Timetable for Upper Eyelid Development in Staged Human Embryos and Fetuses

TAE HO BYUN,[1] JEONG TAE KIM,[1] HYOUNG WOO PARK,[2] AND WON KYU KIM[3]*
[1]Department of Plastic Surgery, College of Medicine, Hanyang University, Seoul, South Korea
[2]Department of Anatomy, College of Medicine, Yonsei University, Seoul, South Korea
[3]Department of Anatomy and Cell Biology, College of Medicine, Hanyang University, Seoul, South Korea

ABSTRACT

In this study, we examined the development of the upper eyelids to provide a basic understanding of gross anatomical structures and information relative to mechanisms of congenital anomalies in the upper eyelids. We studied the upper eyelids by external and histological observation in 48 human embryos and in fetuses from 5 to 36 weeks postfertilization. The upper eyelid fold began to develop at Stage 18. Upper and lower eyelids fused from the lateral cantus at Stage 22, and fusion was complete by 9 weeks of development. Mesenchymal condensations forming the orbital part of the orbicularis oculi (OO), tarsal plate, and the eyelashes and their appendages, were first seen at Week 9. Definite muscle structures of the upper eyelid, such as the orbital part of the OO and the levator palpebrae superioris and its aponeurosis, and the Müller's muscle were observed at 12 and 14 weeks, respectively. In addition, orbital septum, arterial arcade and orbital fat pad, and tarsal gland (TG) were apparent at 12, 14, and 18 weeks, respectively. Opening of the palpebral fissure was observed at Week 20. In addition, we defined the directional orientation between the levator aponeurosis and orbital septum and the growth pattern of the TG. Our results will be helpful in understanding the normal development of the upper eyelid and the origins of upper eyelid birth defects. Anat Rec, 294:789–796, 2011.

Key words: upper eyelids; normal development; staged human embryos; fetuses

INTRODUCTION

The three temporal features of eyelid development in humans are eyelid growth, eyelid differentiation, and eyelid maturation (Andersen et al., 1965). The eyelids are first evident as folds at 7 weeks of development, and they are expanded during the eighth week by rapidly proliferating mesenchymal tissue. The upper and lower eyelids fuse with each other around Week 9, and they separate again at around 6 months (Doxanos and Anderson, 1984). Various congenital anomalies of the eyelids can result from abnormal tissue proliferation, fusion, and reseparation of the eyelids. Abnormal eyelid fold formation leads to cryptophthalmos, microblepharon, and coloboma. Several eyelid anomalies such as ectropion, entropion, epicanthal fold, and epiblepharon are induced by abnormal differentiation of the eyelid margin. In addition, abnormal differentiation of eyelid tissue results in nevus, hemangioma, and dermoid (Doxanos and Anderson, 1984).

The anatomical structures of eyelids have been well studied; however, embryological studies are very limited. Most of the developmental studies were performed in the late nineteenth and early twentieth centuries and are briefly described in some textbooks of embryology and ophthalmology. Moreover, most developmental

*Correspondence to: Won Kyu Kim, MD, PhD, Department of Anatomy and Cell Biology, College of Medicine, Hanyang University, Seoul, South Korea. Fax: 82-2-2281-7841. E-mail: kimwg@hanyang.ac.kr

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TABLE 1. Timetable for development of the upper eyelid

Early development		
Eyelid formation	Stage 18	
Eyelid fusion beginning	Stage 22	
Eyelid fusion completion	Week 9	
Differentiation of structures inside upper eyelid		
Orbital part of orbicularis oculi	Mesenchymal condensation (Week 9)	Definite structure (Week 12)
Tarsal plate	Mesenchymal condensation (Week 9)	Definite structure (Week 14)
Eyelash and its appendages	Mesenchymal condensation (Week 9)	Definite structure (Week 14)
Orbital septum	Definite structure (Week 12)	
Levator aponeurosis	Definite structure (Week 12)	
Levator palpebrae superioris	Definite structure (Week 12)	
Arterial arcade	Definite structure (Week 14)	
Müller's muscle	Definite structure (Week 14)	
Orbital fat pad	Definite structure (Week 16)	
Tarsal gland	Definite structure (Week 18)	
Re-separation of eyelids	Week 20	

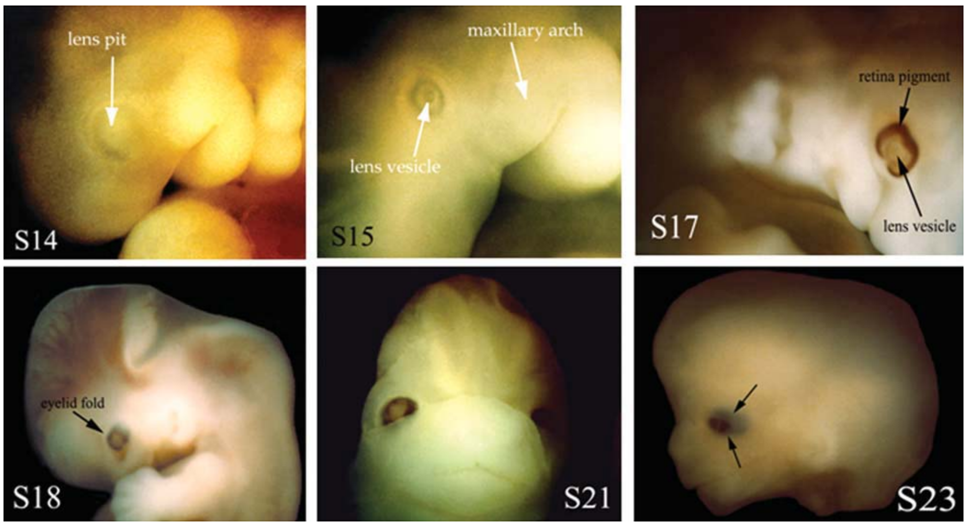

Fig. 1. External appearance of the developing eyelid in human embryos. The upper eyelid fold (arrow) is first seen in Stage 18 (S18) embryos. The eyelids (arrow) begin to fuse from the lateral side in Stage 23 (S23).

investigations of the upper eyelids in humans were limited to the fetal period (Andersen et al., 1965, 1967).

In this work, we studied both early and later developmental events involving the upper eyelids of 48 human embryos and fetuses using external, histological, and histochemical observations. The resulting observations provide a basic understanding of the gross anatomical structures and offer a foundation for understanding the origins of congenital anomalies.

MATERIALS AND METHODS

We examined 16 human embryos and 32 human fetuses from the Park Collection in the Department of Anatomy, College of Medicine, Yonsei University. The use of human specimen was approved by the ethics committee of the Yonsei University. The 16 embryos were grouped into Stage 14 (2 cases), Stage 15 (2), Stage 17 (1), Stage 18 (2), Stage 19 (1), Stage 20 (2), Stage 21 (2), Stage 22 (2), and Stage 23 (2) according to the Carnegie staging system (O'Rahilly and Müller, 1987). The weeks and numbers of fetuses are as follows: Week 9 (3), Week 12 (3), Week 14 (3), Week 16 (3), Week 18 (3), Week 20 (3), Week 24 (3), Week 28 (3), Week 30 (3), Week 32 (2), and Week 36 (3).

All the embryos were fixed in 10% neutral buffered formalin, and the fetuses were fixed in the same fixative by heart perfusion. Before making tissue section, the external appearance of all embryos and fetuses was photographed with a stereoscope (Leica M10, Leica Instruments GmbH, Nussloch, Germany) and a digital camera (Nikon D100, Japan). The embryos and 9-week-old fetuses were serially sectioned. The 12-week and older fetuses were fixed again in 80% alcohol and decalcified for 4 weeks in 5% ethylene diaminetetra-acetic acid (EDTA) solution (pH 6.0) in 10% neutral buffered formalin. The upper eyelids, including eyeballs and orbital bones, were excised, and all tissues were washed, dehydrated in ethanol, and embedded in paraffin. Then, 6-μm-thick sections were cut through the longitudinal median plane of the upper eyelid, and all the sections were deparaffinized, cleared, and hydrated to phosphate buffered saline (PBS) using a descending series of ethanol concentrations; they were stained with hematoxylin and eosin for routine morphological observation.

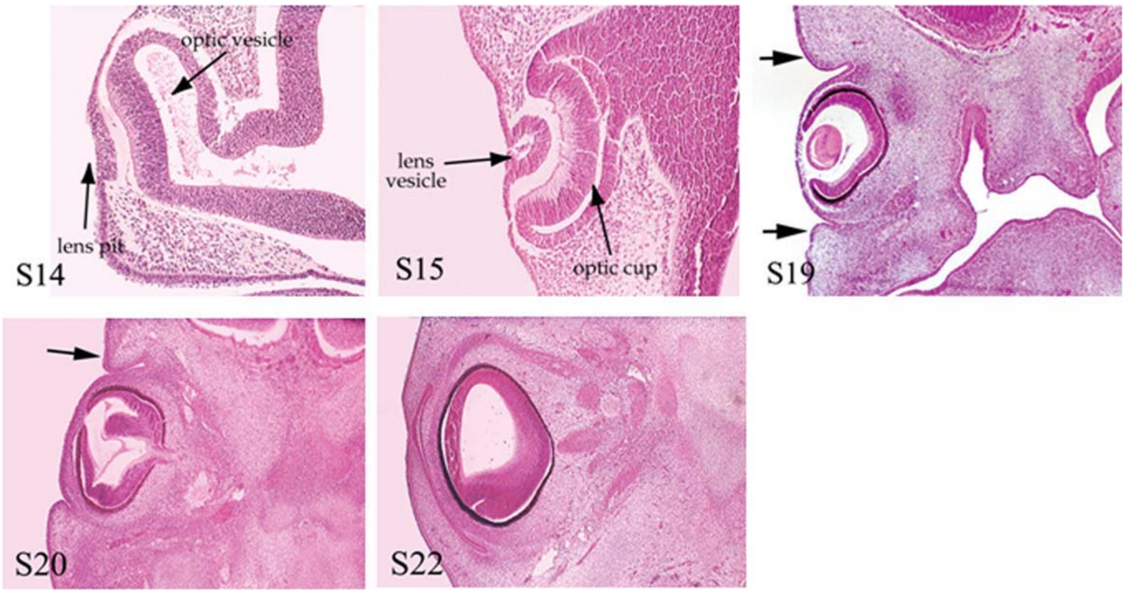

Fig. 2. Histological observations of developing eyelids in human embryos. Distinct upper and lower eyelid folds (arrows) are observed in Stage 19 (S19) embryos. Fused eyelids are seen at the lateral side at Stage 22 (S22).

The formation and distribution of collagen fibers within the upper eyelid were observed with Gomori and Masson's trichrome stains.

RESULTS

Based on our results, the timetable for upper eyelid development in humans is summarized in Table 1.

Early Development of the Eyelids: Formation of Eyelids

External appearance. At Stage 14 (approximately 32 postovulatory days; pd. 32), the lens pit could be seen. The lens vesicle and retinal pigment were developed at Stage 15 (pd. 33). At Stage 17 (pd. 41), the retinal pigment was more distinct, and the lens development was underway. However, the upper eyelid fold was still not visible. It was evident at Stage 18 (pd. 44) and was more distinct at Stage 21 (pd. 52). At Stage 23 (pd. 57), the lateral parts of the upper and lower eyelids were fused (Fig. 1).

Histological findings. At Stage 14, the lens placode was proliferating exterior to the optic vesicle. The invaginated lens vesicle and a small groove above the eye could be seen at Stage 15. However, eyelid folds were not yet present. Distinct upper and lower eyelid folds were formed by Stage 19, and the lateral regions of the eyelids were seen to be fused by Stage 22 (Fig. 2); however, external observation revealed fusion only at Stage 23, as seen above. During the embryonic period, the developing upper eyelid was composed of mesenchymal cells surrounded by periderm, without tissue differentiation.

Differentiation of the Upper Eyelids: From Fusion to Reseparation of the Eyelids

Week 9. The eye was completely closed (Fig. 3A), and the upper and lower eyelids were connected by periderm. Mesenchymal cells had condensed to form the primordia of the eyelash follicles and their appendages, the tarsal plate (TP), and the orbicularis oculi (OO). Blood vessels were evident within the mesenchymal tissue (Fig. 3B); however, no collagen fibers were detected with Masson's trichrome stain (Fig. 3C).

Week 12. Many eyelash follicles could be seen just under the skin, and the orbital part of the OO was already formed. The orbital septum originating from the periosteum of the orbital roof and the levator aponeurosis ran downward to attach to the orbital part of the OO and neighboring connective tissue (Fig. 3D,E). At higher magnification, the levator palpebrae superioris (LPS) could be observed, but not Müller's muscle (Fig. 3F).

Week 14. The upper eyelid was clearly divided into skin and subcutaneous, muscular, and tarsofascial layers. Many blood vessels and collagen fibers were present in the subcutaneous layer. Eyelashes and sebaceous and sweat glands could be seen in the lower part of the upper eyelids. The primordium of the TP had formed by condensation of mesenchymal cells (Fig. 4A). The marginal and peripheral arterial arcades were visible anterior to the lower and upper parts of the TP, respectively. The orbital septum and the levator aponeurosis ran downward separately. Müller's muscle had made an appearance and was attached to the upper border of the TP (Fig. 4B).

Week 16. The TP was clearly visible in the upper eyelid. The orbital septum and the levator aponeurosis

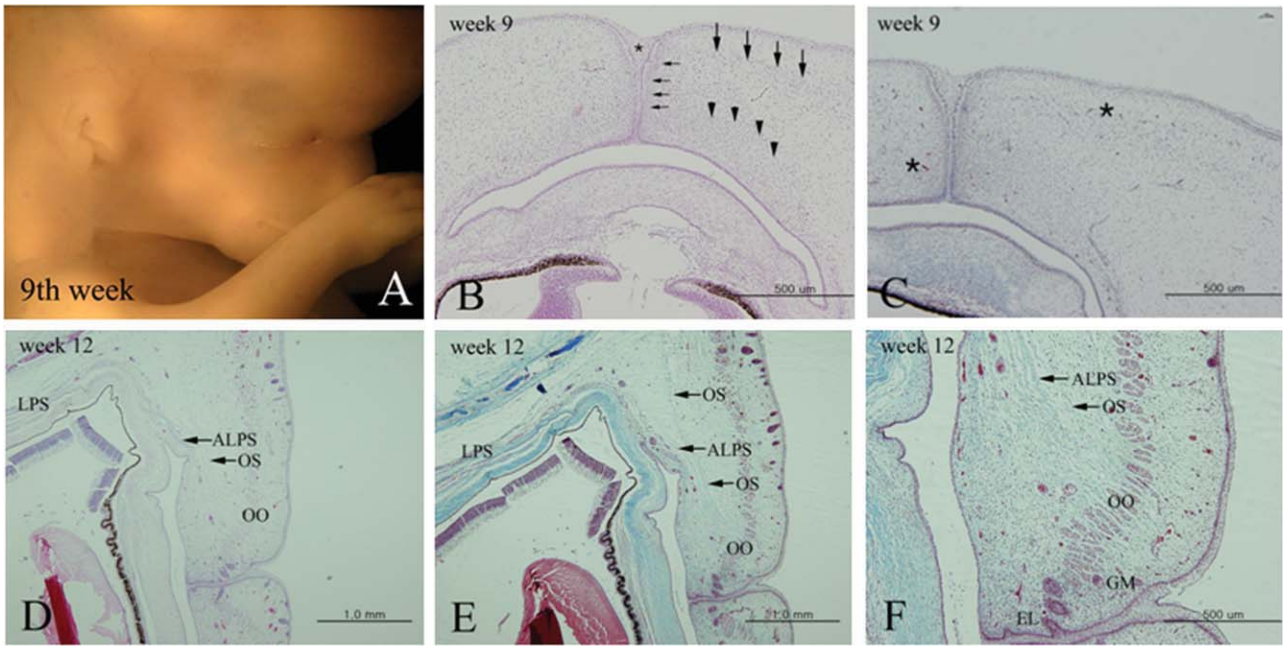

Fig. 3. Developing upper eyelids in the ninth (**A**–**C**) and the twelfth (**D**–**F**) week human fetuses: (B, D) HE stain and (C, E, F) Masson's trichrome stain. The ninth week fetus has closed eyes (A). The primordia of the orbicularis oculi (large arrows), tarsal plate (arrowheads), and accessory glands of the eyelash (EL; small arrows) are visible as mesenchymal condensation. The eyelids are connected by swollen epitrichial cells (asterisk) (B). Rudimentary arterial arcades can be seen (asterisks) (C). The orbital septum runs toward the orbital part of the orbicularis oculi (OO), parallel to the aponeurosis of the levator palpebrae superioris (ALPS) from the levator palpebrae superioris muscle (LPS; D, E). The follicle of the EL and the gland of Moll (GM) can be seen (F).

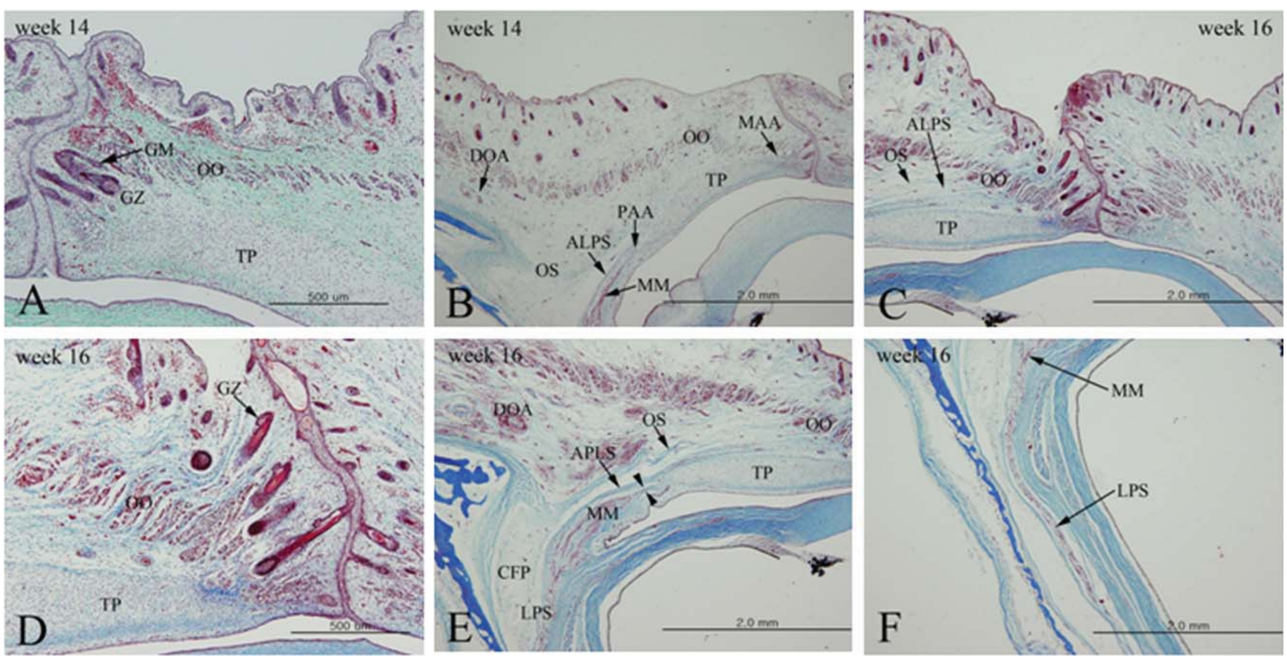

Fig. 4. Developing upper eyelids in the fourteenth (**A**, **B**) and the sixteenth (**C**–**F**) week human fetuses: (A) Gomori's trichrome stain and (B–F) Masson's trichrome stain. The upper eyelid is composed of skin, a subcutaneous layer, a muscular layer, and a tarsofascial layer. A developing eyelash, gland of Zeis (GZ), gland of Moll (GM), and the orbital part of the orbicularis oculi (OO) can be seen. The mesenchymal tissue of the tarsal plate (TP) is more condensed (A). Müller's muscle (MM), which is dorsal to the aponeurosis of the levator palpebrae superioris (ALPS), is first seen in the fourteenth week. Marginal (MAA), peripheral (PAA), and dorsal orbital arterial arcades (DOA) can be seen (B). The TP is surrounded by a fibrous capsule (C). An eyelash and its appendage glands and orbicularis oculi can be seen (D). The central fat pad (CFP) is located between the orbital septum (OS) and the periosteum of the orbital roof. MM is continuous with the LPS, and its tendon (between arrow heads) is attached to the upper border of the TP (E, F).

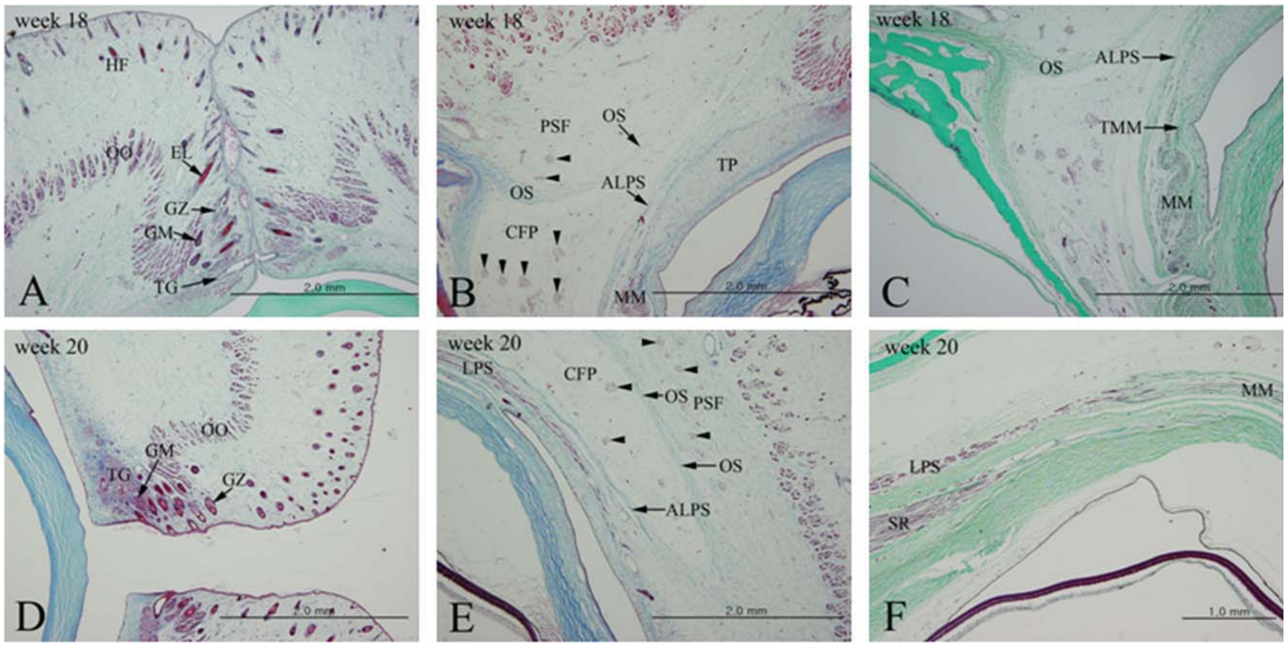

Fig. 5. The developing upper eyelid in the eighteenth (**A**, **B**) and the twentieth (**C**–**F**) week human fetuses: (A, F) Gomori's trichrome stain and (B, D, E) Masson's trichrome stain. The tarsal gland (TG) has grown toward the tarsal plate (TP) in the eighteenth week fetus (A). Brown fat (arrowhead) is scattered within the central fat pad (CFP) and separated from the preseptal fat (PSF) pad by the orbital septum (OS; B). Although from the outside, the eyes of the twentieth week fetus appear closed (C), histology reveals that the eyelids have already separated (D). The TG has penetrated further into the TP (D). The levator palpebrae superioris (LPS) is connected to Müller's muscle (MM). The aponeurosis of the levator palpebrae superioris (ALPS) and OS surround the CFP and fuse just beneath the CFP (E). The extraocular muscle and the superior rectus (SR) can be seen (F).

could be seen anterior to the TP (Fig. 4C). At higher magnification, the marginal arcade was seen to lie on the anterior surface of the lower portion of the TP (Fig. 4D). The orbital septum connected to the orbital periosteum passed anterior to the levator aponeurosis and was attached to the neighboring portion of the OO. Müller's muscle was visible on the upper part of the TP, and the central fat pad (CFP) was located between the orbital septum and the periosteum of the orbital roof (Fig. 4C,E). The LPS had expanded to the superior part of the eyeball underneath the orbital roof (Fig. 4E,F).

Week 18. The tarsal gland (TG) was now apparent and had grown into the TP, which was surrounded by the capsule (Fig. 5A). The general arrangement and orientation of the orbital septum, the levator aponeurosis, and the Müller's muscle were similar to those observed in Week 16. The preseptal fat (PSF) pad was located between the orbital septum and the orbital part of the OO, and the dorsal orbital arcade was posterosuperior to the orbital septum (Fig. 5B,C).

Week 20. From the outside, the eye appeared to be closed; however, histological observation revealed that the eyelids had separated (Fig. 5C). The TGs were now branching. White fat was present in the fat tissues posterior to the orbital septum, whereas brown fat was present deep within the PSF pad, anterior to the orbital septum (Fig. 5E). The orbital septum and the levator aponeurosis were joined just below the CFP, and the LPS muscle was connected to Müller's muscle (Fig. 5F).

Maturation of the Upper Eyelids: Continuous Growth and Maturation of the Various Structures of the Upper Eyelids

Week 24. The TP had lengthened, almost reaching the conjunctival sac, and the TG within the TP was more branched (Fig. 6A,B). The orbital septum and the levator aponeurosis approached each other near the middle of the TP (Fig. 6B), and the levator aponeurosis passed anterior to Müller's muscle, which was attached to the upper part of the TP (Fig. 6C).

Week 28. The TG, which had the appearance of a simple branched tubular gland, was more developed and occupied almost half the length of the TP. In addition, the ciliary part of the OO had developed in the subcutaneous layer of the upper eyelid (Fig. 6D). The levator aponeurosis, which passed anterior to the TP to join the orbital septum, was attached to the tendon of the orbital part of the OO and neighboring collagen bundles in the connective tissue. The tendon of Müller's muscle was attached to the upper part of the TP (Fig. 6E), and the levator aponeurosis passed anterior to Müller's muscle (Fig. 6F).

Week 32. The TG occupied nearly two-third of the length of the TP, and the tendon of Müller's muscle was attached to the upper margin of the TP. The levator aponeurosis, the orbital septum anterior to Müller's muscle, and the TP ran toward the OO (Fig. 7A). The CFP was located between the levator aponeurosis, and the orbital septum was thicker than in Week 28. The PSF pad was anterior to the orbital septum (Fig. 7B,C).

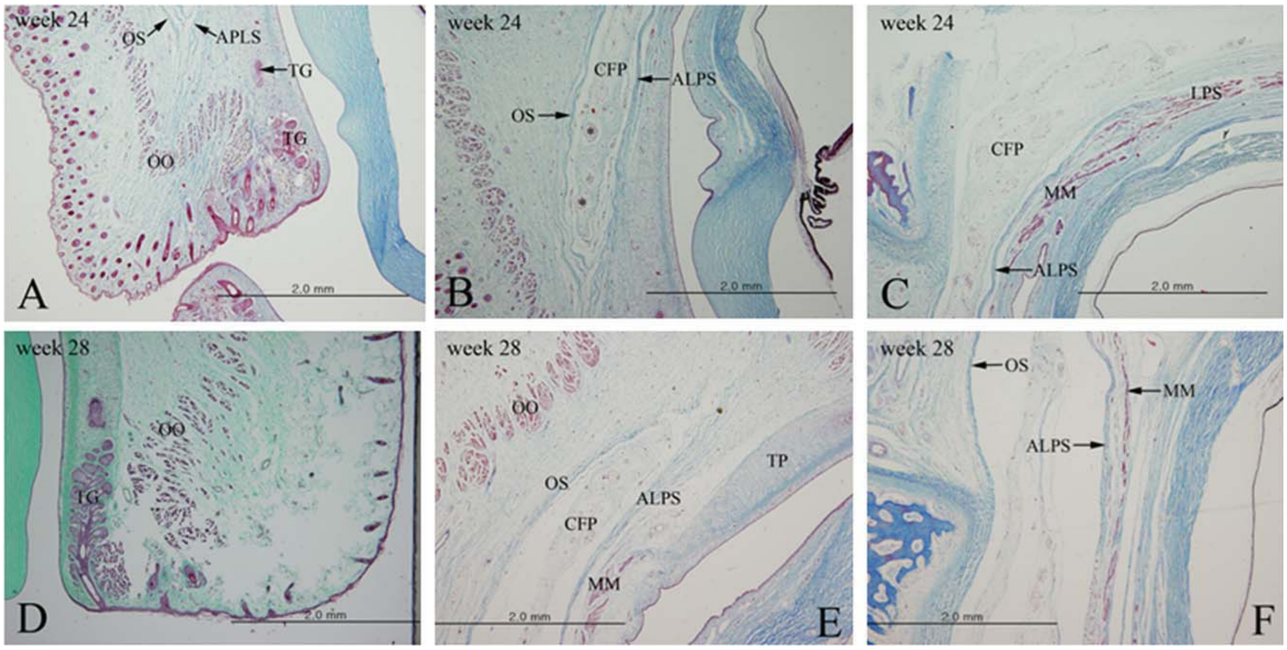

Fig. 6. The developing upper eyelid in the twenty-fourth (**A**–**C**) and the twenty-eighth (**D**–**F**) week human fetuses: (D) Gomori's trichrome stain and (A–C, E, F) Masson's trichrome stain. The general histological findings on the twenty-fourth week fetus are very similar to those on the twentieth week fetus (A–C). The tarsal gland (TG) occupies almost half of the tarsal plate (TP). The other structures are similar to the twenty-fourth week fetus (D–F).

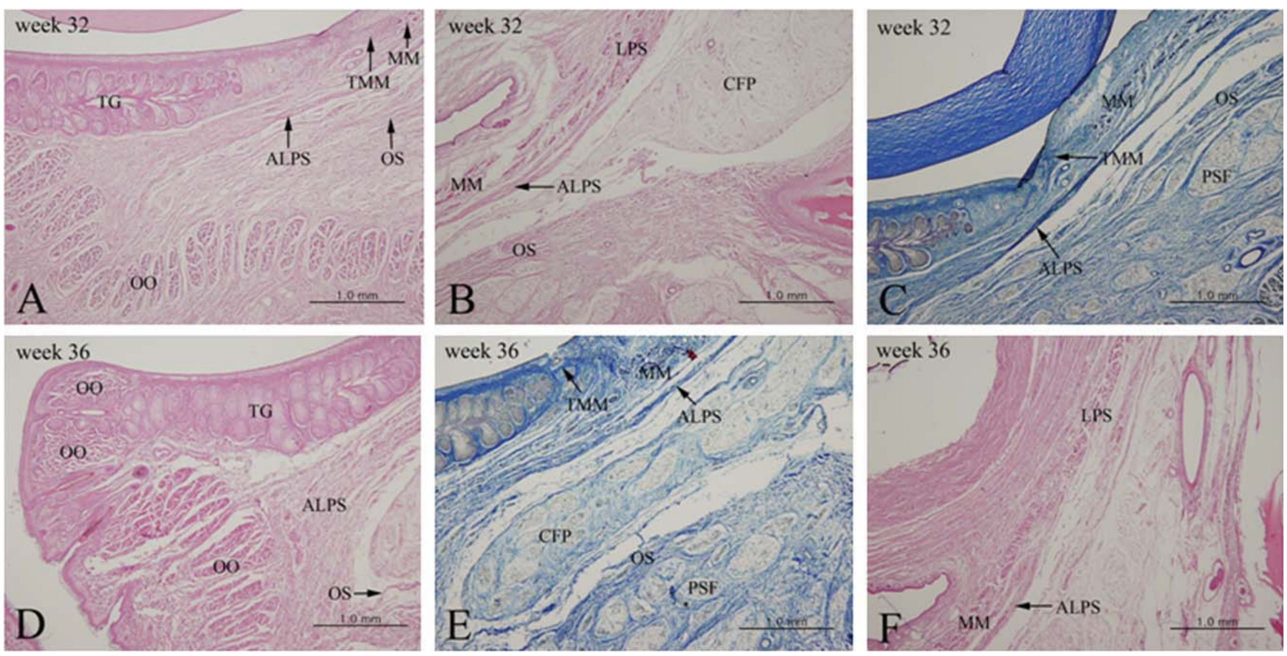

Fig. 7. The developing upper eyelid in the thirty-second (**A**–**C**) and the thirty-sixth (**D**–**F**) week human fetuses: (A, B, D, F) HE stain and (C, E) Masson's trichrome stain. The tarsal gland (TG) has extended into the upper two-third level of the tarsal plate (TP). Fibers of the orbital septum (OS) have fused with the aponeurosis of the levator palpebrae superioris (ALPS) and are inserted into the orbicularis oculi (OO; A). The tendon of Müller's muscle (TMM) inserts into the upper border of the TP (A, C). Distinct muscle bundles of the ciliary part of the OO lie in the subcutaneous layer (D). The TG occupies the entire length of the TP. TMM is attached to the capsule of the upper border of the TP. Anterior to the OS is preseptal fat (PSF) pad, and the central fat pad is also seen (E). MM is continuous with the LPS (F).

Week 36. The orbital part of the OO reached into the subcutaneous layer. The TP was encapsulated by a thick membrane, and the TG within the TP occupied almost the whole length of the plate (Fig. 7D). The levator aponeurosis near the OO had expanded to attach to the ciliary part of the OO and its neighboring connective tissue. The tendon of Müller's muscle posterior to the levator aponeurosis was inserted into the upper part of the TP. A thick CFP could be seen between the orbital septum and the levator aponeurosis, and the PSF pad composed of white fat was located anterior to the orbital septum (Fig. 7E). The LPS muscle was connected to the Müller's muscle (Fig. 7F).

DISCUSSION

The Early Development of the Eyelids

During the embryonic period, eyelid development accompanies eye development. After the lens placode invaginates to form the lens vesicle, mesenchymal tissue surrounding the developing eye begins to proliferate to form the eyelid folds, forming a circular palpebral aperture. These events occur in the seventh week of gestation (Doxanos and Anderson, 1984). von Kölliker (1861) reported that eyelids were formed in the second month, and Mann (1928) studied eyelid formation in 16-mm stage human embryos. Pearson (1980) reported that the first indications of eyelid development were a small depression above the eye and another below it, visible at Stage 16. In addition, the upper eyelids were less distinct than the lower eyelids at Stage 17 (approximately 41 postovulatory days or at the end of the sixth week). However, Mann (1963) and Whitnall (1932) reported that both the upper and lower eyelids could be seen in the seventh week. During the embryonic period, we employed the widely used Carnegie staging system, which is accepted as the standard for human embryos (O'Rahilly and Müller, 1987). Histologically, we observed a small groove above the developing eye at Stage 15; from the outside, we could discern the upper eyelid fold only at Stage 18; and by Stage 19, distinct upper and lower eyelid folds had formed. We observed a similar process of development of the early eyelid folds to that suggested by other studies. However, we did not detect any notch or depression in the upper eyelid (Pearson, 1980).

There are a number of discrepancies between the accounts of different researchers concerning the fusion between upper and lower eyelids. Although Whitnall (1932) suggested that eyelid fusion took place from the ends of the palpebral fissure and continued toward the middle, Corner and Smelser (1950) stated that the eyelids first meet at the outer canthus, and Pearson and Weleber (1975) reported that eyelid fusion occurs at the inner cantus. Moreover, Pearson (1980) reported that the eyelids meet at the lateral canthus in Stage 19 and in the medial canthus at Stage 20 and that eye closure is completed during Stage 23. On the other hand, in this work, we found that fusion began from the lateral side in Stage 22 and continued toward the medial side in Stage 23.

Formation and Differentiation of Structures Within the Upper Eyelid

Fusion of the eyelid folds is essential for differentiation of the structures inside the eyelid, which takes place in the ninth week. Failure of eyelid fusion inhibits the development of the eyelid margin and results in a coloboma or localized defect of the eyelid (Doxanos and Anderson, 1984). At the ninth week, the eyelid is mainly composed of mesenchymal tissue. The first differentiated structure is the orbital part of the OO. The primordium of this muscle is first observed at the 40-mm stage (early ninth week) in human fetuses, and the definitive muscle is formed by the 55 mm stage (eleventh week; Duke-Elder and Cook, 1963). The next structures to form are the TP and TG, which form at almost the same time (Doxanos and Anderson, 1984). Contino. v. Graefes (1907) and Klee (1920) reported that the TP and TG were first observed at the 70- to 80-mm stage (approximately fourteenth week). However, Goto (1951) stated that the TP was visible as a mesenchymal condensation by the third month and was more distinct by the seventh month. The eyelashes and their appendages (glands of Moll and glands of Zeis) begin to form just after TP formation. Further development of the eyelash follicles separates off a portion of the orbital part of the OO to form Riolan's muscle (Doxanos and Anderson, 1984). In this study, we observed the definitive TG in the eighteenth week. This is quite different from the previous reports, which stated that the TG is formed by the fourteenth week (Contino. v. Graefes, 1907; Klee, 1920). We also worked out the sequence of development of the TG: branching in the twentieth week, covering half the length of the TP by the twenty-eighth week, two-thirds by the thirty-second week, and almost the whole length by the thirty-sixth week.

The orbital septum lies posterior to the OO and forms the anterior barrier to the orbital contents. The septum and the levator aponeurosis form compartments for the orbital fat pads, that is, the preaponeurotic or postseptal and CFPs (Barker, 1977). Meanwhile, arterial arcades form and supply the upper eyelids. In this study, the orbital septum began to form in the twelfth week. It originated from the periosteum of the developing frontal bone and attached to the orbital part of the OO together with the levator aponeurosis. In addition, we were able to define the relationship between the levator aponeurosis and the orbital septum: the two structures traveled downward separately until the eighteenth week, then intermingled and expanded to insert into the ciliary part of the OO. The CFP was first noted under the roof of the orbit. Interestingly, we observed that the PSF pad containing brown fat tissues lay anterior to the orbital septum. This fat pad is not clearly seen in Caucasians, whereas both the preseptal and pretarsal fat pads exist in Asians, who do not have double eyelids (Jeong et al., 1999), as in our specimens. In addition, the brown fat tissue deep within the PSF decreased and was finally replaced by white fat tissues as development proceeded. We did not observe the pretarsal fat pad described by Jeong et al. (1999). The marginal, peripheral, and dorsal arterial arcades were distinguishable from Week 14.

The eyelid retractor muscles consist of the LPS and Müller's muscle, which are responsible for eyelid elevation (DiFrancesco et al., 2004). Views concerning the insertion of the LPS differ. The levator aponeurosis is reported to insert into the upper TP in Caucasians (Anderson and Beard, 1977; Collin et al., 1978; Landolt, 1985), but into the septum of the OO (Kuwabara et al., 1975) or the ciliary part of the OO in Japanese (Haramoto et al., 2001), or into a white line about 2–5 mm above the upper margin of the TP in Koreans (Bang et al., 1998). Müller's muscle is a smooth muscle innervated by the sympathetic nervous system. It originates at the level of Whitnall's ligament posterior to the levator aponeurosis and inserts on the superior TP (McCord, 1995). Kuwabara et al. (1975) and Stasior et al. (1993) also stated that Müller's muscle is inserted into the superior border of the TP by a short tendon. However, Bang et al. (1998) considered that it is unlikely that it is inserted in the Müller's muscle, because Müller's muscle is a smooth muscle. Unfortunately, there are few reports, if any, of the development of the LPS and Müller's muscle except for the variation of the origin and insertion of the LPS in the human fetus (Plock et al., 2005). In this study, we first observed the definitive LPS at Week 12. Its aponeurosis, the levator aponeurosis, then expanded to insert into the ciliary part of the OO by Week 32. In addition, we also detected a definite blue-colored tendon of Müller's muscle attached to the capsule of the TP in Week 16, even though it is a smooth muscle.

Reseparation of Eyelids

Reseparation of the eyelids is known to begin in the sixth month (Doxanos and Anderson, 1984), and desquamation of the keratinization of the eyelid epidermis is one of the possible mechanisms responsible for this reseparation (Findlater et al., 1993). Recently, it has been suggested that apoptosis (Mohamed et al., 2003) and the development of the cornea (Zieske, 2004) are involved. Although we were not able to identify the mechanism of reseparation of the eyelids in this study, we observed keratin between the fused eyelids and keratinization at the margin of the upper eyelid. In addition, we established that reseparation of the eyelids occurs in the twentieth week when keratinization of the upper eyelid has completely disappeared; this result is quite different from another report according to which reopening in Caucasians generally occurs in the sixth month (Doxanos and Anderson, 1984).

In conclusion, on the basis of our analysis of human embryos and fetuses, we have generated a timetable for development of the upper eyelid and have noted some differences from previous reports. We have described the directional relation between the levator aponeurosis and the orbital septum, and the growth of the TG within the TP, as well as the times at which the various definite structures within the upper eyelid are established. Our findings should provide a useful basis for understanding congenital anomalies of the upper eyelid.

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