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Development of the Lip and Palate in Staged Human Embryos and Early Fetuses

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Abstract

Cleft lip and palate are the most common congenital malformations in humans. Using 43 staged human embryos and early fetuses ranging from the 4th to 12th week of development, we investigated the development of the lip and palate in order to provide the basic developmental concepts required for managing these anomalies. The lower lip appeared as bilateral mandibular arches at Carnegie stage 11, and these were completely merged at stage 15. The components of the upper lip, medial nasal prominence and maxillary process, appeared at stage 16, and completely merged at stage 20. The median palatine process appeared at stage 16, and the lateral palatine process, at stage 17. The palatine processes and the nasal septum started to fuse abruptly at stage 23, and from external observation seemed to be fused at the 9th week. However, complete fusion did not take place until the 12th week of development. The tongue was prominent at stage 16, showed differentiation of the muscular tissue at stage 21, and was located superior to the lateral palatine process before stage 23. These results may be used in understanding the different mechanisms present in the formation of various congenital anomalies in this region.

Key Words: Upper lip, lower lip, palate, human embryo, fetus, Carnegie stage, cleft lip, cleft palate, tongue

INTRODUCTION

It is known that the lower lip develops from the mandibular arches at the cranial portion of the embryo in an early stage, and the upper lip from the maxillary processes, the lateral nasal prominences and the intermaxillary segment. On the other hand, the palate develops from the median and lateral palatine processes originating from the intermaxillary segment and the maxillary processes, respectively.[1-11] The developing structures in the embryo are influenced both structurally and functionally by various surrounding tissues. Therefore, for an understanding of the precise developmental process of the lips and the palate, it is necessary to analyze the sequential changes in the entire structure of the craniofacial region. However, there have been no such studies using Korean embryos or fetuses.

In this study, we investigated the normal development of the lips and the palate in Korean embryos and fetuses ranging from the 4th to the 12th week of development.

MATERIALS AND METHODS

Materials

We used 43 cases of human embryos and fetuses between the 4th and 12th week of development from the human embryo collection at Yonsei University College of Medicine (Table 1). Embryos within the 8th week were staged by the Carnegie stage system[12] and the fetal ages were estimated by Iffy, et al.[13]

Methods

We observed the characteristic features of the craniofacial region using a stereoscope at a magnification of 5-60. The processes and prominences that contribute to the formation of the upper and lower lips were observed carefully. Some materials were cut

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horizontally at the angle of the mouth for observation of the developing palate and nasal septum. After stereoscopic observations, the materials were fixed in 10% formalin, embedded in paraffin, cut serially, and stained with hematoxylin and eosin.

RESULTS

Upper and lower lips

Stage 11: This embryo had 19 pairs of somites and two pairs of branchial arches. The mandibular processes defined the inferior boundary of the stomatodeum (Fig. 1A). The central portion of the upper margin of the stomatodeum was protruded by the developing prosencephalon, although any structures associated with the formation of the upper lip were not recognized. The oropharyngeal membrane, which separated the stomatodeum from the foregut, was perforated at this stage.

Table 1. List of the Embryos and Fetuses Used in This Study

Age (week)	Carnegie stage	Number of cases
4	11	1
	12	3
	13	2
5	14	3
	15	3
6	16	3
	17	3
7	18	3
	19	3
	20	3
	21	3
	22	2
	23	3
9		2
10		2
11		2
12		2
		43

Stage 12−13: The embryos of stage 12 had three pairs of branchial arches, and four pairs were seen at stage 13. The stomatodeum became a transverse, slit-like structure between the enlarged mandibular arch and prosencephalon. The caudal two-thirds of the mandibular arches were merged at the midline, except for the still-separated rostral one-third (Fig. 1B). In microscopic observations, there were no necrotic changes at the merged midline region where both mandibular arches were joined. The primordia of the upper lip were not yet distinguished on the upper margin of the stomatodeum.

Stage 14: The mandibular and hyoid arches became markedly enlarged, and a prominent cervical sinus was observed between the hyoid arch and the epipericardial ridge. The surface ectoderm covering the optic vesicle was slightly depressed to form a lens pit. The merging of the mandibular processes proceeded further, remaining as only a shallow depression on the rostral surface of the lower margin of the stomatodeum (Fig. 2A). Lateral to the stomatodeum, the maxillary process became recognizable as an elevation.

Stage 15: The cervical sinus became smaller. Both mandibular processes were merged completely to form the lower lip (Fig. 2B). The nasal pits became prominent and were open laterally. Lateral to the nasal pit, the lateral nasal prominence was discernible. At this stage, the maxillary process occupied the upper lateral region of the stomatodeum. Therefore, the lateral nasal prominence and the maxillary process formed components of the upper lip. However, the

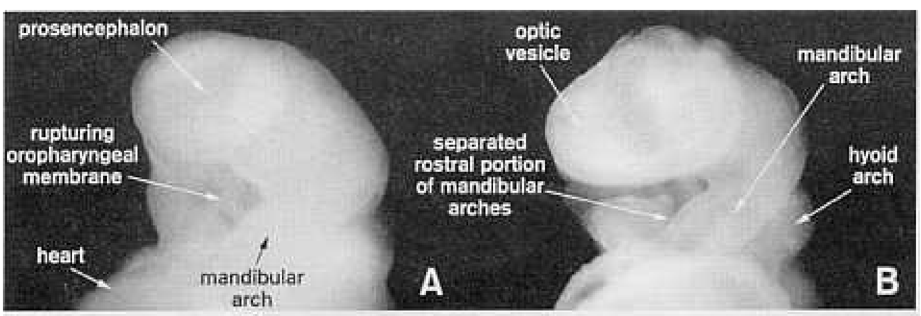

Fig. 1. Ventral view of 4 week old embryos. A: stage 11, B: stage 12.

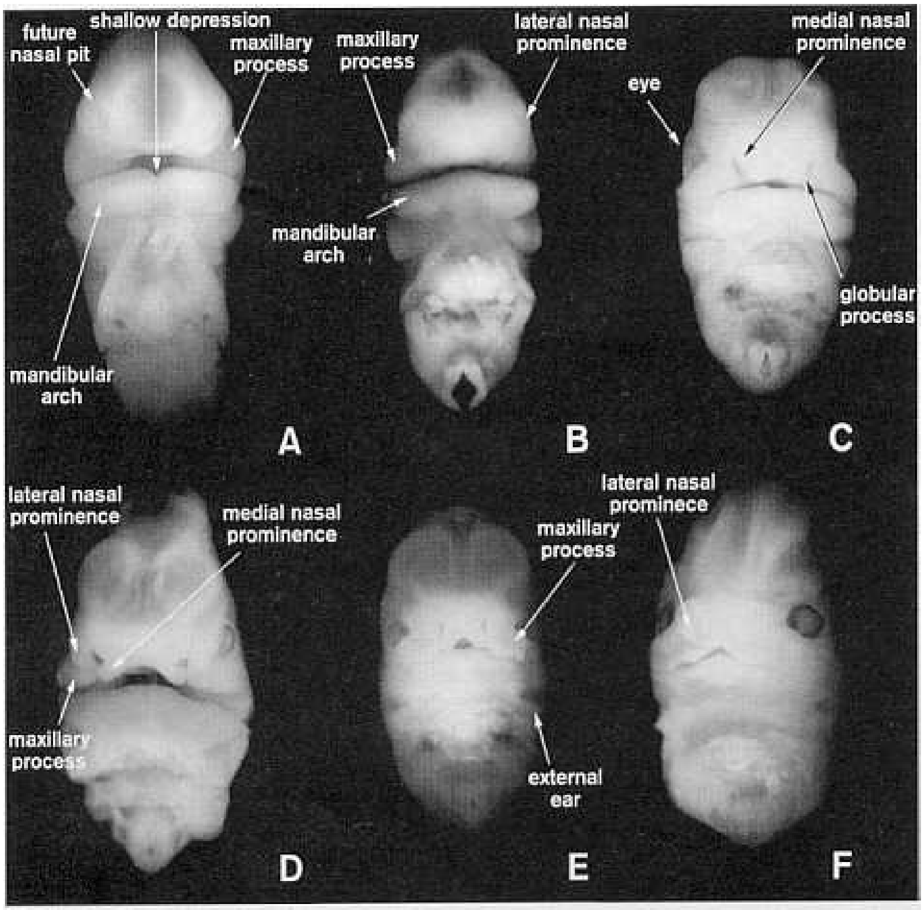

Fig. 2. Ventral view of embryos between week 5 and 7. A: stage 14, B: stage 15, C: stage 16, D: stage 17, E: stage 18, F: stage 19.

enlarged maxillary process was widely separated from the lateral nasal prominence.

Stage 16: The cerebral vesicle was prominent and the retinal pigment was observed externally. The cervical sinus disappeared almost completely and three auricular hillocks were able to be distinguished on the hyoid arch. The nasal pits were directed medially. Less distinct medial nasal prominences were be observed medial of the deep nasal pit (Fig. 2C). The caudal end of the medial nasal prominence was expanded to form the globular process of His. The medial and lateral nasal prominences were, in fact, a continuous protrusion separated by the nasal pit, and open at the inferior margin. The maxillary processes extended further medially of the groove between the medial and lateral nasal prominences, but was separated by a deep groove from them. At this stage, the upper lip was composed of the maxillary process, the medial nasal prominence and the intermaxillary segment between them.

Stage 17: The cervical sinus disappeared completely. The nasofrontal groove between the maxillary process and the lateral nasal prominence became apparent. On the mandibular and hyoid arches, six auricular hillocks were found. The nasal pit was moved further medially and deepened to form a nasal sac (Fig. 2D). The maxillary process extended medially close to the medial nasal prominence. Additionally, the groove between the maxillary process and the medial and lateral nasal prominences became more prominent.

Stage 18: The conjuctival groove appeared and the auricular hillocks began to merge. The eyes and nasal pits were moved to the medial portion of the face (Fig. 2E). The maxillary processes were enlarged to constitute more than two-thirds of the upper lip. The nasofrontal groove and the groove between the medial and lateral nasal prominences and the maxillary process became less apparent. Both medial nasal prominences became closer, forming the future nose, although the deep groove between them still remained.

Stage 19: As the maxillary process merged with

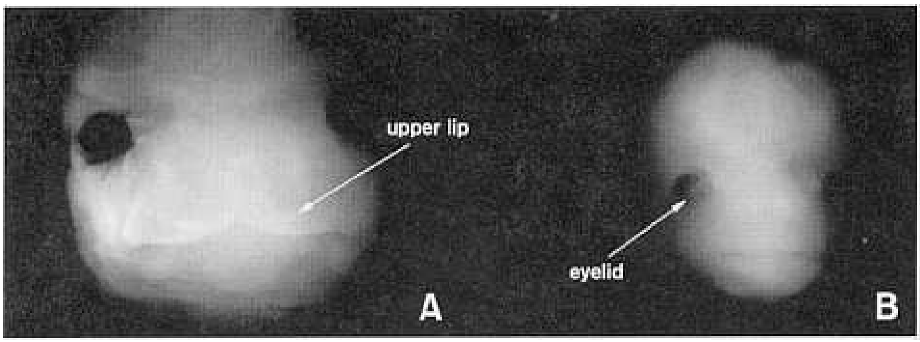

Fig. 3. Ventral view of 8 week old embryos. A: stage 20, B: stage 23.

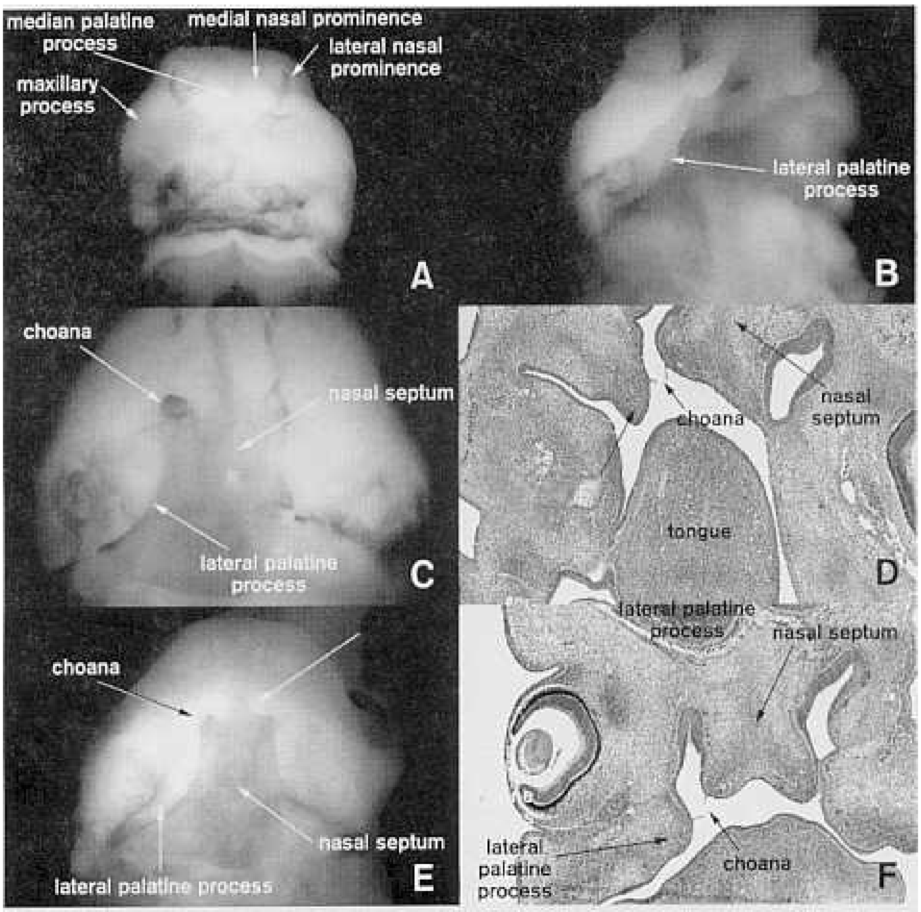

Fig. 4. Development of the palate during week 6 and 7 of development. A: stage 16, B: stage 17, C, D: stage 18, E, F: stage 19.

the medial nasal prominence, the groove between them became indistinct. However, the groove between both medial nasal prominences and that between the maxillary process and the lateral nasal prominence remained (Fig. 2F).

Stage 20: The nasal cavity was packed by a nasal plug. The external ears resembled those of an adult, but were placed dorsocaudal to the mouth. The formation of the upper lip was almost complete (Fig. 3A).

Stage 21-fetal period: The upper lip and its surrounding structure developed into an adult form gradually without any remarkable changes, and the groove between the medial nasal prominences disappeared at stage 23 (Fig. 3B). The upper lip of the fetus was nearly identical to that of an adult. The nasal tip became prominent, the eyes moved medially and the external ears moved upward with aging.

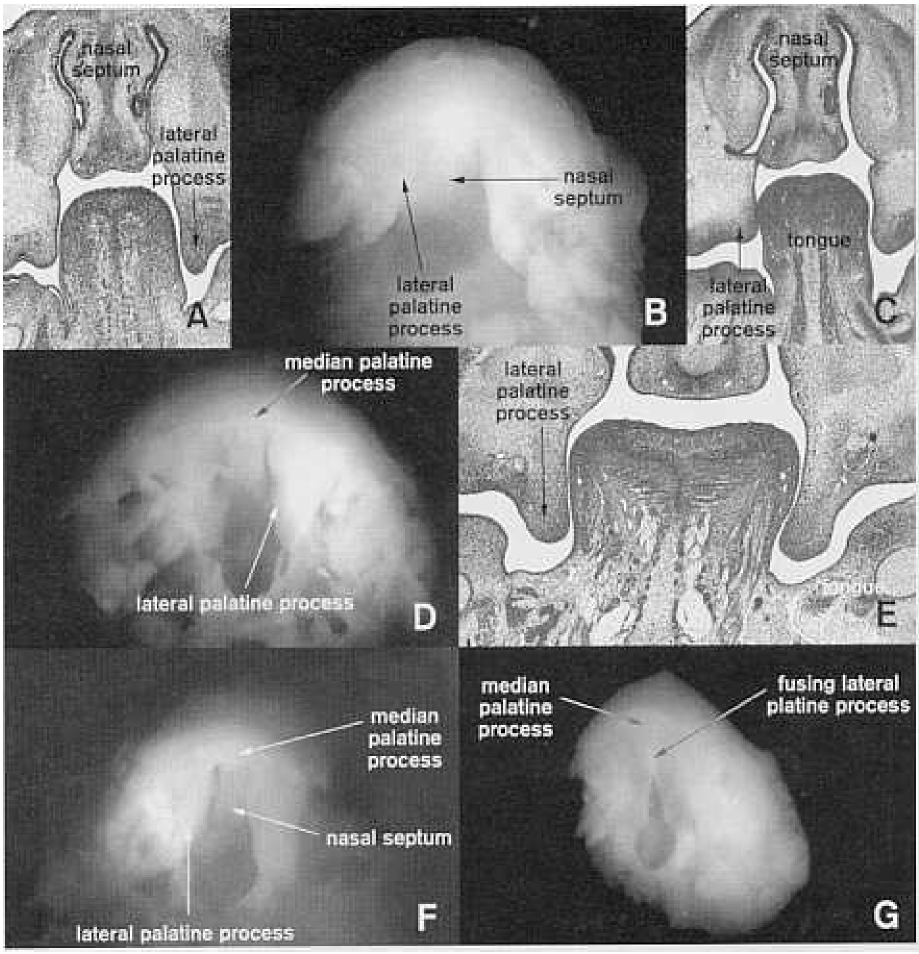

Fig. 5. Development of the palate during week 8 of development. A: stage 20, B, C: stage 21, D, E: stage 21, F: stage 22, G: stage 23.

Palate

Stage 16: The intermaxillary segment was demarcated between the medial nasal prominences, but the lateral palatine processes could not be found (Fig. 4A). The cells in the tongue began to show an ordered arrangement.

Stage 17: The enlarged nasal sac was separated from the primitive oral cavity by a thin oronasal membrane. The lateral palatine processes projected downward from the medial surface of the maxillary process (Fig. 4B).

Stage 18: The primitive choana appeared with a perforation of the oronasal membrane (Fig. 4C). The nasal septum between the primitive nasal cavities was composed of condensed mesenchymal tissue. The lateral palatine process was a continuous downward projection from the vicinity of the auditory tube to the caudal portion of the choana, but the projection did not extend medially beyond the lateral margin of the choana (Fig. 4D). The lateral palatine process was composed of condensed mesenchymal tissue with occasional blood vessels. A shallow depression was observed at the middle of the median palatine process. The tongue, which had abundant innervations, showed a well-ordered arrangement of cells, although differentiated muscle fibers could not be found.

Stage 19: A vertically arranged lateral palatine process appeared in a crescent form and extended medially to cover the lateral half of the choana (Fig. 4E and 4F).

Stage 20: The lateral palatine process extended further medially to cover the choana entirely. Mesenchymal condensation was observed within the inferior portion of the lateral palatine process (Fig. 5A). The downward growth of the nasal septum was more advanced, and early differentiation of the muscle fibers could be found in the tongue.

Stage 21: At this stage, the midline depression on the median palatine process disappeared almost entirely. The lateral palatine process protruded further, resulting in the formation of a U-shaped free margin

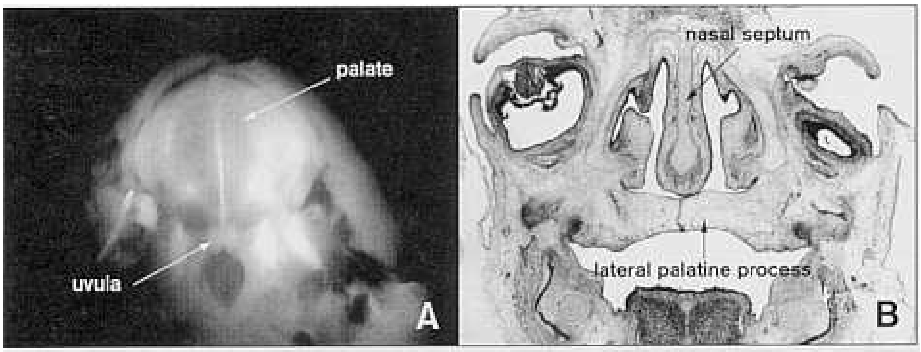

Fig. 6. Development of the palate during week 9 of development. A: Inferior surface of palate, B: Coronal section through nasal cavity.

(Fig. 5B). The mesenchymal tissue at the inferior portion of the lateral palatine process became more compact. Muscle fibers were found in the tongue (Fig. 5C).

Stage 22: The lateral palatine process projected further downward (Fig. 5D), and in one embryo it was transformed horizontally. The nasal septum also protruded further downward. The differentiation of the tongue was further advanced (Fig. 5E).

Stage 23: The lateral palatine processes of all embryos at this stage appeared to have completed horizontal transformation. In one embryo, both lateral palatine processes were widely separated (Fig. 5F). In other embryos, the lateral palatine processes began to fuse with each other at the anterior portion, and to fuse with the primary palate (Fig. 5G).

9−12th week: At the 9th week of development, both the lateral palatine processes and the median palatine process merged completely, but the uvula was remained as bifid (Fig. 6A). However, incomplete fusion of the nasal septum and the lateral palatine processes was found in the histological sections (Fig. 6B). At the 12th week, the fusion between the palatine processes and the nasal septum was completed and the uvula showed an adult-like configuration.

DISCUSSION

Many processes and prominences appear in the head and neck region during the early stages of development, and subsequently disappear. In general, a process is defined by a groove that disappears by the fusion of the surrounding processes. On the other hand, if the groove between the prominences disappears by the proliferation of the underlying mesenchyme, that is a merging.[14] Therefore, the failure of the mesenchymal proliferation in the processes or under the groove between neighboring prominences, with or without secondary degenerative changes, may result in the formation of a cleft between them.

The mandibular process can be observed at stage 11 (ca. 22 days), and is merged at the ventral midline by stage 15 (late in the 5th week) to form the lower lip. Consequently, a cleft involving the lower lip, which is rare, occurs at the midline in most cases. On the other hand, the upper lip develops from the maxillary processes, the medial nasal prominences and the intermaxillary segment. The primordia of the upper lip begins to appear at stage 16 (early in the 6th week) and comes to resemble an adult-like structure during subsequent development. Due to the temporary subdivision of the developing upper lip and the groove on the intermaxillary segment, a cleft involving the upper lip is relatively frequent. The groove between the maxillary process and the medial nasal prominence normally disappears by stage 20. However, a failure in this merging process due to insufficient proliferation or degeneration of mesenchyme, causes a lateral cleft lip. Because of the positional interrelationship, a lateral cleft involving the upper lip is frequently accompanied by a cleft palate. Sometimes, a partially degenerated mesenchyme in the groove remains to form a Simonart's band. The median cleft lip develops from the groove on the intermaxillary segment, by the same processes in the lateral cleft formation. Iizuka[15] proposed that the critical period of upper lip formation is between stages 17 and 18. He also reported that bilateral asymmetrical development of the upper lip might be due to the differential time of exposure to teratogens. The palate develops from the median palatine process, lateral palatine processes, and nasal septum. The median palatine process, a part of the intermaxillary segment, is also called the primary palate, and the lateral palatine process is called the secondary palate. When the coordinated development of these processes and the tongue is disturbed, a cleft between the processes can be generated.

Many investigators have reported the time of palatine closure, but the results have been quite variable. The inconsistency of the data may be attributed to differences in race or sex, or incompatible methods used in fixation, measurement and staging of the embryos. The racial differences in the incidence of the cleft palate shows the possible relationships between race and the time of palatine closure. In the female, cleft palate is more frequent and severe because the closure of the lateral palatine processes proceeds slowly, and consequently, the susceptible period of susceptibility to teratogen is prolonged.[16-18] The critical period in palate formation was reported to be the 7th week [crown rump length (CRL): 25 mm] in the male and the 8th week (CRL: 30 mm) in the female.[17] In addition, the individual differences seen in palate formation cannot be ignored. The variable definition of "closure" also may cause confusion in the interpretation of the results. Fulton[1] reported that embryos measuring 29−33 mm in CRL were in the critical period of palatine closure and those embryos larger than 33 mm had completed palate formation. Iizuka[19] reported the variable extent of palatine closure in embryos measuring 28−33.9 mm in CRL and complete closure in embryos larger than 46 mm. On the other hand, Wood and Kraus[20] reported that the critical period is between the 8th and 11th week. In this study, a definitive determination of the palatine closure time was difficult because of the small number of cases. However, we observed that the palate began to close at stage 23, and this result is compatible with other reports.[1,17,19,20]

The mechanisms concerning the horizontal transformation of the lateral palatine processes and their fusion are controversial. The lateral palatine processes appear at stage 17 as small projections on the medial surface of the maxillary processes and grow vertically during subsequent development. They are transformed horizontally and contact each other at the end of the 8th week, and fuse rapidly thereafter.[1,3,17,20] The transformation of the lateral palatine processes may occur by intrinsic changes of the processes themselves or by extrinsic forces from the surrounding structures. Walker and Fraser[21] reported that the transformation of the lateral palatine processes is due to an internal force. However, it is doubtful that the lateral palatine processes can actively transform their own shape because no motile component is found in them. Many investigators have suggested that an internal force could be generated by the hydration of hyaluronic acid in the mesenchyme, changes of the vascularization and blood flow, increased tension in the tissue, sudden changes of the mitotic activity, etc.[22]

The protrusion of the mandible, which was retracted before the 7th week,[23] and the simultaneous anterior movement of the tongue are believed to help the horizontal transformation of the lateral palatine processes.[18] The extension of the head changes the position of the tongue inferiorly, and this may play some role.[24] Diewert[9] also reported the differential growth rate of each portion of the head in the sagittal section of the embryos, which may be a factor of external forces. On the other hand, Humphrey[25] reported the beginning of the mouth opening reflex before the transformation of the lateral palatine processes. He suggested that this positional change of the tongue is due to the mouth opening generating a pressure gradient between the oral and nasal cavity, and this force transformed the lateral palatine processes horizontally. Our histological observation of the differentiation of the muscle fibers in the tongue is consistent with this report.

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