

Early fetal development of the medial canthal ligament and Horner's muscle: a histological study

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SUMMARY

Horner's muscle is a well known structure that accelerates lacrimal drainage. However, the fetal topographical relationship between this muscle and the medial canthal ligament (MCL) seems to differ from the adult morphology because a fetal connective tissue band toward the eyelids has never been demonstrated.

We examined horizontal and frontal sections of 15 specimens (20-30 weeks of gestation) from the large collection of human fetuses stored at the Complutense University in Madrid (Spain). Frontal sections demonstrated the orbicularis oculi muscle inserting to a raphe-like structure along the horizontal parts of the lacrimal canaliculi. In horizontal sections, the raphe-like structure corresponded to a fibrous tissue mass sandwiched by the superior and inferior lacrimal canaliculi. The tendons of Horner's muscle were divided into 1) the so-called "reflection tendon" that included the typical myotendinous junction at the insertion into the maxilla, and 2) the so-called "direct tendon" in the roof of the lacrimal sac. However, Horner's muscle did not insert into the canaliculi, but was simply attached to, or embedded in, the fibrous sheath around them.

Notably, none of these connective tissue structures was attached to the tarsi. Horner's muscle and its tendon might contribute to formation of the bony attachment of the future MCL, but the main part of the MCL most likely originates from the raphe-like structure. The connection between the MCL and the tarsi seems to be established after birth due to the growth of connective tissue along the lacrimal canaliculi. Although congenital entropion is a rare condition among Westerners, the present study demonstrated that the tarsus is unlikely to be fixed at a late stage in Western fetuses.

Key words: Horner's muscle – Orbicularis oculi muscle – Medial canthal ligament – Medial palpebral ligament – Lacrimal canaliculus – Human fetus

INTRODUCTION

According to classical studies (Jones, 1958; Nagashima and Araki, 1963; Robinson and Stranc, 1970; Jones and Wobig, 1977), the medial canthal ligament (MCL) extends deeply into both the upper and lower eyelids, although the major focus of interest in those studies was

Horner's muscle (i.e., the nasal terminal part, or the so-called lacrimal part of the orbicularis oculi muscle). Ritleng et al. (1983) reviewed the classical concepts and presented an excellent diagram of the MCL, indicating that it connects the nasal ends of the upper and lower tarsus with the bony orbit. Gray's Anatomy (Williams, 1995) clearly describes the medial palpebral ligament (synonymous with the MCL) as extending between the maxilla and tarsus, the lower edge of the ligament being separated from the lacrimal sac by some fibres of the orbicularis oculi muscle. The MCL has recently been regarded as the tendon(s) of Horner's muscle (Yamamoto et al., 2001; Kakizaki et al., 2005, 2008, 2010), although Jones and Wobig (1977) provided the first description of the term "medial canthal tendon". Notably, according to Kakizaki et al. (2008, 2010), in adult cadavers the nasal end of the tarsus is not fixed to the maxilla by the MCL but by Horner's muscle itself. Therefore, a better understanding of Horner's muscle seems to be required when considering the structure and function of the MCL.

The fetal MCL has been previously described by Fernandez-Valencia et al. (Fernandez-Valencia and Gomez-Pellico, 1990; Fernandez-Valencia et al., 2001; Cuadra-Blanco et al., 2006). Although those authors did not demonstrate the topographical relationship between the primitive MCL and the tarsus, they considered the MCL to be tendons of Horner's muscle (see above). The largest fetus used in their studies had a crown-rump length (CRL) of 210 mm, at approximately 25 weeks of gestation (Fernandez-Valencia and Gomez-Pellico, 1990). Does the fetal tendon of Horner's muscle really develop into a distinct MCL attached to the tarsus? We hypothesize that the anatomy of the fetal medial canthus is quite different from the adult morphology, since no connection between the fetal MCL and the tarsus has ever been demonstrated. Therefore, it seems likely that the tendons of the fetal Horner's muscle do not correspond to the primitive form of the MCL.

Consequently, the aim of the present study was to re-examine fetal medial canthal anatomy using large human fetuses. Previous research on Horner's muscle has largely focused on the muscle insertion to the lacrimal drainage apparatus (Ritleng et al., 1983; Kakizaki et al., 2005). Thus, using silver staining we also focused on the morphology of

the striated muscle insertion, where the muscle endomysium (rich in collagen types 3 and 4) is continuous with the actual tendon (rich in collagen type 1; Osanai et al., 2011a, b).

MATERIALS AND METHODS

This study was performed in accordance with the provisions of the Declaration of Helsinki 1995 (as revised in Edinburgh 2000). All specimens were part of the large collection housed at the Embryology Institute of Complutense University in Madrid (Spain). They had been obtained from women suffering miscarriages and ectopic pregnancies at the Department of Obstetrics of the University. Although -because of the nature of the specimens- we were unable to rule out the presence of pathology, our aim was to describe the morphology that was commonly evident in the fetuses at each stage. Approval for the study was granted by the ethics committee of the University.

We examined the paraffin-embedded histology of 15 fetuses with an estimated gestational age of 20-30 weeks (CRL 185-250 mm; 5 fetuses each at approximately 20, 25 and 30 weeks). At each stage, four fetuses were used for horizontal sections, and one fetus for frontal sections. Sections 10- μ m thick were cut at 0.5-mm intervals, and stained with hematoxylin and eosin (HE), Masson trichrome, or silver impregnation, as described previously (Osanai et al., 2011a, b). The current silver staining so-called glitter staining) allows discrimination of types-3 and -4 collagen fibers (coloured black) from type-1 collagen fibers (coloured red or brown). Types-3 and -4 collagen fibers are abundant in the endomysium and basement membrane of striated muscle fibers, while the usual connective tissue fibers such as those in tendon, ligament and periosteum are composed of type-1 collagen (Duance et al., 1980). The black-colored epimysium is continuous with the red-colored tendon at the typical myotendinous junction (Abe et al., 2010; Osanai et al., 2011a).

RESULTS

Silver staining provided a clear discrimination of the muscle epimysium (types 3 and 4 collagen) from usual connective tissues (type 1

Common abbreviations for figures

- AA, angular artery from the facial artery
- AX, artery at the medial angle of the eye (possibly, the medial palpebral artery)
- CLC, common lacrimal canaliculus
- CJ; conjunctiva or conjunctival space
- EA, anterior ethmoidal artery
- FB, frontal bone
- H1 and H2, posterior and anterior bundles of Horner’s muscle (lacrimal part) of the orbicularis oculi muscle
- ILC, (P or H), inferior lacrimal canaliculus (perpendicular or horizontal part)
- LB, lacrimal bone
- LS, lacrimal sac
- MCO, primitive medial check ligament
- MX, maxilla
- OO (P), OO (O), orbicularis oculi muscle (palpebral or orbital part)
- SC, sclera
- SLC (P), SLC (H), superior lacrimal canaliculus (perpendicular or horizontal part)
- SO, superior oblique muscle and its pulley

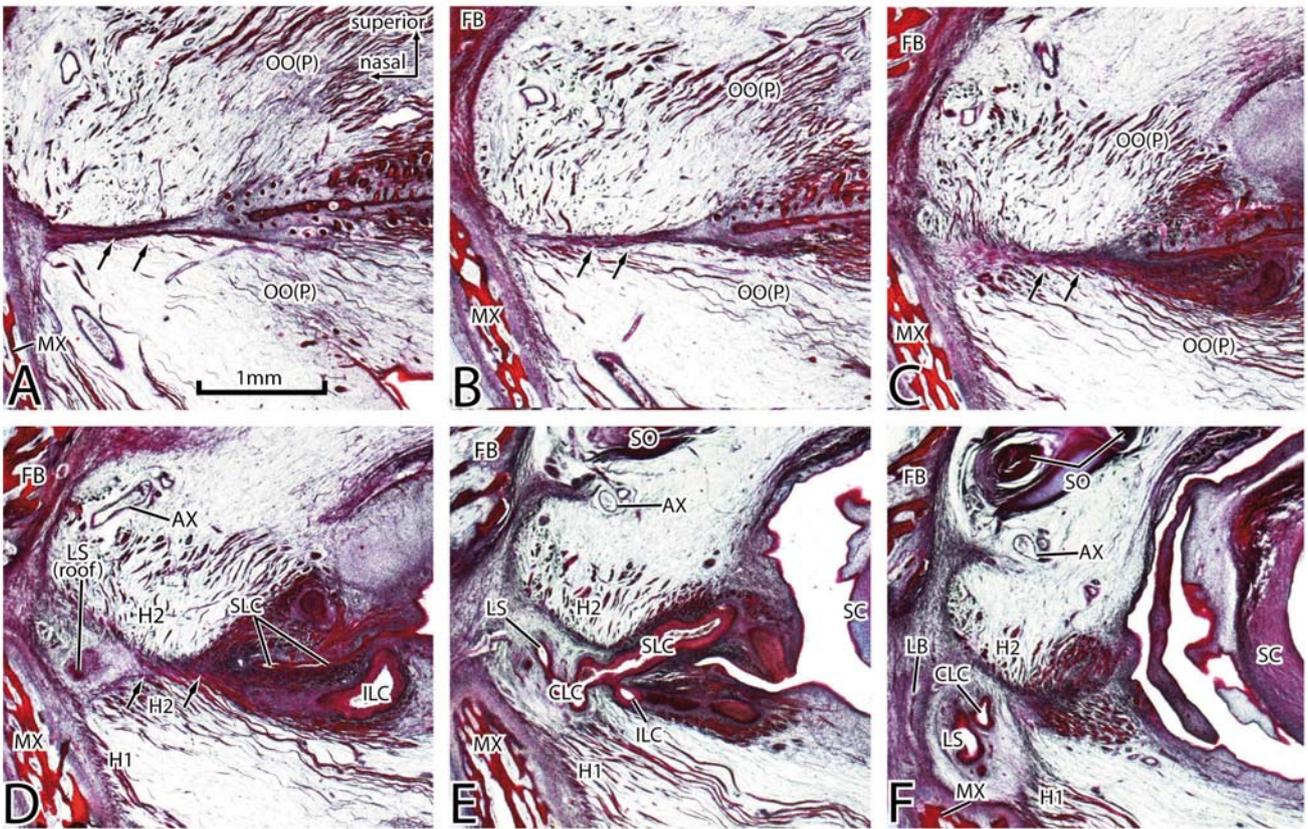


Figure 1. A raphe receiving muscle fibers from both the upper and lower eyelids in a 190-mm fetus (approximately 20 weeks). Frontal sections. HE staining. Panel A (or Panel F) is the most anterior (or posterior) side of the figure. Intervals between panels are 0.5 mm (A-E) and 1.0 mm (E-F), respectively. Arrows in panels A-D indicate a raphe-like structure that receives orbicularis oculi muscle fibers from both the upper and lower eyelids. The raphe is connected with the fused eyelids (panels A-C) as well as with the lacrimal canaliculi (panels C and D). H1 and H2 are the lacrimal part of the orbicularis oculi muscle (or Horner’s muscle); H2 is located on the anterior side of H1 (see Figs. 2 and 4). In panel F, a small part of the lacrimal bone (LB) is evident on the nasal side of the lacrimal sac (LS). Other abbreviations: see the list of common abbreviations. All panels are prepared at the same magnification (scale bar in panel A).

collagen). However, Masson trichrome failed to stain striated muscle red, possibly because of the less mature state of the muscle fibers. We readily identified the posterior bundle of Horner’s muscle (H1 in the present figure), in which the tendons corresponded to the so-called “reflection tendon”, as well as the anterior bundle (H2 in the figures), in which the

tendons corresponded to the so-called “direct tendon” (Ritleng et al., 1983).

Observations of frontal sections

At the medial canthus, the palpebral part of the orbicularis oculi muscle inserted along a raphe-like connective tissue structure (Fig. 1), which was oriented horizontally and connected

the conjunctiva to the maxilla. It was also continuous with connective tissue sheaths around the lacrimal canaliculi, but located in front of the lacrimal canaliculi and sac. The raphe-like structure was sandwiched by the horizontal parts of the superior and inferior canaliculi, but located behind the common canaliculus. The muscle fibres from the lower eyelid were identified as a group of continuous linear structures, but those from the upper eyelid were cut obliquely and the terminal fibres appeared short in frontal sections. Thus, the muscle fibres did not run in the frontal plane in the upper eyelid. In terms of fibre direction as well as location, the long muscle fibres inserting into the raphe-like structure were different from Horner's muscle, surrounding, and attached to, the lacrimal canaliculi.

Observations of horizontal sections

At 20 weeks, the posterior bundle of Horner's muscle in the orbicularis oculi muscle (H1, see above) was well developed and extended horizontally between the lacrimal canaliculus and maxilla (Fig. 2). In contrast, the anterior bundle muscle fibres of Horner's muscle (H2, see above) were cut obliquely at most levels other than that including the common lacrimal canaliculus (Fig. 2E,F), where they formed a bundle extending between the lacrimal bone and the superior or inferior canaliculus. At most levels, the H2 muscle fibres inserted into the lacrimal sac (Fig. 2B,C,D,G,H). The lacrimal diaphragm, a distinct fascia covering the lacrimal sac (Kakizaki et al., 2010), was absent. In both the upper and lower eyelids, the palpebral part of the orbicularis oculi muscle was sep-

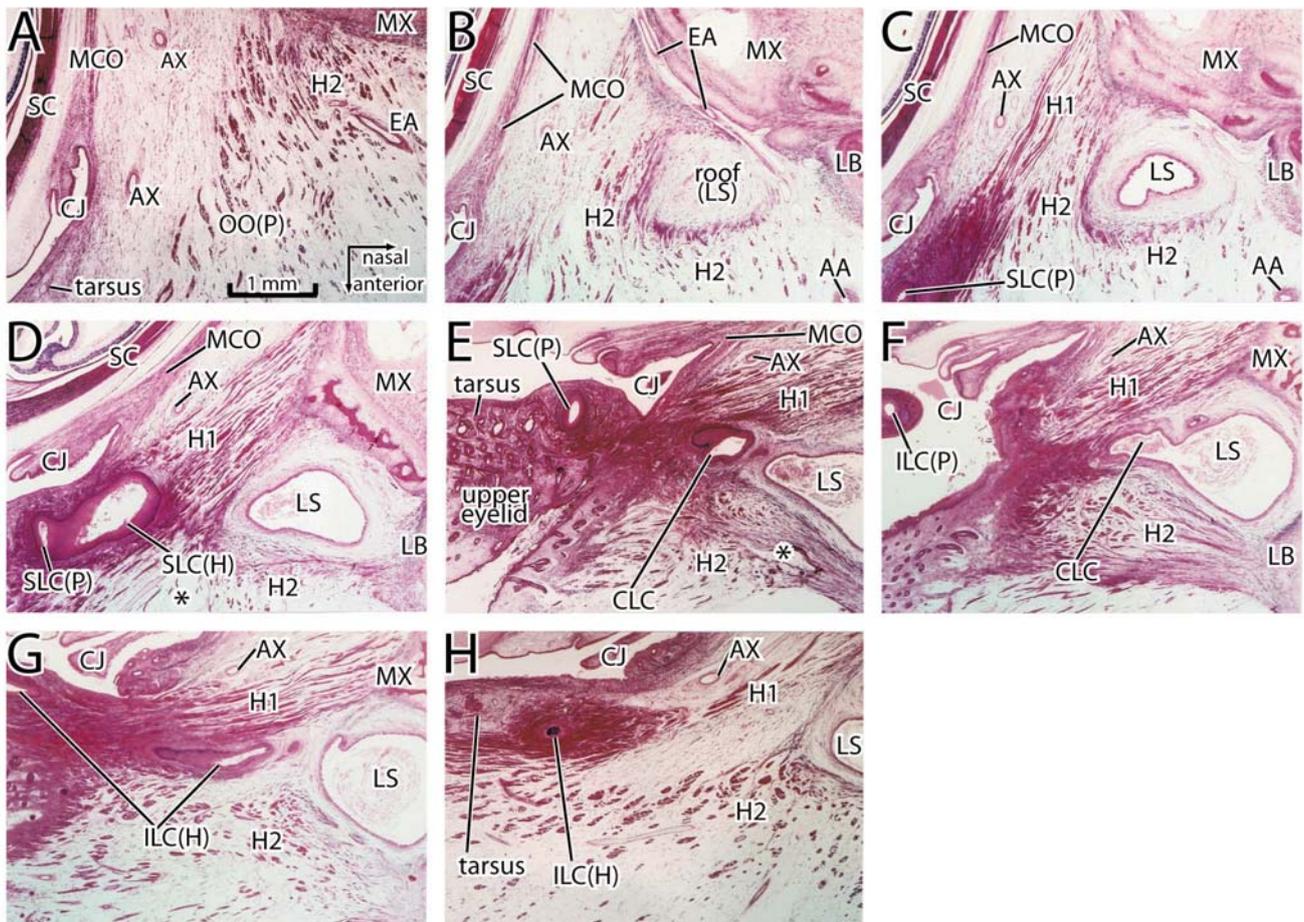


Figure 2. Striated muscle development at the medial angle of the eye in a 185-mm fetus (approximately 20 weeks). Horizontal sections. HE staining. Panel A (or Panel H) is the most superior (or inferior) side of the figure. Intervals between panels are 4.0 mm (A-B), 1.0 mm (B-C, C-D, D-E, E-F, F-G) and 2.0 mm (G-H) Panel A displays the early-stage development of the tarsal plate (tarsus). In panel A, the palpebral part of the orbicularis oculi muscle or OO (P) extends longitudinally in the upper eyelid and inserts to the maxilla (MX), but is separated from the tarsus by loose tissue containing an artery (AX) Panel B shows the roof of the lacrimal sac (LS). The anterior bundle of Horner's muscle (H2) inserts to both the sac (panels B and C) and the lacrimal bone (panels F and G). In contrast to the sparsely distributed H2 fibers, the posterior part of Horner's muscle (H1) forms a distinct bundle. In panels C and D (or panel G), the posterior bundle of Horner's muscle (H1) connects the superior (or inferior) lacrimal canaliculus (SLC, ILC) with the maxilla. In panels E and F, no striated muscle is attached to the common lacrimal canaliculus (CLC). Asterisk in panels D and E indicates artifactual damage to the tissue. Panel E (or Panel H) displays the tarsus attaching to the lacrimal canaliculus in the upper (or lower) eyelid. Asterisk in panels D and E indicates artifactual damage to the tissue. Panel E (or Panel H) displays the tarsus attaching to the lacrimal canaliculus in the upper (or lower) eyelid. Other abbreviations: see the list of common abbreviations. All panels are prepared at the same magnification (scale bar in panel E).

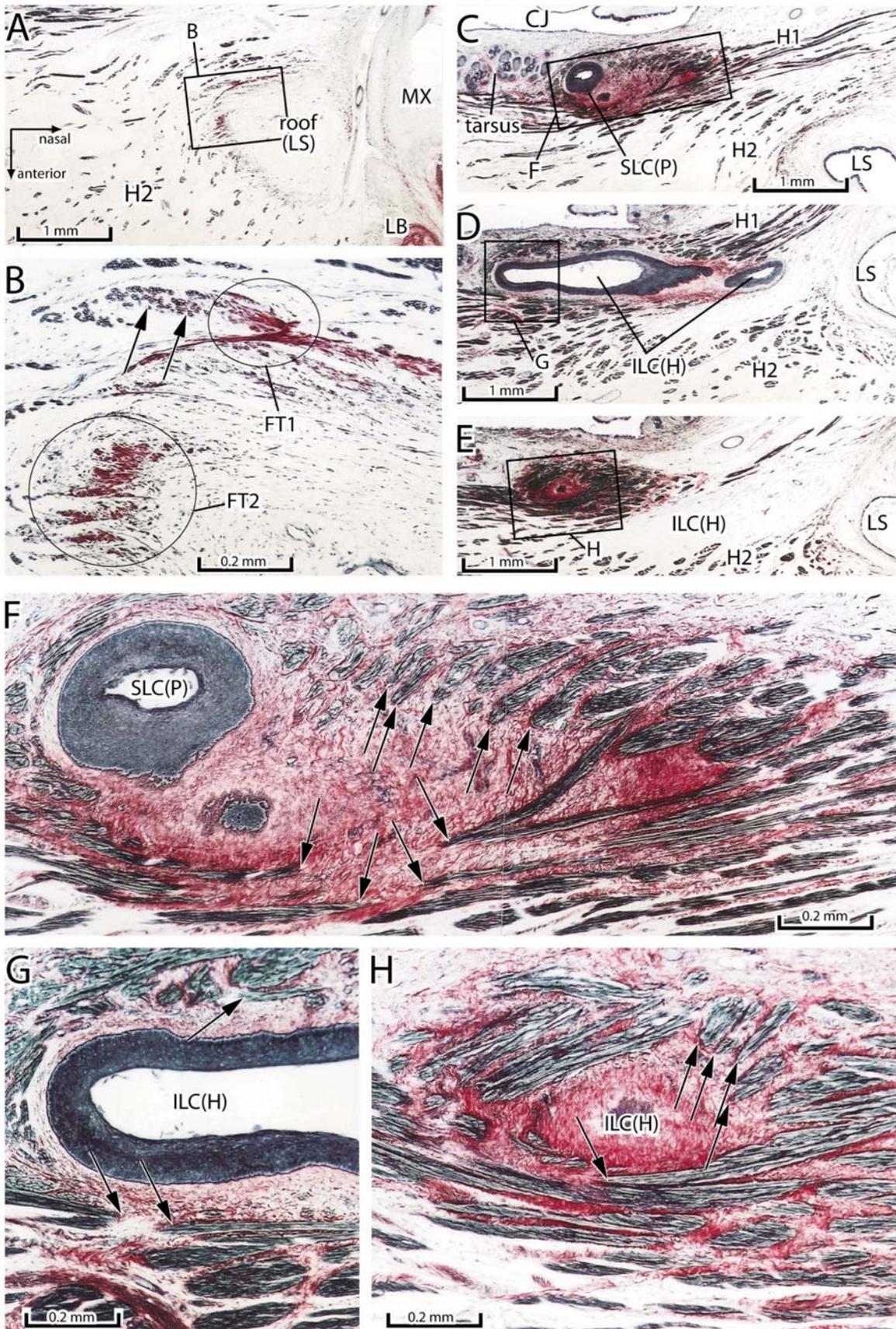


Figure 3. Silver staining of the smallest specimen.

Same specimen as that shown in Fig. 4. Horizontal sections. Panel A (or Panel E) is the most superior (or inferior) side of the figure. Panels A, C and E correspond to the levels shown on Figs. 2B, 2C and 2H, respectively. Panel D represents a level between Figs. 2G and H. Panels B, F, G and H are higher-magnification views of the squares in panels A, C, D and E, respectively. Panel B shows fibrous tissues (FT1, FT2) at the attachment of the anterior bundle (H2 in Fig. 2) of Horner's muscle to the lacrimal sac. The FT2 appears to be the actual insertion, in contrast to FT1, because of the transverse sectioning of the muscle fibers (arrows). Note that in panels F-H, muscle fibers end suddenly (arrows) without continuation to red-colored type 1 collagen fibers. The endomysium (collagen types 3 and 4) of the striated muscle fibers is stained black. Other abbreviations; see the list of common abbreviations.

arated from the tarsus by loose connective tissue (Fig. 2A,E,H). Horner's muscle was also not attached to either the tarsus or the common lacrimal canaliculi (Fig. 2E,F,H). Silver staining of a specimen at 20 weeks demonstrated red type-1 collagen fibres around the lacrimal canaliculi, and also at some muscle fibre insertions to the bone (Fig. 3). In contrast to the canaliculi, the sac carried few type 1 collagen fibres (Fig. 3A,B). Notably, the striated muscle fibres of Horner's muscle did not connect with collagen fibres around the canaliculus but were simply attached to, or were embedded in, the

collagen meshwork (Fig. 3F,G,H). Moreover, the muscle fibre direction differed depending on the location around the canaliculus.

The muscle fibre configuration was maintained at 30 weeks (Fig. 4): Horner's muscle did not insert into the lacrimal canaliculi, but was attached to, or surrounded, the latter (Fig. 4C,D). Site-dependent differences in muscle fibre direction around a canaliculus were easily identified at 30 weeks (Fig. 4D). A tendon- or ligament-like collagenous band was limited to 1) the long tendons of the H2 bundle of Horner's muscle inserting into the lacrimal sac, especially

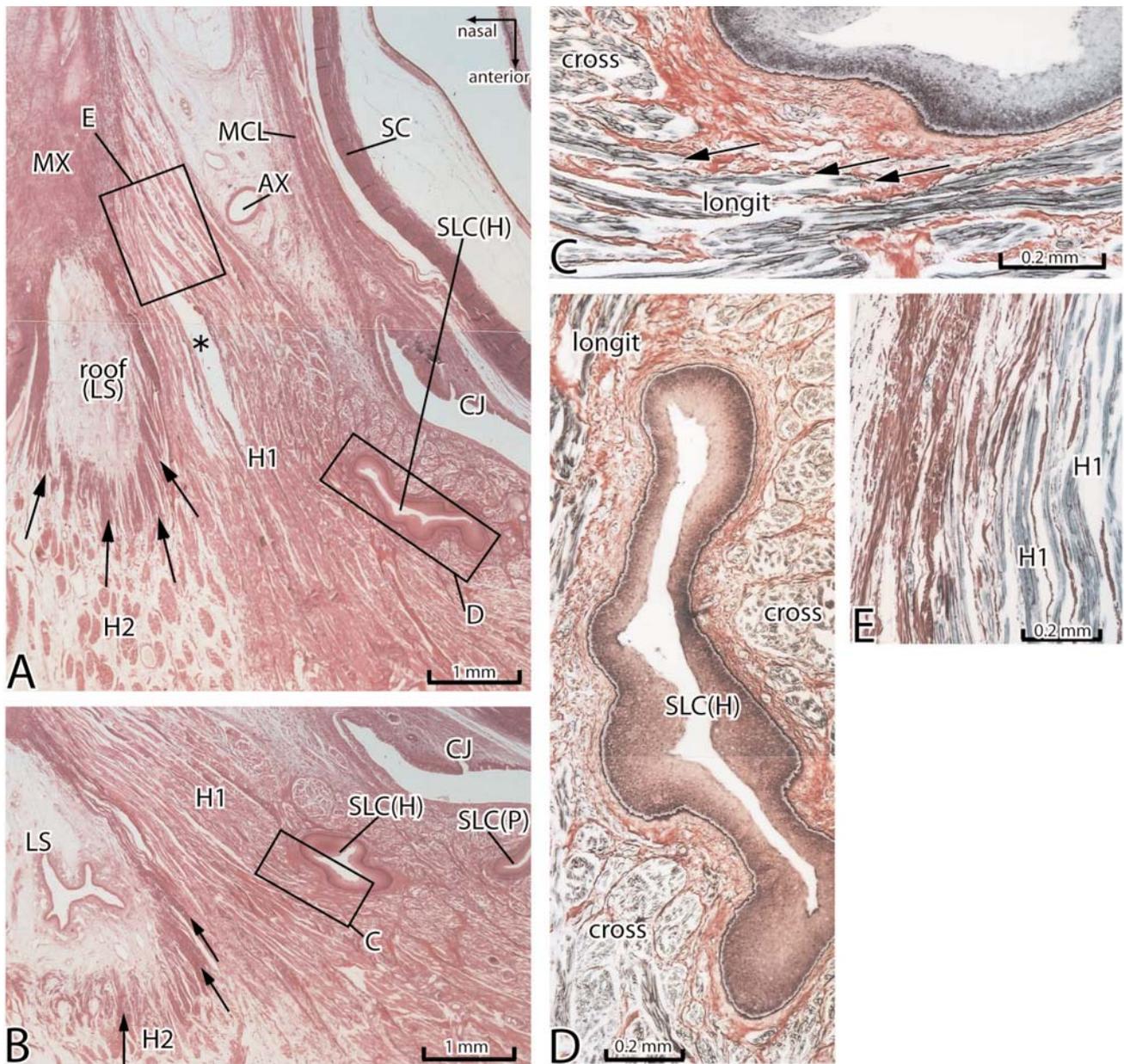


Figure 4. Striated muscle development at the medial angle of the eye in a 250-mm fetus (approximately 30 weeks). Largest specimen examined in this study. Horizontal sections. HE staining (panels A and B) and silver staining (panels C-E). Panels A is 2 mm superior to panel B. Panel C is a higher-magnification view of the square in panel B, while panels D and E correspond to the squares in panel A. Panels A and B display thick tendons (arrows) at the insertion of the anterior bundle (H2) of Horner's muscle to the lacrimal sac, in contrast to the typical muscle-tendinous junction of the posterior bundle (H1) of Horner's muscle (panel E). In panels C and D, "longit" or "cross" indicates longitudinal or transverse sections of the striated muscle fibers. Note, in the longitudinal sections, no striated muscle fibers (arrows) connect with red colored type 1 collagen fibers, but end suddenly.



Figure 5. Medial end of the upper tarsus in the largest specimen. Same specimen as that shown in Fig. 4. Horizontal sections of the upper eyelid. Silver staining. Panel A (or panel B) is 12 mm (or 6 mm) superior to Fig. 4A. An insert at the right upper angle of panel A, corresponding to the square in panel A, displays the typical muscle-tendonous junction of the orbicularis oculi (OO). The orbital septum is not evident at the medial angle of the eye (panels A and B). Note absence of any definite connection of the medial end of the upper tarsus with fascial structures. H1 and H2 indicate the posterior and anterior bundles of Horner's muscle, respectively (see Fig. 4A). The posterior bundle (H1) is absent in this superior level. A candidate for the medial palpebral artery (AX) reaches the superior tarsus in panel B. Other abbreviations: see the list of common abbreviations. Panels A and B are prepared at the same magnification (scale bar in panel B).

the sac roof or fornix (H2 muscle bundle; Fig. 4A,B); 2) the primitive but thick medial collateral ligament inserting to the limbus of the conjunctiva (Fig. 4A), and 3) the short tendons of the H1 bundle of Horner's muscle inserting to the maxilla (Fig. 4A). In the last case, i.e., the bony insertion of Horner's muscle, there was the typical musculotendinous junction in which the black-colored endomysium of muscle fibres was

connected with red-colored type-1 collagen fibres (Fig. 4E). At all stages examined, a candidate for the medial palpebral artery ran along the supero-inferior axis lateral to the H1 bundle of Horner's muscle.

At 30 weeks, the tarsus was well developed, but no fascial structure was connected with the tarsus (Figs. 5 and 6). In the upper eyelid, the preseptal layer of the palpebral part of the

orbicularis oculi muscle was clearly separated from the tarsus by loose connective tissue, and the pretarsal layer of the muscle was clearly identified in horizontal sections of all 4 specimens at 30 weeks (Fig. 5B). In the loose connective tissue, several fascial structures were seen running along and within the superficial side of the tarsus: one of them, the posterior-most or deepest fascia, was connected with the orbital septum more superiorly or laterally. A candidate for the medial palpebral artery extended to the superior tarsus (Fig. 5B). In the lower eyelid, although the laminar architecture was basically the same as that in the upper eye-

lid, the pretarsal layer of the palpebral part of the orbicularis oculi muscle was absent, possibly due to the fact that it did not lie within the plane of section. Notably, in horizontal sections of 3 out of 4 specimens at 30 weeks, a connective tissue mass was evident along and within the superior side of the horizontal part of the inferior lacrimal canaliculus (Fig. 6A). This fibrous tissue, a candidate for the perspective medial canthal ligament (MCL), issued thick collagen fibres into the H1 bundle of Horner's muscle. In the lower eyelid, the orbital septum was evident on the inferior side of the tarsus (Fig. 6C). The medial rectus sheath was not

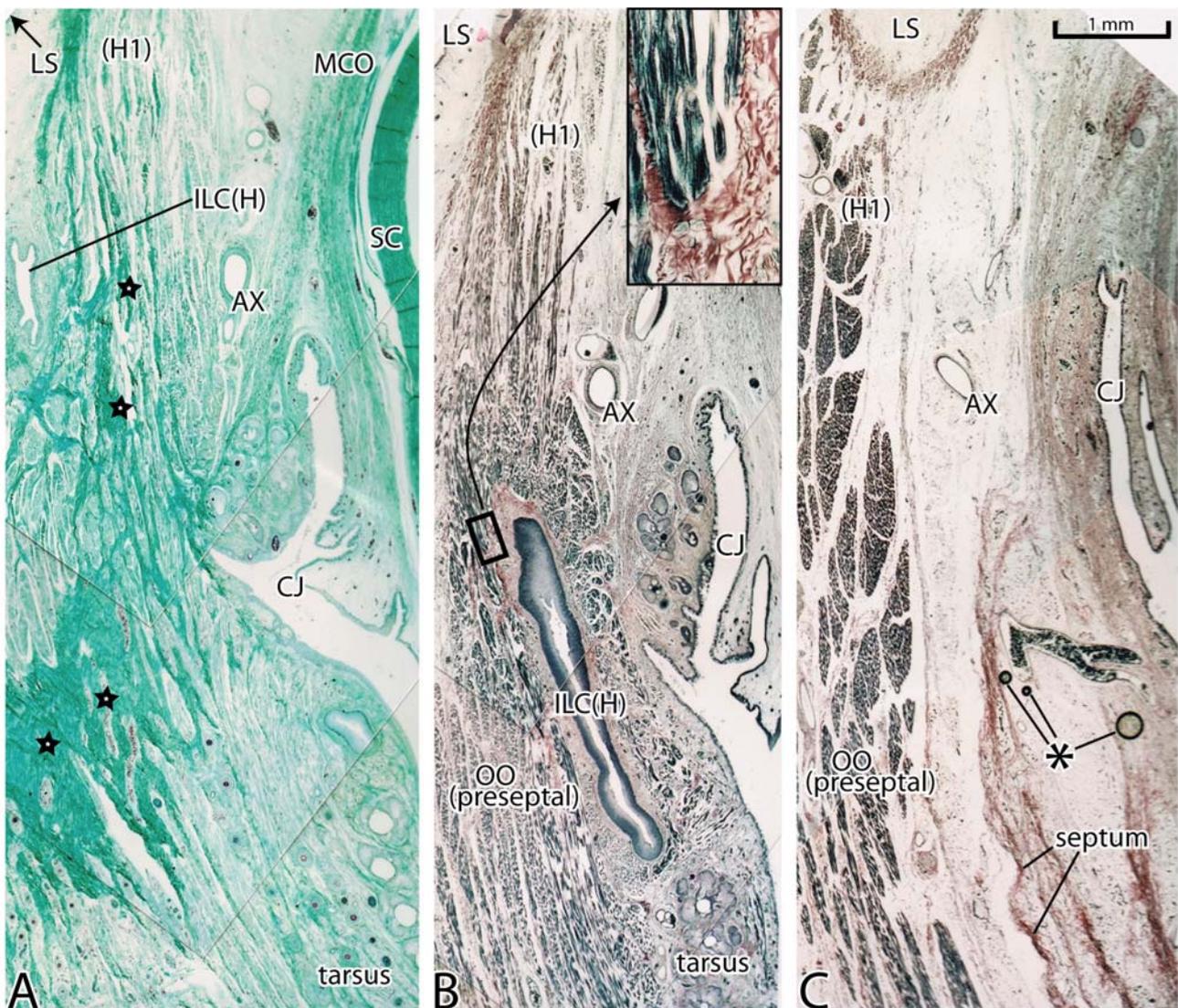


Figure 6. A candidate for the primitive medial canthal ligament seen in the largest specimen. Same specimen as that shown in Figs. 4 and 5. Horizontal sections of the lower eyelid. Masson trichrome staining (panel A) and silver staining (panels B and C). Masson trichrome fails to stain striated muscle red. Panel A (Panel C) is the most superior (inferior) side of the figure. Panel A is 4 mm inferior to Fig. 4B. Intervals between panels are 1 mm (A-B) and 2 mm (B-C), respectively. Panel A exhibits a mass of collagen fiber bundles (green color, clear stars) and the perspective medial canthal ligament at a site sandwiched between the superior and inferior lacrimal canaliculi (the inferior canaliculus is shown in Fig. 6B). An insert at the right upper angle of panel B, corresponding to the square in panel B, displays sudden ending of muscle fibers along the inferior lacrimal canaliculus (ILC). Panel C shows the margin of the developing orbital septum (septum). The pretarsal part of the orbicularis oculi muscle is thin in the lower eyelid and located between panels B and C. Note that, in panels A and B, there is no definite connection of the medial end of the upper tarsus with any fascial structures. Other abbreviations: see the list of common abbreviations. All panels are prepared at the same magnification (scale bar in panel C).

connected with fascial structures in the lids, but with the primitive medial collateral ligament. Thus, the capsulopalpebral ligament had not yet developed. A primitive mass of fatty tissue was evident on the medial side of the conjunctival space (Fig. 5).

Consequently, the tarsus was not connected with either the bony orbit at the medial canthus or any parts of the lacrimal drainage route. The tendons of Horner's muscle were divided into two groups: 1) those including the typical myotendinous junction of the posterior bundle (H1) located along the maxilla, and 2) multiple thick tendons of the anterior bundle (H2) located along the lacrimal sac. From a histological viewpoint (see Materials and Methods), it was confirmed that the muscle fibres did not insert into the canaliculi. In frontal sections, a perspective MCL was found in a raphe-like structure extending between the conjunctiva and maxilla in front of the lacrimal sac, corresponding to a fibrous mass sandwiched by horizontal parts of the superior and inferior lacrimal canaliculi in horizontal sections.

DISCUSSION

The present study revealed that a raphe-like connective tissue structure received the palpebral part of the orbicularis oculi muscle. This structure was difficult to identify because of its specific location, sandwiched by horizontal parts of the superior and inferior lacrimal canaliculi. Thus, in horizontal sections, the raphe-like structure appeared as a fibrous mass with an irregular shape, but in terms of tissue architecture it differed from the connective tissue sheath of the canaliculi. This raphe-like structure was independent of, and separated from, Horner's muscle and its tendons, and we considered it to be the most likely candidate for the perspective medial canthal ligament (MCL). As described in the Introduction, the tendons of Horner's muscle are divided into 1) the so-called "reflection tendon", which includes the typical myotendinous junction at the insertion into the maxilla, and 2) the so-called "direct tendon" at the roof of the lacrimal sac. Notably, no connective tissue structures, including the raphe and Horner's muscle tendons, are attached to the tarsi. As discussed by Robinson and Stranc (1970), the "tensor tarsi" muscle (part of the orbicularis oculi muscle) seems to be absent. A connection

between the MCL and the tarsi, if present in adults, seems to be established after birth due to connective tissue growth along the lacrimal canaliculi. Although we did not use complete serial sections, in the lower eyelid we did not find the pretarsal layer of the palpebral part of the orbicularis oculi muscle, this being consistent with observations in adults by Yamamoto et al. (2001).

In this context, it is necessary to bear in mind that the fetal medial collateral ligament (medial check ligament) is quite different from the adult morphology: in fetuses it originates from the medial rectus and inserts to the conjunctiva without any bony attachment (Osanai et al., 2011a). According to Kakizaki et al. (2010), in adult cadavers the medial collateral ligament supports the posterior aspect of Horner's muscle and is inserted into the medial orbital wall. Thus, in the MCL as well as the medial collateral ligament, the adult morphology is not consistent with the fetal primitive form, but seems to change depending on functional demands after birth. Kakizaki et al. (2008) considered that, in adults, medial fixation of the tarsi should be performed by the medial rectus capsulopalpebral fascia and Horner's muscle, rather than by the MCL. In fact, Horner's muscle and its tendon may contribute to the formation of the bony attachment of the MCL, but the major part of the MCL most likely originates from the fetal raphe-like structure.

From a histological viewpoint (see Materials and Methods), it was confirmed that Horner's muscle does not insert into the lacrimal canaliculi, but is simply attached to, or embedded in, the fibrous sheath around the canaliculi. Thus, direct transfer of tensile stress from the muscle to the canaliculus is difficult. However, such "attachments" seem to be sufficient for maintaining the mutual topographical relationship of the lacrimal canaliculus, MCL and bony orbit. The difference in muscle fibre direction according to the location around the canaliculi (i.e., differences in the strength of contraction to the canaliculi) may be effective for stabilizing the anatomy against rolling of the palpebral margin. Without Horner's muscle in fetuses, horizontal parts of the canaliculi, especially the inferior canaliculus, would become mechanically too weak relative to the adjacent raphe-like structure receiving the muscle contraction from the strong orbicularis oculi.

In their beautifully prepared sagittal sections of fetuses at CRL stages between 100 mm and 165 mm, Fernandez-Valencia and Gomez-Pellico (1990) identified the MCL as a dense connective tissue band extending horizontally between the lacrimal sac and skin dermis at the medial canthus. Likewise, in their frontal sections of two 100-mm CRL fetuses (Fernandez-Valencia et al., 2001), they demonstrated a similar fibrous band originating from the superior and inferior lacrimal canaliculi, joining together and inserting into the lacrimal sac. This also extended horizontally, and the horizontal plane of the fibrous structure corresponded to the line formed by the fused eyelids. In their figures, the palpebral part of the orbicularis oculi muscle appeared to attach to the fibrous band. Parts of the structure they described appeared to correspond to the present raphe-like structure or primitive MCL to varying degrees. However, we found no evidence that the foetal MCL connected with either the skin dermis or the lacrimal sac. Likewise, in the present fetuses, the fibrous sheath around the lacrimal canaliculi was different from the primitive MCL, and located distant from it. In addition, at the level of the lower eyelid Fernandez-Valencia and Gomez-Pellico (1990) described an insertion of the preseptal part of the orbicularis oculi muscle to the maxilla (i.e., the anterior dilator muscle of Hyrtl). We also observed a thick muscle layer in this location (Fig. 5A).

Congenital entropion is a rare condition among Westerners, who have little epiblepharon (Crawford, 1984, Yang et al., 1996). However, in mongoloids, where epiblepharon is often present, congenital entropion occurs fairly frequently (Crawford, 1984). In Japan, congenital entropion is found in 46% of newborn babies, but this percentage decreases with the growth of the child, and reaches 24% at 1 year of age, 20% at 2 years, 7% at 3 to 4 years, and 2% at 5 to 6 years (Noda et al., 1989). Many cases of entropion are associated with a family history (O'Donnell and Collin, 1991). This epidemiological observation suggests a difference in the foetal anatomy of the tarsus and associated connective tissues among human populations. Nevertheless, the present study has demonstrated that the tarsus is unlikely to be fixed in late-stage caucasian fetuses. The difference between human populations may be formed after birth.

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