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Development of the deep flexor tendons and lumbricalis muscle in the hand and foot: a histological study using human mid-term foetuses

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To revisit foetal development of the deep flexor tendons of the hand and foot, we examined the paraffin-embedded histology of 20 mid-term foetuses at 8–15 weeks of estimated gestational age (35–118 mm crown-rump length or CRL). At 8–9 weeks, in front of the metacarpal bones, the flexor pollicis longus and flexor digitorum profundus (FDP) muscles provided a plate-like, common tendon from which the lumbricalis muscles originated. However, in the foot, we had no evidence of such a common tendon. The flexor pollicis tendon was separated from the common tendon at 9–10 weeks possibly due to mechanical stress from the laterally growing thumb. Notably, at the lumbricalis muscle origins at 10–12 weeks, the FDP and flexor digitorum longus tendons remained undifferentiated and the primitive tenocytes were dispersed from them. The dispersed cells seemed to develop into an interface tissue between the lumbricalis muscle fibre and the deep tendon. In 3 of 5 specimens at 15 weeks, we found an excess number of the FDP tendons (5–7) in the proximal side of the lumbricalis muscle origin. However, the excess tendons dispersed in the lumbricalis muscle origin. The development of the lumbricalis muscle origin might follow the tendon splitting for four fingers. However, conversely, we hypothesised that the developing lumbricalis muscles re-arranged the deep flexor tendons to provide a configuration of "one deep tendon per one finger (or toe)". The quadrates plantae muscle seemed not to contribute on the re-arrangement. (Folia Morphol 2012; 71, 3: 154–163)

Key words: flexor digitorum profundus muscle, flexor digitorum longus muscle, lumbricalis muscles, carpal tunnel, tendon arrangement, human foetus

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INTRODUCTION

The flexor digitorum profundus muscle (FDP) of the fingers is divided into four or more muscle slips continuous with just four tendons for each of the fingers, at a level proximal to the carpal tunnel, whereas the flexor digitorum longus muscle of the toes carries a very long, single tendon that finally divides into four tendons at the level of the metatarsal bone in spite of variations in the quadrates plantae muscle slips [11]. Dylevsky [8] reported, in contrast to the flexor digitorum superficialis muscle, a single, common tendon of the FDP and the flexor pollicis longus (FPL) muscles. Thus, in both the hand and foot, the deep flexor tendons can make a common tendon. Does the two-layered arrangement of the superficial tendons in the hand prohibit formation of the common superficial tendon? Leijnse (1997) [14] considered that, in his excellent dissection study of the FDP and lumbricalis muscle in the adult human hand, the tendon splitting depends on mechanical stress from the finger movements in utero. Leijnse [14] also demonstrated that, in adult cadavers, crisscrossing of the FDP tendon fibres frequently occurs at the lumbricalis muscle origin because of oblique insertion of the latter muscle to the FDP tendons. Thus, the development of the lumbricalis muscle attachment to the FDP may follow the splitting of the FDP tendons.

In the extremities, both a muscle belly and its tendon are considered to develop at the same time, according to recent developmental biology studies [5, 7, 17, 22]. Likewise, the small hand muscles as well as the long flexor muscles seem to develop together in the human foetuses [8]. Foetal development of the muscle-tendon interface has been one of the leading topics of anatomical research [1, 3]. Do the lumbricalis muscle fibres really establish the proximal attachment after differentiation and splitting of the FDP tendons? If so, what happens at the surface of the foetal tendon is of interest because rearrangement of the tenocytes and collagen fibrils is likely to be required to for the interface. Previous studies of finger tendons did not consider lumbricalis muscle attachment [6, 8]. Consequently, the aim of this study was to clarify the foetal morphology of the lumbricalis muscle origins at and along the deep flexor tendons in the hand and foot.

MATERIAL AND METHODS

The study was performed in accordance with the provisions of the Declaration of Helsinki 1995 (as revised in Edinburgh 2000) [10]. We examined the paraffin-embedded histology of 20 mid-term foetuses at 8–15 weeks of estimated gestational age (CRL 35–118 mm): 5 foetuses each at 8––9 weeks (CRL 35–40 mm), 10 weeks (CRL 50–58 mm), 12 weeks (CRL 71–80 mm), and 15 weeks (CRL 102––118 mm). With the agreement of the families concerned, these specimens had been donated to the Department of Anatomy, Chonbuk National University, Korea, and their use for research had been approved by the university ethics committee. Without contravening any of the university or hospital rules, authors other than those affiliated to Chonbuk University did not need to supply details of this research project to the corresponding committee in Japan. The foetuses had been obtained by induced abortions. After abortion, each of the mothers had been personally informed by an obstetrician about the possibility of donating the foetus for research: no attempt was made to encourage donation. Because of randomisation of the specimen numbering, it was not possible to trace any of the families concerned.

The donated foetuses had been fixed with 10% w/w formalin solution for more than three months. After division into the head and neck, thorax, abdomen and pelvis, and the four extremities, all parts were decalcified by incubating them at 4°C in 0.5-mol/L EDTA solution (pH 7.5; Decalcifying solution B; Wako, Tokyo) for 1–3 days, depending on the size of the material. The scapula and its associated muscles were included in the upper extremity segment, whereas the hip joint was included in the abdomen and pelvis segment. Routine procedures for paraffin-embedded histology (to yield sections 5 μm thick) were conducted. Parts other than the hand and foot were used for our recent studies, most of which have already been published. Most specimens of the hands and feet were processed for cross sections, while one hand and one foot in each of the four stage groups was used for tangential sections along the palmar or plantar aspect. The interval of sections was 20–100 microns depending on the size. Most sections were stained with haematoxylin and eosin (HE) or silver impregnation according to Lillie et al. [16], while some were used for immunohistochemistry (see below).

The silver impregnation used here is usually referred to as "Gitter (reticular network) staining" and differs from both the well-known Gomori method [23] and periodic acid methenamine silver staining [25]. Using the Gitter staining (silver im-

pregnation) method, we recently discriminated the black-coloured fibrous tissue comprising collagen type 3 and 4 fibrils from the red-brown or brown--violet-coloured tissue of collagen type 1 fibrils [1, 19, 20]. The primary antibodies used were (1) mouse monoclonal anti-human desmin (dilution, 1:50; Dako, Glostrup, Denmark); (2) mouse monoclonal anti-human striated muscle actin (dilution, 1:100; Dako, Glostrup, Denmark); and (3) mouse monoclonal anti-human CD34 (dilution, 1:100; Dako, Glostrup, Denmark). The second antibody (Dako Chem Mate Envison Kit, Dako, Glostrup, Denmark) was labelled with horseradish peroxidase (HRP), and antigen-antibody reactions were detected using the HRP-catalysed reaction with diaminobenzidine (with haematoxylin counterstaining). We recently demonstrated that the desmin (or CD34) antibody is useful for identification of skeletal muscle insertions (or primitive connective tissues) in human foetus paraffin sections [1, 2]. The present immunohistochemistry for the striated muscle actin is available for specimens that do not react with the desmin antibody according to our experience in a study of the oesophagus [13] although it is not specific for the muscle insertion but positive in entire parts of striated muscle fibres.

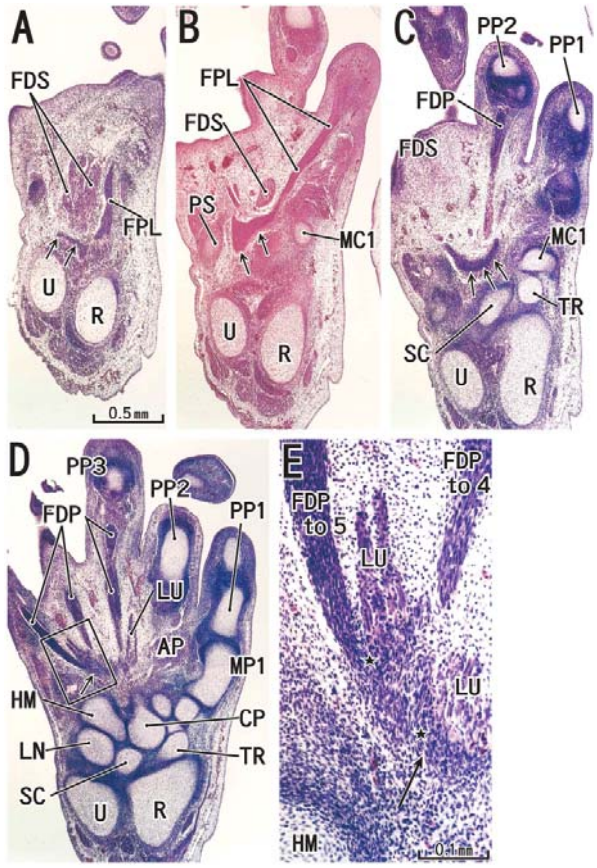

Figure 1. Tangential sections of flexor tendons of the hand in an 8-week foetus. HE staining. The smallest specimen in this study (CRL 35 mm). The upper side of each panel corresponds to the distal side of the hand. Panel **A** (or **D**) is the most palmar (or dorsal) side of the figure, **E** is a higher magnification view of a square in panel **D**. Intervals between panels are 0.05 mm (**A**–**B**, **B**–**C**) and 0.1 mm (**C**–**D**), respectively. Notably, tendons of the flexor pollicis longus muscle (FPL) and the flexor digitorum profundus muscle (FDP) provide a common primitive tendon (arrows in panels **A**–**D**). The common tendon is composed of mesenchymal cells and the density is lower than the distal tendons (**F**). The lumbricalis muscles (LU) originate from the mesenchymal condensation of the common tendon. The flexor digitorum superficialis tendons (FDS) divides into two slips and they sandwich the deep tendon (i.e. a primitive chiasm; **C**, **D**). Panels **A**–**D** are prepared at the same magnification (scale bar — **A**); AP — adductor pollicis muscle; CP — capitate bone; HM — hamate bone; LN — lunate bone; PP — proximal pharynx; PS — pisiform bone; R — radius bone; SC — scaphoid bone; TR — trapezium bone; U — ulna bone; other abbreviations: I — interosseous muscles (dorsal and ventral); LU — lumbricalis muscles; MC1–MC5 — first-fifth metacarpal bone; MT1–MT5 — first-fifth metatarsal bone; MN — medianus nerve; UA — ulnar artery.

RESULTS

Foetal palmar flexor tendons and the lumbricalis muscles

The present figures include tangential sections (sections along and in parallel with the skin surface) of only one hand at eight weeks (Fig. 1). Although tangential sections are good for demonstration of the topographical anatomy of the hand and foot, especially of the bone arrangement, the lumbricalis muscle origins from the FDP tendons were difficult to show because we did not use serial sections but semi-serial sections. Consequently, the other six figures are based on cross sections.

The carpal tunnel, extending from the level of the proximal row of carpal bones to the level of the proximal part of the metacarpal bones, was surrounding by a distinct fibrous band or sheath after nine weeks (Figs. 2–5). In 3 of 5 foetuses at 8––9 weeks, the band appeared to be restricted in the palmar aspect (Fig. 2), while the band was not evident in another 2 specimens. The connective tissue band completely surrounded the long tendons at ten weeks (Fig. 3). This fibrous band or sheath was positive for CD34 (Fig. 4E, H), as we reported recently [2]. However, the band or sheath did not attach to the pisiform bone (Fig. 4C), and the ulnar nerve and artery were exposed to the subcutaneous tissue containing the developing palmar aponeurosis (Figs. 4C, 5B, C). The dorsal aspect of the sheath of the carpal tunnel was thick and continuous with

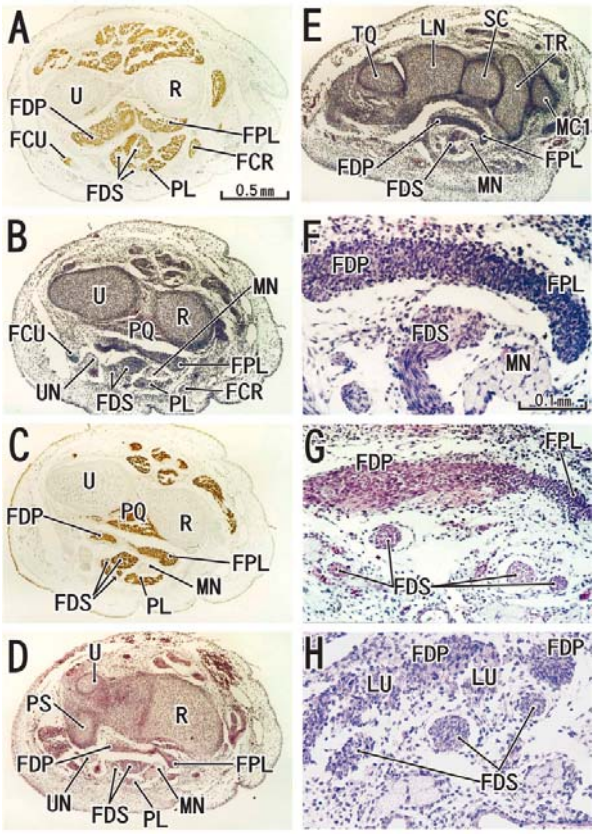

Figure 2. Cross sections of flexor tendons of the hand in a 9-week foetus. HE staining (**B**, **D**–**H**) and immunohistochemistry for striated muscle actin (**A**, **C**). The smallest-second specimen in this study (CRL 38 mm). The upper side of each panel corresponds to the dorsal side of the hand. Panel **A** (or **H**) is the most proximal (or distal) side of the figure, **F** is a higher magnification view of the central part of panel **E**. Intervals between panels are 0.15 mm (**A**–**B**), 0.01 mm (**B**–**C**), 0.2 mm (**C**–**D**), 0.3 mm (**D**–**E**), and 0.2 mm (**E**–**G**, **G**–**H**), respectively. In the proximal site (**A**), the flexor pollicis longus muscle (FPL) and the flexor digitorum profundus muscle (FDP) are clearly separated. However, these muscles are connected in panels **B** and **C** and these tendons provide a plate-like common tendon in the carpal tunnel (**E**). The lumbricalis muscles first appear in panel **G** (eosinophilic parts in the common tendon) and occupy between tendons in panel **H**. Panels **A**–**E** (**F**–**G**) are prepared at the same magnification (scale bar — **A** or **F**); FCU — flexor carpi ulnaris muscle; LN — lunate bone; PL — palmaris longus muscle; PQ — pronator quadrates muscle; PS — pisiform bone; R — radius bone; SC — scaphoid bone; TQ — triquetral bone; TR — trapezium bone; U — ulna bone; UN — ulnar nerve; other abbreviations — see Figure 1.

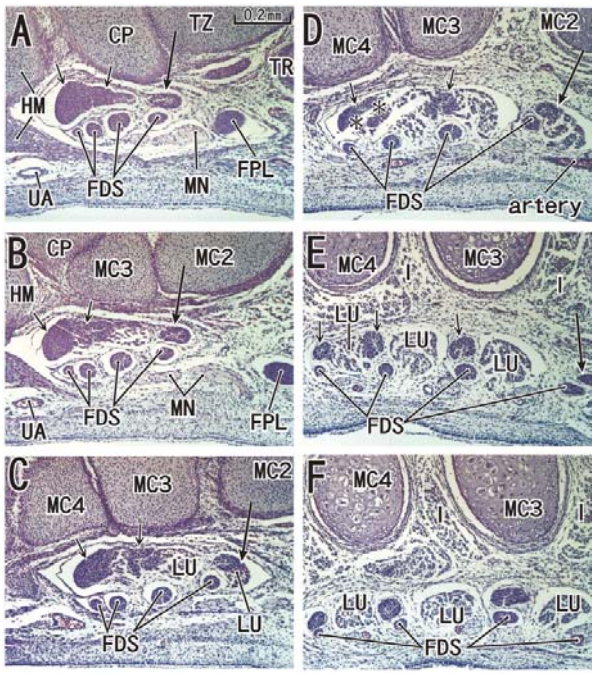

Figure 3. Deep flexor tendon to the index finger is located away from the other tendons: a 10-week foetus. HE staining. Cross sections. The upper side of each panel corresponds to the dorsal side of the specimen. Panel **A** (or **F**) is the most proximal (or distal) side of the figure. Intervals between panels are 0.15 mm (**A**–**B**, **B**–**C**) and 0.3 mm (**C**–**D**, **D**–**E**, **E**–**F**), respectively. In panel **A**, the deep tendons to the third–fifth fingers are fused to make a mass (short arrows). In panels **B**–**D**, in contrast to tendons of the flexor digitorum superficialis (FDS) with clear demarcation, tendons of the flexor digitorum profundus (arrows) appear to terminate at the margin to form anlagen of the lumbricalis muscles (LU). In this specimen, the deep flexor tendon to the index finger (long arrow) is located away from the other tendons (**D**, **E**). Asterisks in panel **D** indicate an artifactural tissue loss during the histological procedure. All panels are prepared at the same magnification (scale bar — **A**); CP — capitate bone; HM — hamate bone; TR — trapezium bone; TZ — trapezoid bone; other abbreviations — see Figure 1.

the perichondrium of the metacarpal bones, while the palmar aspect was relatively thin and faced the subcutaneous tissue. The thin palmar aspect was connected with the flexor pollicis brevis muscle (Fig. 4D). Therefore, the foetal sheath corresponded to the deep layer of the flexor retinaculum or the deep transverse carpal ligament, although there is some confusion in the definition of these terms (reviewed by Isogai et al. [12]). There was a space, large or small, around each of the tendons in the tunnel. The ulnar nerve and artery were also accompanied by a space around them, but at 15 weeks the covering band or the pisohamate ligament was not yet evident (Fig. 5A).

In the carpal tunnel and on the more proximal side, the tendons of the FPL muscle and FDP provided a common primitive tendon or a plate-like mesenchymal cell cluster (Figs. 1B, C, 2E–G). The cell density was lower than the distal part of the tendons (Fig. 1E), but it is higher than the digitorum superficialis tendons (Fig. 2F). The lumbricalis muscles originated from the common tendon (Fig. 1E). The clear demarcation between the lumbricalis muscle and deep tendon became bro-

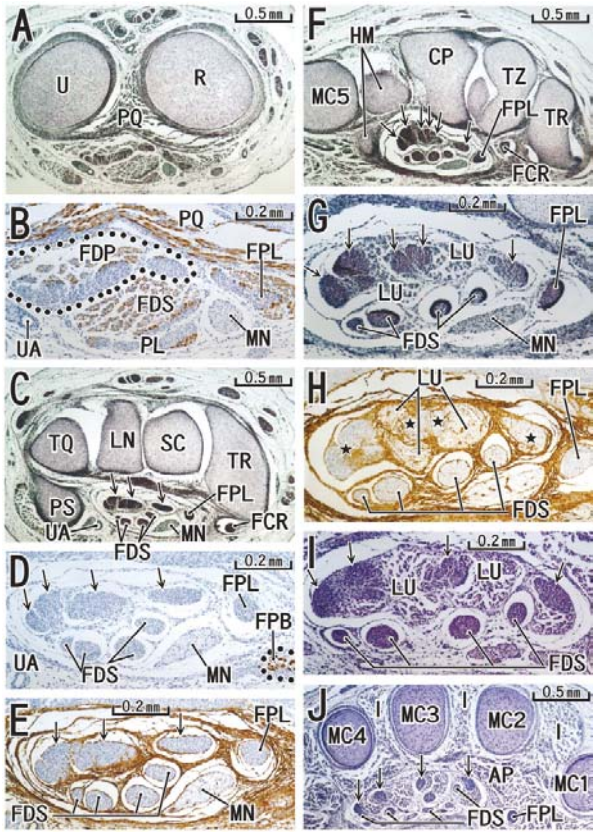

Figure 4. Flexor tendons of the hand in a 12-week foetus. HE staining, silver impregnation and immunohistochemistry. Cross sections. The upper side of each panel corresponds to the dorsal side of the specimen. Panel **A** (or **J**) is the most proximal (or distal) side of the figure; **A** and **B** are cross sections of the wrist, **C**–**E** display the level across the proximal row of carpal bones and, **F**–**I** exhibit the level across the distal row of carpal bones. Intervals between panels are 0.05 mm (**A**–**B**), 1.0 mm (**B**–**C**), 0.05 mm (**C**–**D**), 0.01 mm (**D**–**E**), 1.2 mm (**E**–**F**), 0.1 mm (**F**–**G**), 0.05 mm (**G**–**H**), 0.1 mm (**H**–**I**), and 0.2 mm (**I**–**J**), respectively. Panels **A**, **C**, **F** and **G** — silver impregnation; **B**, **D** — desmin immunohistochemistry; **E**, **H** — CD34 immunohistochemistry; **I**, **J** — HE staining. The primitive carpal tunnel is enclosed by a thick fibrous band (**C**, **F**) that is positive for CD34 (**E**, **H**). Each tendon of the flexor digitorum superficialis muscle (FDS) carries an independent sheath, whereas tendons of the flexor digitorum profundus (FDP, arrows) lose the independent sheath at and near the origins of the lumbricalis muscles (LU). Striated muscle fibres of the long flexors disappear until the level shown in panel **D**. Note, in contrast to the tendons in the proximal level (**E**), CD34-positive mesenchymal tissues invading into the primitive tendons (stars — **H**) in the level of LU origins. The magnification is shown by a scale bar in each panel; AP — adductor pollicis muscle; CP — capitate bone; FPB — flexor pollicis brevis muscle; HM — hamate bone; LN — lunate bone; PL — palmaris longus muscle; PQ — pronator quadrates muscle; PS — pisiform bone; R — radius bone; SC — scaphoid bone; TR — trapezium bone; TQ — triquetral bone; TZ — trapezoid bone; U — ulna bone; other abbreviations — see Figure 1.

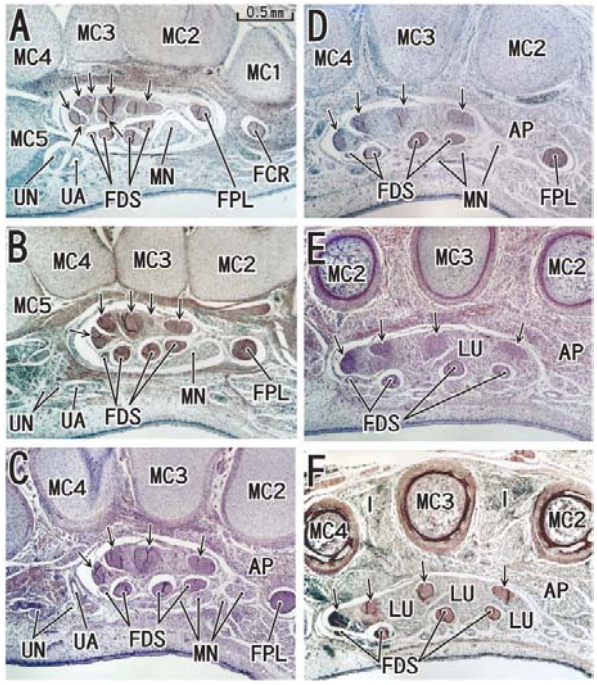

Figure 5. Excess numbers of deep tendons at the wrist are rearranged into the 4-tendon pattern in the level of the lumbricalis muscle origin: a 15-week foetus. HE staining and silver impregnation. Cross sections. The upper side of each panel corresponds to the dorsal side of the specimen. Panel **A** (or **F**) is the most proximal (or distal) side of the figure. Intervals between panels are 1.5 mm (**A**–**B**), 0.5 mm (**B**–**C**), 0.3 mm (**C**–**D**), 0.5 mm (**D**–**E**), and 0.3 mm (**E**–**F**), respectively. Panels **A**, **B**, **F** are silver impregnation; **C**–**E** are HE staining. Tendons of the flexor digitorum profundus (arrows) are counted 7 or more in the level shown in panel **A**, but these tendons are fused and destroyed in the level of origins of the lumbricalis muscles (LU). Note, in contrast to tendons of the flexor digitorum superficialis (FDS), a pale staining and unclear demarcation of the deep tendons at the process of re-arrangement in silver impregnation (**D**, **E**). All panels are prepared at the same magnification (scale bar — **A**); AP — adductor pollicis muscle; UN — ulnar nerve; other abbreviations; other abbreviations — see Figure 1.

ken at 10–12 weeks (see below). Even in the smallest specimen in this study (CRL 35 mm; 8 weeks), the flexor digitorum superficialis tendons divided into two slips and they sandwiched the deep tendon in the finger (Fig. 1C, D). The flexor carpi radialis and ulnalis muscles had already made definite tendons at 8–9 weeks.

The developing FDP tendons were identified as dense mesenchymal cell clusters that were stained brown-violet by silver impregnation (i.e. a bundle of type-1 collagen fibrils; Figs. 4, 5). By HE staining as well as silver impregnation, the colour was paler in the FDP tendons than in both the superficial tendons and the FPL tendon. At 10 and 12 weeks of gestation, on the proximal side of the wrist (a level at which the pronator teres muscle was present), the FDP tendons were composed of two masses: one large mass on the ulnar side and another small mass on the radial side (Figs. 3, 4). The

latter, radial tendon progenitor was continuous with a deep tendon for the index finger. The deep and superficial tendons for the index finger were sometimes (2 of 15 specimens examined) enclosed by a sheath distant from the other tendons (Fig. 3D). At 15 weeks, the large ulnar mass of the deep tendon anlagen was divided into 3 or more tendons on the proximal side. Thus, in 3 of 5 specimens at 15 weeks, the FDP tendons were counted 5–7 in number in the proximal side of the lumbricalis muscle origin. Figure 5A demonstrates a specimen containing the greatest number of tendons. We found no distinct difference in tendon morphology between stages, except for that at the lumbricalis muscle origins (see below).

The morphology of the lumbricalis muscles differed between stages: an origin from a FDP tendon was identified as "dispersed cells from margins of the tendon anlagen" at 10–12 weeks (Figs. 3B, C, 4G), while the muscle and tendon became again clearly demarcated at 15 weeks (Fig. 5C, D). The FDP tendons at the lumbricalis muscle origins contained abundant CD34-positive fibrous tissues (Fig. 4H), in contrast to the proximal or distal parts of the tendon. The lumbricalis muscle origin was consistently located on the distal side of the distal end of muscle fibres of the FDP, i.e. at the level of the pisiform bone (Fig. 4C). Thus, we found no continuation of muscle fibres between the lumbricalis and FDP muscles. The lumbricalis muscle origin was attached to a specific morphological feature of the developing FDP tendon, i.e. dense mesenchymal cell clusters with relatively pale staining (see above). Notably, at 15 weeks, the pale coloured tendons at the lumbricalis muscle origins were thicker than other parts of the corresponding tendons (Fig. 5C–E). Therefore, the FDP tendons were "dilated" and changed the structure specifically at the lumbricalis muscle origins, and not throughout the carpal tunnel. The tendons of the flexor digitorum superficialis were located on the superficial side of the lumbricalis muscle at any proximodistal level of the hand. We found no tendinous interconnections in either the FDP or the flexor digitorum superficialis muscle at or near the carpal tunnel.

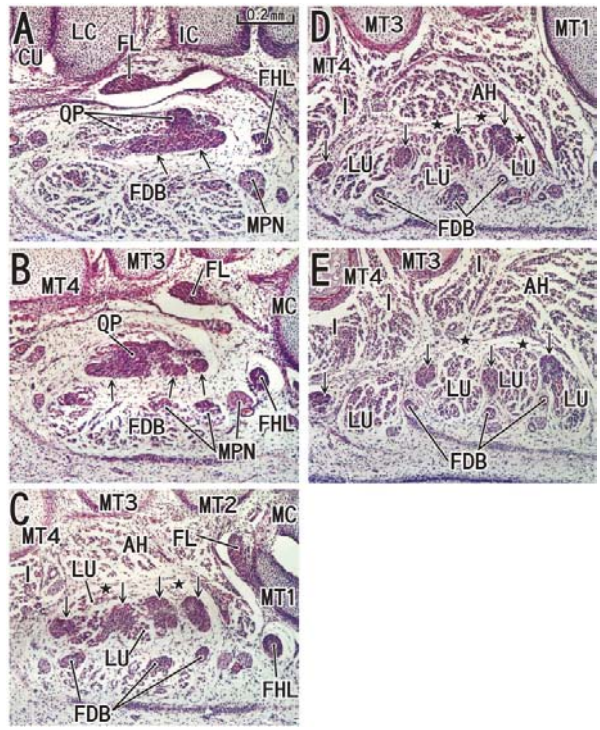

Figure 6. Flexor tendons to the toes: a 12-week foetus. HE staining. Cross sections. The upper side of each panel corresponds to the dorsal side of the specimen. Panel **A** (or **E**) is the most proximal (or distal) side of the figure. Intervals between panels are 0.3 mm. **A.** The quadrates plantae muscle and its tendon (QP) attach to the plate-like tendon of the flexor digitorum longus (arrows). **B**, **C.** The plate-like single tendon divides into 4 tendons to the toes (arrows) with origins of the lumbricalis muscle (LU) interposed between deep tendons. The flexor digitorum brevis muscle (FDB) loses the muscle fibres in panel **C** and **D** and all 4 superficial tendons appear in panel **E**. A distinct fascia or sheath is limited to that between the adductor hallucis muscle (AH) and flexors (stars — **C**, **D**). All panels are prepared at the same magnification (scale bar — **A**); CU — cuboid bone; FL — tendon of the fiburalis longus muscle; IC — intermediate cuneiform bone; LC — lateral cuneiform bone; MC — medial cuneiform bone; MPN — medial plantar nerve; other abbreviations — see Figure 1.

Foetal plantar flexor tendons and the lumbricalis muscles

A sheath and tunnel were evident for both tendons of the flexor hallucis longus and fibularis longus muscles, but not for the flexor digitorus longus (Fig. 6A–C). The quadrates plantae muscle contained a single thick tendon at the distal part of the muscle. Muscle fibres as well as the tendon of the quadrates plantae muscle inserted into a thick tendon of the flexor digitorum longus muscle (Fig. 6A). We found no connection between the quadrates plantae and flexor hallucis longus muscles. Immediately distal to the insertion, the flexor tendon divided into 4 parts (Fig. 6B). When the lumbricalis muscle fibres appeared between the divided flexor tendons, the tendons changed their morphology from fibrous tissue to a cluster of mesenchymal cells (Fig. 6C). Thus, similarly to the hand muscles, the lumbricalis muscle appeared to originate from the dispersed tendon-

-composite cells (Fig. 6C, D). However, the tendon for the second toe was darkly stained, in contrast to other tendons (Fig. 6C). During these changes from the proximal to the distal area of the foot, the tendons of the flexor digitorum brevis muscle became evident in the developing lumbricalis muscle (Figs. 6A–D). However, in contrast to the morphology in the hand, the superficial flexor tendons were embedded between the lumbricalis muscles distally (Fig. 6E, F). We found no distinct difference in plantar tendon morphology between stages. Even in all 5 specimens at 8––9 weeks, we had no evidence of a common tendon between the flexor hallucis longus and the flexor digitorum longus muscles in the levels of the metatarsal bones.

Foetal fingers and toes

In both the hand and foot, a pair of deep and superficial flexor tendons consistently acquired a common sheath at the level of the transverse head of the adductor pollicis or hallucis muscle (Fig. 7). At this distal level, the lumbricalis muscle fibres were still evident between the tendon sheaths. The tendon sheath was separated from the perichondrium of the metacarpal bone a short distance away, partly due to the interposed deep transverse metacarpal ligament (Fig. 7B, C), but it was fused with the metatarsal perichondrium immediately distal to the adductor hallucis muscle (Fig. 7I). Distally, however, the finger tendon sheath was also incorporated into the perichondrium. Thus, the dorsal aspects of the flexor tendons finally faced, or were attached to, the phalangeal bone. In both the hand and foot, splitting of the superficial flexor tendons occurred, providing a chiasm at the level of the proximal phalangeal bones (Fig. 7C, J). The dorsal aponeurosis of the finger or toes connected between the interosseous muscle and extensor tendon. Overall, the tendon morphology of the toe was a mini-version of that in the finger, with a slight difference.

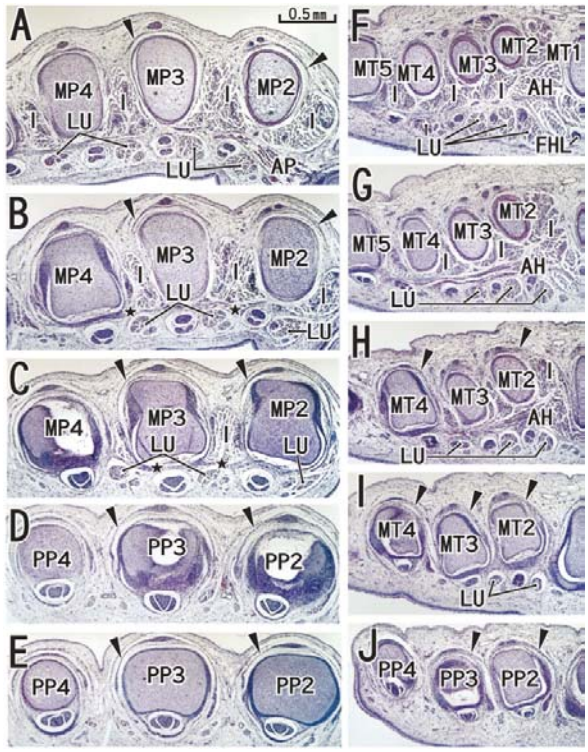

Figure 7. Distal portion of the flexor tendons in the hand and foot: a 12-week foetus. HE staining. Cross sections. The upper side of each panel corresponds to the dorsal side of the specimen. The left-hand (or the right hand-side) column shows the fingers (or the toes). Panels **A**, **F** (or **E**, **J**) are the most proximal (or distal) side of the figure. A basic configuration of the flexor pulley system appeared the same between the hand and foot. At a level including the transverse head of the adductor pollicis or hallucis (AP, AH), both in the hand and foot (**A**, **G**, **H**), the superficial tendon is not yet divided into two parts for the chiasma. The dorsal aponeurosis of fingers (arrowheads) connects between the extensor tendon and interosseous muscle (**I**). Stars in the left-side column indicate the deep transverse metacarpal ligament.
All panels are prepared at the same magnification (scale bar — **A**); FHL — flexor hallucis longus; PP — proximal pharynx; other abbreviations — see Figure 1.

DISCUSSION

The present histological study demonstrated a specific morphology of the foetal tendons in and around the carpal tunnel and plantar region: attachment sites for the lumbricalis muscles to the deep flexor tendons were characterised by (1) pale staining with both silver impregnation and HE and (2) primitive tenocytes dispersing along the interface between the tendon and the lumbricalis muscle. This staining feature appeared to suggest that maturation and accumulation of type 1 collagen fibrils is more "delayed" than in other parts of the tendon. According to Chaplin and Greenlee [6], the primitive tenocytes produce the collagen, and the fibrils accumulate between these cells. A tendon of the quadrates plantae, that joins the tendon of the flexor digitorum longus immediately proximal to the lumbricalis origin, showed dark staining, in contrast to the flexor tendons at the origin of the lumbricalis muscle. The colours produced by the present silver impregnation method correspond to the types of collagen fibrils (see Material and Methods). The colour of the deep

flexor tendons at the origin of the lumbricalis muscle differed from that of the superficial flexor tendons. Instead of collagen fibril deposits, abundant tenocytes appeared to remain undifferentiated at the lumbricalis muscle origins, and appeared to disperse from the original site (i.e. the tendon itself) to form an interface between the tendon and the lumbricalis muscles. CD34-positive mesenchymal tissues invaded into the tendon at the lumbricalis muscle origins, also suggesting an increase of multipotential stem cells at the site. Notably, dispersing cells from the FDP tendons were evident at 10 and 12 weeks rather than 8––9 weeks. Thus, chronologically, a separation of the FPL tendon from the common tendon (possibly at 9–10 weeks) was followed by a division of the FDP tendons (Fig. 8; see also below).

The FDP muscle fibres reached the distal level at which the pisiform bone was seen. Thus, the distal end of the antebrachial flexor muscle fibres may move from the distal side to the proximal side, as seen in adults. Conversely, the tendon would appear to become longer. Lewis [15] described this change as the "recession" of the flexor muscle bellies onto the forearm. The deep tendons sometimes numbered more than 5 on the proximal side of the lumbricalis muscle origin. Here, however, the tendons changed into 2 or 3 undifferentiated tissue masses. Thus, the lumbricalis muscles divided the mass again and appeared to "bundle" each tendon of the FDP at the hand and wrist. Therefore, the lumbricalis muscles are likely to contribute to long tendon morphogenesis through "rearrangement" of the deep flexor tendon configuration. Conversely, if the FDP tendons number more than 5 at the level of the carpal tunnel and more distally, such variations may interfere with the smooth and independent function of each finger. In the foot, one factor that decides the site of division of the flexor digitorum longus tendon may also be the lumbricalis muscle rather than the quadrates plantae muscle. The morphology of the deep tendons and lumbricalis muscles in the foot was almost same as that in the hand. The cells dispersing from the tendon to the lumbricalis muscle interface were restricted to the distal side of the joining quadrates plantae muscle.

The morphology of the deep tendons and lumbricalis muscles in the foot was almost the same as in the hand. The dispersing cells from the tendon to the lumbricalis muscle interface were restricted in the distal side of the joining of the quadrates plantae muscle. Thus, the hypothetical role of the foetal lumbricalis muscles for re-arrangement of the deep flexor tendons seemed to be the same in both the hand and foot. We do not rule out the possibility that cell death occurs at the lumbricalis muscle origin for re-arrangement of tendons. However, rather than cell death, the dispersed cells from the deep tendons suggested a differentiation of the interface tissue for the lumbricalis muscle origin. In contrast, a mechanism for separation of the FPL tendon from the hand common tendon seemed to be different from the tendon re-arrangement because the dispersed cells

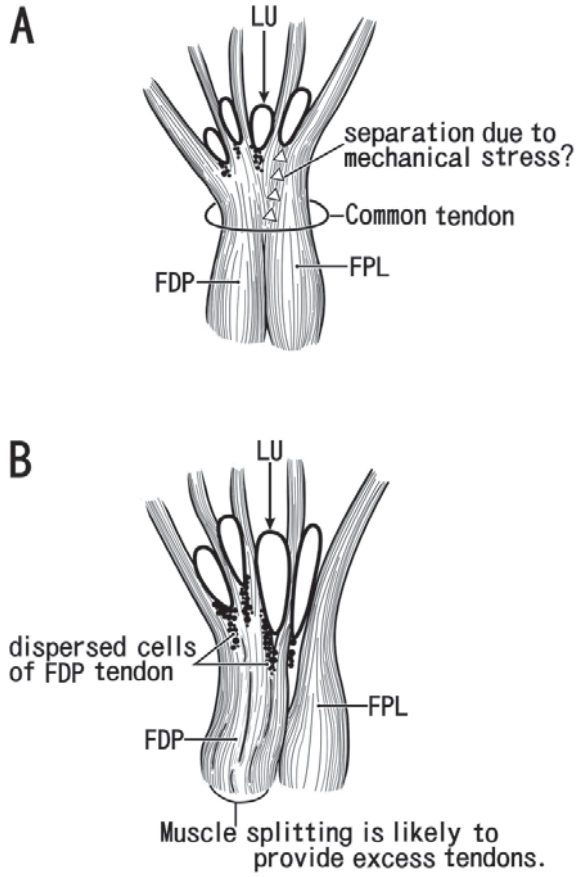

Figure 8. Schematic representation of a splitting process of the deep flexor common tendon in the foetal hand. **A.** The common tendon of the flexor digitorum profundus and flexor pollicis longus muscles (FDP, FPL) at 8–9 weeks. A separation of the FPL tendon (triangles) occurs at 9–10 weeks; **B.** Displays a dividing into 4 tendons in association with dispersed cells along the tendons at the origins of the lumbricalis muscles (LU). In the proximal side of the LU muscle origin, the splitting of the flexor digitorum profundus muscle is likely to provide more than 4 bellies and tendons. We hypothesize a re-arrangement process into the 4-tendon-pattern by the dispersed cells at the LU muscle origin.

were not seen along the FPL tendon. We speculate that mechanical stress due to lateral growth of the first finger is committed to the separation (Fig. 8A). Enoki et al. [9] hypothesised a role of the hand lumbricalis muscles as a mechanoreceptor rather than a helper of the finger extension and flexion, because of abundant muscle spindles in the muscles. We also agree with the hypothesis, but the morphology may establish depending on functional demands during postnatal growth. As we discussed above, the more essential role of the lumbricalis muscles seemed to be found in clear separation of the four deep tendons for fingers in foetal development.

Finally, we discuss the differences between the hand and the foot. The most essential difference seems to be found in a fact that, in the foetal foot, we had no evidence of a common tendon between the flexor hallucis longus and the flexor digitorum longus muscles. The long courses of these tendons from the ankle to the toes are likely to provide a clear separation. The tendon sheath in the fingers and toes, including the annular pulley, showed a similar morphology, although previous descriptions appear to be limited to the finger [4, 24]. Thus, the morphology of the tendon sheath in the toes was a mini-version of that in the finger. The deep flexor tendon of the second or index finger was distant from the others, but the specific morphology at the origin of the lumbricalis muscle was common. However, in contrast to other tendons in the foot, the deep flexor tendon for the second toe was darkly stained, as was the case for tendons of the longus hallucis muscle. This fact, suggesting delayed or insufficient development of the muscle-tendon interface, seems to be related to a higher incidence (around 10%) of absence of the second lumbricalis muscle in the foot [18, 21]. In contrast to the hand and wrist, the foot had no area in which muscle fibres were absent along the deep flexor tendons: muscle fibres of either the quadrates plantae muscle or the lumbricalis muscles were associated at any proximodistal level. Moreover, the lumbricalis muscles were thicker than those in the hand, and the fibres of the flexor digitorum brevis muscle extended distally to the level at which the adductor hallucis muscle was evident. This morphology (i.e. co-existence between tendons and muscle fibres) seemed to interfere with the development of a space and sheath along the tendon. In addition, because of the simple configuration of the merging with the flexor tendon, variations of the quadrates plantae muscle slips [11] seemed to arise in the later foetal stages or after birth.

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