Fetal Check Ligament Connected between the Conjunctiva and the Medial and Lateral Recti

Hajime Osanai,1 Jose Francisco Rodríguez-Vázquez,2 Hirosi Abe,3 Gen Murakami,4 Hirosi Ohguro,1 and Mineko Fujimiya5

PURPOSE. There seems to be little or no information about the morphology of the fetal eye check ligament.

METHODS. The authors examined longitudinal and cross-histologic sections from the large collection of human fetuses at Universidad Complutense, Madrid.

RESULTS. In longitudinal sections from 20 fetuses (four at each of 12, 15, 20, 25, and 30 weeks of gestation), a distinct connective tissue band was found connecting the medial and lateral recti and the limbus of the conjunctiva (sites at and around the lateral and medial angles of the conjunctival space). Silver impregnation revealed that the muscle endomysium (type 4 collagen dominant) was connected with composite fibers of the band (type 1 collagen). The cross-sections from three fetuses (20 weeks) exhibited a site-dependent difference in the rectus sheaths: the orbital-sided sheath suddenly increased in thickness when it tightly attached to the muscle bundles. The attaching orbital-sided muscle bundles reached 14% to 15% (or 18%–20%) in the cross-sectional area of the MR (or the LR).

CONCLUSIONS. Taken together, the distinct connective tissue band extending to the conjunctiva was “originated from” the MR and LR rather than from a part of the muscles inserting into the connective tissue band. This band was most likely a primitive form of the check ligament. The authors hypothesize that the primitive check ligament conducts muscle tension to the conjunctiva to coordinate growth patterns between the anterior and posterior sides of the eyeball. This hypothesis may support en bloc recession for infantile esotropia. (or the LR).

The mediolateral and check ligaments are well-known concept, and these structures are identified as triangular attachment parts of the anterior extensions of sheaths of the medial and lateral recti to, respectively, the lacrimal and zygomatic bones. However, to our knowledge, fetal development has not been examined. In view of the wide range of discussions regarding the active pulley hypothesis,1-5 we asked whether the fetal check ligament should be included in the developing pulley. On the assumption that the primitive check ligament independently develops before establishment of the pulley and sleeve system, we conducted the present study using both longitudinal and cross-sections of the recti obtained from human fetuses.

Sevel9,10 considered that the fetal tendon insertion of the medial and lateral rectus muscles “moves posteriorly” from the “limbus” of the conjunctiva (sites at and around the lateral and medial angles of the conjunctival space) to the site along the equator of the eye that is present in adults. It was noteworthy that the primitive tendon he described inserts into the limbus of the conjunctiva and is very similar to the adult platelike structure or the medial rectus pulley identified by Ruskell et al.11 Consequently, to provide a better understanding of the pulley and check ligament, the latter of which has been ignored recently, we reexamined the fetal morphology of the Sevel’s rectus insertions into the conjunctiva.9,10 In the present histologic study, in addition to the usual staining methods for connective tissues (e.g., Masson trichrome), we chose silver impregnation to demonstrate a connection between the muscle endomysium (type 4 collagen-dominant) and the usual connective tissues (type 1 collagen) according to Abe et al.13

MATERIALS AND METHODS

The study was performed in accordance with the provisions of the Declaration of Helsinki 1995 (as revised in Edinburgh 2000). We examined the paraffin-embedded histologic sections of 23 midterm fetuses at 12 to 30 weeks of estimated gestational age (80- to 250-mm crown-rump length). For longitudinal sections of the recti, four fetuses each were used at ages 12, 15, 20, 25 and 30 weeks. Thicknesses of the sections were 5 μm (fetuses earlier than 15 weeks) or 10 μm (fetuses later than 20 weeks), at intervals of 50 μm (early stage) or 100 μm (late stage), and were stained with hematoxylin and cosin (HE) or silver impregnation according to Lillie et al.14 In addition, three fetuses (20 weeks) were used for cross-sections of the recti (10-μm thick; 200-μm interval) with Masson trichrome staining or silver impregnation. After tracing of muscle bundles on the cross-sections with Masson trichrome, the first author (HO) measured the cross-sectional area using an image analysis program (ImageJ software; developed by Wayne Rasband, National Institutes of Health, Bethesda, MD; available at http://rsb.info.nih.gov/ij/index.html). Two sections were used for measurement of one medial or lateral rectus.

We chose specimens that did not show obvious deformation of the eyeball resulting from histologic procedures, such as shrinkage and irregularity of shape. All the specimens were part of the large collection kept at the Embryology Institute of Universidad Complutense, Madrid. They were obtained from women who had miscarriages and ectopic pregnancies and were treated 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 fetuses at each stage. Approval for the study was granted by the ethics committee of the University.

RESULTS
In 20 specimens for longitudinal sections of the recti, we consistently observed a connective tissue band inserting into the limbus of the conjunctiva9,10 (see Introduction) and a tendon of the medial rectus (MR) and lateral rectus (LR) muscles. This “limbus” was located far lateral or medial to the corneal limbus that has recently been noted as a site of the epithelial cell progenitors.15,16 The connective tissue band for the MR was much thicker than the LR at all the stages examined. Type 1 collagen fibers in the connective tissue band, stained red in the present silver impregnation, were much less dense than those in the tendon. The anterior end of the muscle belly was located at the level of the lens until 15 weeks, but it moved posteriorly after 20 weeks. Muscle fibers in the MR and LR appeared not to be heterogeneous, and we found no morphologic demarcation between the orbital and global parts of the muscle in the longitudinal sections (Figs. 1, 2).

Figure 1A shows the topographic anatomy of the anterior part of the MR at 15 weeks, and Figure 1B shows that at 30 weeks. Although the eyeball and its surrounding structures grew in size more than twofold during this period (almost threefold from 12 to 30 weeks of gestation), the anterior part of the MR and LR showed the same combination of structures: a thin tendon slip from the muscle belly to the eyeball and a thick connective tissue band extending from the orbital surface of the rectus to the conjunctiva. The connective tissue band appeared to originate from the orbital surface of the medial or lateral recti. The attaching site of the band to the MR was at or in the anterior side of the equator of the eyeball, whereas the band attached to the LR in the posterior side of the equator. Thus, the lateral connective tissue band was longer than the medial band.

Figure 2 displays higher magnification views of Figure 1. We noted a connection between the muscle endomysium (rich in type 4 collagen; indicated by black in silver impregnation) and the connective tissue band (type 1 collagen; indicated by red in silver impregnation; Fig. 2B). Such fibrous connections increased with age. At the superior and inferior margins, the band was connected with a sheetlike structure for the inferior or superior rectus. Thus, the sheaths of the four rectus muscles, as a whole, appeared to provide a sleeve system surrounding the eyeball, as described by Koornneef.17 Tenon’s sheath or capsule was evident after 25 weeks at the global side of the rectus (Fig. 1B): this “portal” morphology was the same as that in adults.5,18

Cross-sections of the recti from three fetuses at 20 weeks clearly showed a site-dependent difference in morphology of the rectus sheaths. First, a sheath of the MR was not distinct in the posterior site (Fig. 3A); second, the orbital-sided sheath suddenly increased in thickness (Fig. 3B); third, the global-sided sheath gradually became unclear (Fig. 3C); and fourth, only the orbital-sided sheath remained as a thick connective tissue band (Fig. 3D). This sequence was the case with the LR: the connective tissue band suddenly became thick at attachment sites of the orbital-sided muscle bundles. According to our measurement using cross-sections, the MR and LR exhibited the maximum sectional area in the posterior side of the equator of the eye ball (MR, 0.47 mm² at mean; LR, 0.69 mm² at mean). However, at the anterior site in which the muscle bundles attached to the connective tissue band, both muscles were reduced in thickness (MR, 0.25 mm² at mean; LR, 0.55 mm² at mean). The orbital-sided muscle bundles attaching tightly to the connective tissue band reached 14% to 15% (or 18%–20%) in the cross-sectional area of the MR (or the LR). Silver impregnation exhibited abundant type 1 collagen fibers in the orbital-sided muscle bundles (Fig. 3F). However, most of the orbital-sided muscle bundles of the MR and all the LR muscle bundles did not “insert” into the connective tissue band: the insertion was seen in only one or two bundles of the MR (1.5%–1.8% of the cross areas; Figs. 3E–G). Moreover, the limited muscle insertions appeared to contain no or few type 1 collagen fibers for tensile stress (Fig. 3G).

Taken together with results from both of the longitudinal sections and the cross-sections, the distinct connective tissue band to the conjunctiva was “originated from” the orbital surface of the MR and LR rather than the reverse (i.e., the muscles inserted into the connective tissue band).

DISCUSSION
The present study of midterm fetuses demonstrated the coexistence of the tendon of the rectus muscle and the connective tissue band extending between the rectus and the conjunctiva. Although Sevel9 might have considered these structures to be variations of muscle insertions, the latter connective tissue band seemed unlikely to “change” to a real tendon. The connective tissue band is most likely a...
primitive form of the check ligament rather than the pulley because, other than a few orbital-sided muscle insertions, there was no evidence to suggest the morphology of the adult pulley. At the stages examined, the periorbital smooth muscle tissues were restricted to the orbital muscle behind the eyeball and were not present around the rectus.\textsuperscript{18} Osa-
nai et al.\textsuperscript{19} demonstrated that the MR pulley plate is composed almost exclusively of anteroposterior-oriented elastic fibers. The primitive check ligament may be a precursor of the anteroposterior elastic fibers. Generally, in the human fetus, elastic fiber differentiation may occur in the late stage of development or even after birth.\textsuperscript{20}

\textbf{FIGURE 2.} Higher magnification views of a connection between the medial rectus endomysium and the connective tissue band. Longitudinal sections. (A, HE staining; B, silver impregnation) Higher magnification views of the site at and around the circle in Figures 1A and 1B, respectively. (B) The endomysium (type 4 collagen-dominant) is clearly shown as \textit{black lines} along the surfaces of striated muscle fibers, in contrast to type 1 collagen fibers (\textit{red}). The endomysium is connected with the distinct connective tissue band (i.e., primitive check ligament). A few muscle fibers (\textit{stars}) at the medial margin of the medial rectus (MR) appear to insert into the distinct connective tissue band (\textit{arrows}). \textit{Cap}, capillary.
Plock et al. recently interpreted Sevel’s classical description (see Introduction) as “the medial rectus tendon achieves its adult position within 24 months postpartum, after simultaneous growth of the anterior part of the eye and posterior recession through tissue degeneration.” Their interpretation suggests that the rectus pulley develops postnatally. Actually, according to the present observation, Tenon’s capsule also starts development in the late fetal stage. Most kinds of eye movements, especially gaze movement, develop after birth and even in infants. Therefore, the adult morphology of the thick MR pulley seems to develop according to the mechanical demands of the minute eye movements that occur after birth. In fact, in the orbital layer of the rat rectus muscle, the unique isozyme distribution of the myosin heavy chain is established at 11 days postpartum. Using a 17-month-old human specimen, Lim et al. demonstrated a difference in collagen content between the orbital and the global parts of the rectus. Thus, the acquisition of eye movement in the first year of life seems to be critically important for pulley development. Future detailed imaging studies of the eye in children may demonstrate the details of this postnatal development.

The most striking observation in the present study was that the primitive check ligament, rather than a pulley, connected between the conjunctiva and the rectus. Moreover, tensile stress was suggested between the muscle and the ligament because of the abundant type 1 collagen fibers contained in the orbital-sided muscle bundles. However, the tension seemed to be smaller than that along the rectus tendon because type 1 collagen fibers in the primitive check ligament were less dense than those in the tendon. According to the present observations, we concluded that the ligament “originated from” the medial and lateral recti rather than the reverse (i.e., the muscle...
inserted into the ligament). The next question that arose was this: why does the primitive check ligament connect to the conjunctiva rather than to bones? There was no bony attachment of any periocular connective tissues found in fetuses. Thus, the well-known bony attachment of the check ligament in adults seems to be develop after birth. During fetal development, rather than the functional regulation of eye movements, we hypothesize that the primitive check ligament conducts muscle tension to the conjunctiva to coordinate growth patterns between the anterior and posterior sides of the eyeball. This is the usual configuration observed between the skeletal muscle fascia and the collateral ligament of limb joints, which avoids impingement of the ligament into the joint.26,27 Thus, it is better to consider that, at least in fetuses, the check ligament plays a role different from that of the adult pulley and sleeve system. However, we do not deny the possibility that the fetal check ligament, which we described, develops into part of the pulley after birth.

For surgical treatment of infantile esotropia, Helveston et al.28 postulated posterior recession of the conjunctiva and Tenon’s capsule in combination with the classical recession of the medial rectus insertion. In anomalous topographic relation between the rectus insertion and the limbus of the conjunctiva, the Helveston et al.28 en bloc recession may induce a fibrous connection between the limbus and the rectus insertion and, after surgery, may provide a possibility to coordinate growth patterns between the anterior and the posterior sides of the eyeball.

References