or Carnegie stage 21, and the diverticular lumen is embedded in the actively expanding epithelial structure.^{1,2,4,5} Therefore, it seems difficult to trace the fate of the initial laryngeal lumen from the respiratory diverticulum, especially in horizontal sections.

There are three likely scenarios to explain the change from a double laryngeal lumina to a final single lumen (Figure 2): (1) the entire VR remains in exchange of closure of the uppermost part of the diverticulum (scenario A); (2) the initial diverticular lumen remains in exchange of closure of the upper part of the VR (scenario B); and (3) both the VR and diverticulum remain, fusing together to provide a final laryngeal lumen because of the degeneration of the epithelial lamina. Therefore, scenarios A and B require canalization between the VR and diverticulum (ie, an opening of the vestibulotracheal canal). Zaw-Tun and Burdi¹ ruled out formation of the vestibulotracheal canal and postulated a scheme similar to scenario C, despite the fact that their limited sagittal sections demonstrated a canalization candidate. Viejo et al² seemed to consider that the entire supraglottic larynx is derived from the VR without any contribution of the diverticular lumen, thus apparently corresponding to scenario A.

In addition to the previously mentioned three scenarios, if pronounced inferior migration and absorption of the VR occurs so that the initial opening migrates in far inferiorly, it seems likely that the anterior wall of the larynx could develop from the VR, in contrast to the posterior wall derived from the respiratory diverticulum. According to our interpretation of the detailed 3-D reconstruction provided by Viejo et al² on the basis of horizontal sections, small lumina separated by and embedded

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From the *Department of Anatomy, Tokyo Dental College, Tokyo, Japan; †Department of Otolaryngology and Head and Neck Surgery, Graduate School of Medicine, Tohoku University, Sendai, Japan; ‡Institute of Embryology, Complutense University Madrid, Madrid, Spain; §Division of Internal Medicine, Iwamizawa Asuka Hospital, Iwamizawa, Japan; and the ||Department of Surgery, Faculty of Medicine, Chonbuk National University, Jeonju, Korea.

Address correspondence and reprint requests to Masahito Yamamoto, Department of Anatomy, Tokyo Dental College, 2-9-18 Misaki-cho, Chiyoda-ku, Tokyo 101-0061, Japan. E-mail: yamamotomasahito@tdc.ac.jp

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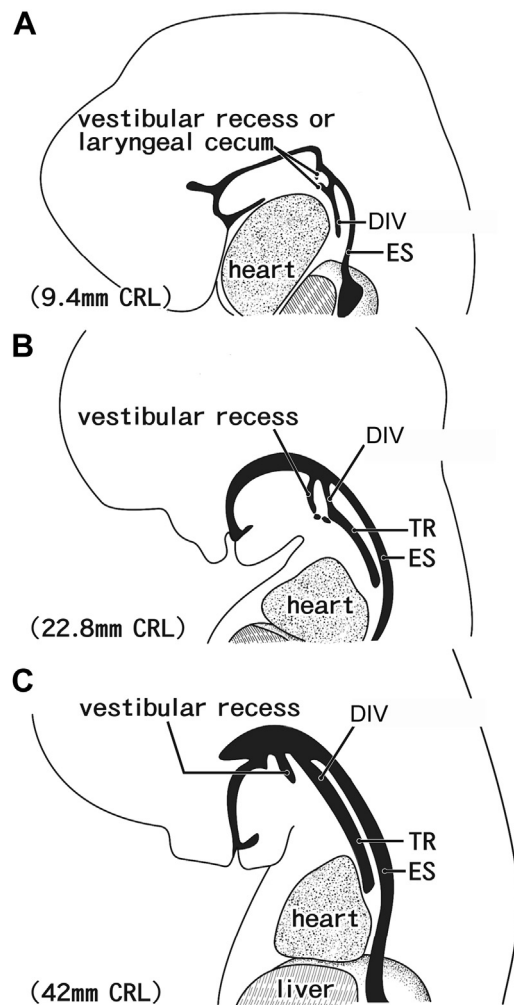


FIGURE 1. Schematic drawings of the vestibular recess or laryngeal cecum. The vestibular recess or laryngeal cecum protrudes anteroinferiorly from a site immediately anterior to the opening of the respiratory diverticulum or trachea (TR) to the pharynx. A temporary communication of the cecum with the trachea is suggested in panels A and B. Panel C indicates no communication of cecum with the trachea. DIV, initial laryngeal lumen from the respiratory diverticulum; ES, esophagus. Modified from Keibel and Mall (1912).

in the epithelial lamina of the embryonic larynx were not discriminated. Conversely, any clear demonstration of changes in the laryngeal cavity would seem to require sagittal sections. Accordingly, using sagittal sections of human embryos and fetuses, the aim of the present study was to demonstrate whether switching of the upper laryngeal cavity from the respiratory diverticulum to the VR occurs.

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 10 embryos and eight fetuses. Five embryos had a crown-rump length (CRL) of 8–12 mm (approximately 5–6 weeks; Carnegie stage 16–17), and the other five had a CRL of 25–35 mm (approximately 7–8 weeks; stage 22–23).

Serial sagittal paraffin sections of 10 embryos (hematoxylin and eosin or silver staining) 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). We had no information on the status of pregnancy or family history in relation to the specimens.

Sagittal sections (hematoxylin and eosin or silver staining) of eight fetuses (180–240 mm; approximately 25–30 weeks) had been prepared for our previous study.⁶ With the agreement of the families concerned, these fetuses had been donated to the Department of Anatomy, Chonbuk National University, Korea, and one of the authors (B.H.C.) had obtained approval for their use in research from the university ethics committee. The fetuses had been obtained by induced abortions, after which each of the mothers had been personally informed by an obstetrician about the possibility of fetal donation for research: no attempt was made to actively encourage donation. The donated fetuses were fixed in 10% w/w formalin solution and stocked in the same solution for >3 months. After trimming of the tissue mass including the tongue and larynx (30 × 20 × 20 mm), the specimens were decalcified in ethylenediaminetetraacetic acid (EDTA) solution at 4°C during 3 days (0.5 mol/L, pH 7.5; decalcifying solution B, Wako, Tokyo). After routine processing, 10- μ m thick sections were prepared at intervals of 20–50 μ m, depending on the size of the embryos and fetuses. Then sections stained with hematoxylin and eosin (H&E). The morphology of laryngeal cavity was observed under the light microscope. Because of randomization of the specimen numbering, it was not possible to trace any of the families concerned.

RESULTS

Embryologic anatomical terms

Several terms for the embryonic laryngeal cavity other than the respiratory diverticulum, such as the primitive laryngopharynx, laryngotracheal sulcus, pharyngoglottic duct, and pharyngotracheal duct,^{1,5} are not used in the present communication. Instead, to distinguish between the VR and the respiratory diverticulum, we use a single term, “diverticular lumen” (or DIV, as abbreviated in the figures). Similarly, we do not use the term “vestibulotracheal duct” for canalization between the VR and the diverticular lumen at the cricoid level and instead used the simple term “canalization.”

Observations of embryos at approximately 5–6 weeks

Sagittal sections at 5 weeks demonstrated the second-fourth pouches as slits or sulci within a long, bulky tissue mass extending from the paravertebral mesenchyme to the tongue (Figure 3). In a single specimen, the lateral or inferior end of the fourth pouch carried a short branch extending inferiorly, that is, a probable fifth pouch (Figure 3E). However, this probable fifth pouch was separated laterally from the midline laryngeal structures. A connection between the fourth pouch and the ectoderm was

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