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ORIGINAL ARTICLE

Lack of striated muscle fibers in the longitudinal anal muscle of elderly Japanese: a histological study using cadaveric specimens

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Abstract
Purpose and methods The aim of this study is to investigate variations in the longitudinal anal muscle (LAM), especially in the meeting pattern between the levator ani and rectum at the origin of the LAM. We examined the histology of the anal canal and the lower rectum of 50 cadavers (25 males, 25 females) of elderly Japanese individuals with the aid of immunohistochemistry.
Results We observed two patterns in the meeting site between the levator ani and the rectum. In type 1, observed in 26 specimens, the smooth muscle-rich fascia lining the internal or medial aspect of the levator ani (i.e., the fascia pelvis parietalis or endopelvic fascia) was connected to the external muscle layer. In type 2, observed in 24 specimens, multiple intramuscular septa of the levator ani were attached to a smooth muscle mass, with the latter joining the external smooth muscle layer of the rectum. However, 21 specimens (6 type 1 and 15 type 2) carried few smooth muscles at the meeting site. We did not find any striated muscle in the LAM, although this might have been the result of age-associated degeneration. Thus, active traction of the pelvic viscera by the LAM seemed unlikely in elderly Japanese.
Conclusions Rather than playing an active role, as suggested by the integral pelvic floor theory, the LAM seemed to be an elastic skeleton that maintains the shape of the anal canal.

Keywords Longitudinal anal muscle · Conjoint longitudinal muscle coat · Smooth muscle · Striated muscle · Levator ani · Integral pelvic floor theory

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Introduction

Anatomical research on the longitudinal anal muscle (LAM) began when the LAM was first noticed on the anterior side of the rectum [1]. The medial anterior smooth muscle fibers of the rectum, called the musculus rectourethralis superior, pass downward to insert into the perineal body. Pelvic magnetic resonance imaging (MRI) revealed rectoperineal bands originating from the anterior surface of the rectum and inserted into the perineal body [2], a muscle also called the rectoperinealis muscle [3]. All of these anterior smooth muscles were regarded as analogous to intermedial fibers of the anterior bundle of the LAM, suggesting that attention should be paid not only to the anterior part but also the laterally located major parts of the LAM, with the latter receiving fibers from the levator ani muscle [4]. The levator ani muscle was found to send muscle fibers to the external longitudinal smooth muscle layer of the rectum (ELR) [5]. Two types of muscle fibers from the levator ani to the ELR or LAM were later described: outer striated muscle fibers and a smaller number of smooth muscle fibers [6]. The joining or meeting of muscle fibers between the levator ani and ELR has been described as the conjoint muscle coat of the rectum [7, 8] and as the conjoined longitudinal muscle layer [9, 10].

Despite this extensive history, to our knowledge, most studies of the LAM did not identify its origin and the meeting site between the levator ani and ELR, but rather concentrated on the LAM morphology in the intersphincteric space. Connective tissues between the levator ani and ELR include a bulky smooth muscle mass receiving all the striated muscle fibers of the levator ani and a smooth muscle-made fascia pelvis parietalis [11]. In contrast or in addition to the smooth muscle-mediated connection, striated muscle fibers from the levator were reported to join the LAM [6]. Special attention is necessary to identify striated muscles using general staining methods, such as hematoxylin and eosin (HE) or Azan-Mallory staining, especially at a lower magnification that cannot determine striation. The integrated pelvic floor theory has suggested that the LAM plays a key role in the static and dynamic support of the pelvic viscera [12, 13], suggesting that the LAM is a striated muscle. Thus, this study was designed to determine the origin and composition of fibers of the LAM using specimens from elderly Japanese individuals.

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 50 donated cadavers (25 males and 25 females), ranging in age at time of death from 75 to 99 years (mean age 89 years). All individuals died of ischemic heart failure or intracranial bleeding. None had undergone surgery, as determined by assessment of their medical records, as well as by macroscopic observation after opening of the abdominopelvic cavity. These cadavers had been donated to Tokyo Dental College for research and education on human anatomy, and their use for research had been approved by the college ethics committee. The donated cadavers had been fixed by arterial perfusion of 10 % *v*/*v* formalin solution and stored in 50 % *v*/*v* ethanol solution for more than 3 months. From each cadaver, we prepared one large tissue block that contained the levator ani muscle, the posterior walls of the vagina and rectum, and any connective tissue around these viscera. Two to five macroslices, each 15 mm thick, including the anterolateral part of the rectum (1–3 o'clock positions), as well as the inferomedial edge of the levator ani, were made from each tissue mass, followed by routine procedures for paraffin-embedded histology. The sectional planes included the oro-anal (proximodistal) axis of the inferior rectum and anal canal.

In the multiple paraffin blocks obtained from each cadaver, to compare an interface morphology between the levator ani and rectum, we chose a paraffin block that included the greatest number of fibrous structures between them: such a site was seen at and 2-3 o'clock positions. From the selected paraffin blocks, we made serial or semiserial 5-6 sections. Most sections were stained with HE, with others used for immunohistochemistry or Elastica-Masson staining (a variation of Masson-Goldner staining [14]). Elastica-Masson staining has been shown to be very useful for the identification of smooth muscles (pink or pale violet), striated muscles (deep red), collagen fibers (green), and elastic fibers (black) [15, 16]. The primary antibodies used for muscle fiber immunohistochemistry included mouse monoclonal anti-human alpha smooth muscle actin (1:100; Dako M0851, Glostrup, Denmark), which is strongly positive for smooth muscles and vascular endothelium [17], and mouse monoclonal anti-rabbit sarcomeric actin (skeletal muscle actin; 1:50; Dako M0874), which is strongly positive for striated muscles [18]. Following washing, the sections were incubated with horseradish peroxidase (HRP)-labeled secondary antibody, and antigen-antibody reactions were detected by the HRP-catalyzed reaction with diaminobenzidine. All samples were counterstained with hematoxylin. As a negative control, primary antibody was omitted. The sections were monitored and photographed with a Nikon Eclipse 80, but photos at ultralow magnification (less than ×1 at the objective lens) were taken with a high-grade flat scanner with translucent illumination (Epson scanner GTX970).

Results

Striated muscles in the LAM

Incubation of sections with antibody to sarcomeric actin showed the distribution of the striated muscle fibers, but this reaction was weaker than with antibody to smooth muscle actin (Figs. 1, 2, 3, and 4). Thus, both antibodies were used to examine the distribution of striated muscle fibers in the LAM. Elastica-Masson staining of the LAM demonstrated striated muscle fibers, including striations, as a bright red color (Figs. 1, 2, and 4), as well as abundant elastic fibers, which were colored dark violet (Figs. 1a and 3a). The LAM was identified as an inferior elongation of the ELR, with both collagen fibers and smooth muscle fibers added to the LAM at the levator-ELR meeting site (Figs. 2a–c and 4a–c). When the entire downward course from the meeting site to the subcutaneous tissue was assessed, the LAM was found to be thickest (2–5 mm) in the space between the external and internal anal sphincters. Inferiorly, the LAM was divided into four to seven branches to insert into the subcutaneous part of the external anal sphincter. As a result, the striated sphincter was divided into several compartments (Fig. 1a). Striated muscle fibers were not observed in the LAM in the

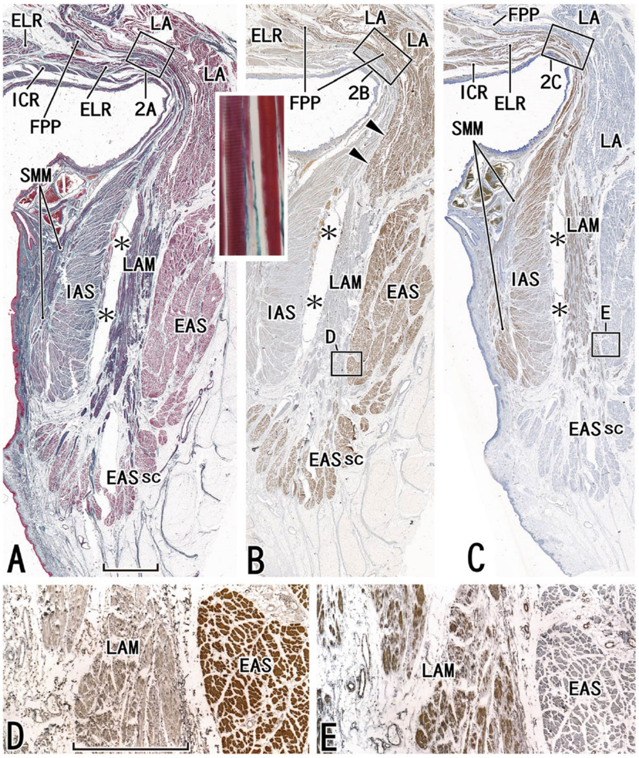

Fig. 1 Smooth muscle-rich fascia pelvis parietalis joining the external longitudinal muscle layer of the rectum. A specimen obtained from an 82-year-old woman was stained with **a** Elastica-Masson stain, **b** antibody specific for striated muscle fibers, and **c** antibody specific to smooth muscle fibers. All samples were assessed at close to the same magnification (*scale bar* in **a**, 5 mm). Assessments included (1) the meeting area (*square with 2A*, *2B*, or *2C*) between the fascia pelvis parietalis (FPP) and the external longitudinal muscle layer of the rectum (ELR) and (2) the LAM running inferiorly between the external and internal sphincters (EAS, IAS) to reach the subcutaneous part of the external anal sphincter (EASsc). At lower magnification (**a**), the LAM was *colored dark violet* due to the abundant numbers of elastic fibers. A bundle of the levator ani (*arrowheads* in **a**) appeared to originate from the fascia pelvis parietalis. The LAM in the intersphincteric space is shown in **d** (immunohistochemistry for striated muscle fibers) and **e** (immunohistochemistry for smooth muscle fibers). **d**, **e** Corresponding to the *squares labeled D and E* in **b** and **c**, and were prepared at the same magnification (*scale bar* in **d**, 1 mm). The *insert* between **a** and **b** shows the striation of the levator ani muscle fibers. The LAM did not contain striated muscles (**d**). *Asterisks* indicate artifactual spaces resulting from the histological procedure. *ICR* internal circular muscle layer of the rectum, *LA* levator ani, *SMM* submucosal muscle and its associated connective tissue plate

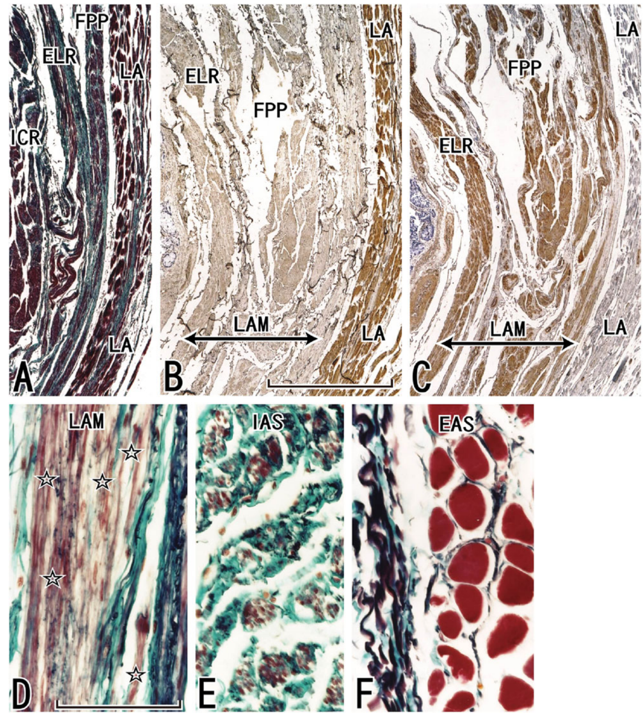

Fig. 2 The meeting area between the fascia pelvis parietalis and the external longitudinal muscle layer of the rectum. The specimen was the same as that in Fig. 1 and was stained with **a**, **d, e, f** Elastica-Masson stain, **b** antibody against striated muscle fibers, and **c** antibody against smooth muscle fibers. All samples were prepared at the same magnification (*scale bar* in **b**, 1 mm) and showed smooth muscle-rich fascia (FPP) joining the rectal muscle (ELR) to provide the longitudinal anal muscle (LAM). **d**, **e**, **f** Higher magnification (*scale bar* in **d**, 0.1 mm) showed the LAM, the internal anal sphincter (IAS), and the external anal sphincter (EAS), respectively, with elastic fibers *colored black*. Smooth muscle fibers in the LAM carry no striation (**d**). *ICR* internal circular muscle layer of the rectum, *LA* levator ani

intersphincteric space (Figs. 1d, e and 3d, e). Likewise, the levator ani sent no striated muscle fibers to the LAM. However, assessment of a specimen with a well-developed sphincter (Fig. 1a) showed a bundle of the levator ani that appeared to originate from the fascia pelvis parietalis and ended in the immediately superior side of the intersphincteric space. The LAM was composed of collagen fibers, elastic fibers, and smooth muscles, with almost all the fibrous components running longitudinally along the supero-inferior axis of the anal canal (Figs. 2d and 4d). This supero-inferior direction clearly differed from that of striated muscle fibers of the levator ani. In several samples, an elastic fiber-rich fascia bundled the LAM (data not shown).

Interindividual morphologic variations at the levator-rectum interface

The ELR decreased in thickness at a site 5–15 mm proximal or oral to its meeting site with the levator ani (Figs. 1a, 3a, and 5). Immunohistochemistry clearly showed the distribution of smooth muscles at the meeting site (Figs. 2c, 4b, and 5). The morphology of the meeting site could be classified into two types (Table 1). In type 1, observed in 26 of the 50 specimens, the smooth muscle-rich fascia pelvis parietalis lining the internal or medial aspect of the levator ani (i.e., the endopelvic fascia [19]) was connected with the ELR (Figs. 1c and 5f). In type 2, observed in the other 24 specimens, multiple intramuscular septa of the levator ani were attached to a smooth muscle mass, with the latter joining the ELR (Fig. 5d). In type 1, the fascia pelvis parietalis was thick and contained abundant smooth muscles at the inferior part 10–30 mm from the

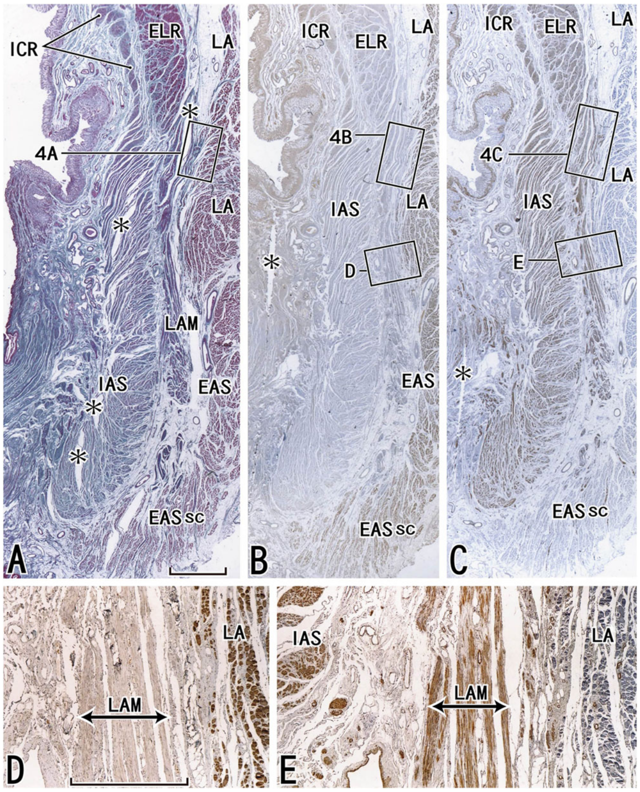

Fig. 3 Multiple intramuscular septa of the levator ani merge with the external longitudinal muscle layer of the rectum. A specimen obtained from an 85-year-old woman was assessed after **a** Elastica-Masson staining, **b** antibody against striated muscle fibers, and **c** antibody against smooth muscle fibers. Sections were prepared at the same magnification (*scale bar* in **a**, 5 mm) and included (1) the meeting area (*squares with 4A*, *4B*, or *4C*) between the intramuscular septa of the levator ani (LA) and the external longitudinal muscle layer of the rectum (ELR) and (2) the longitudinal anal muscle (LAM) running inferiorly between the external and internal sphincters (EAS, IAS) to reach the subcutaneous part of the external anal sphincter (EASsc). At lower magnification (**a**), the LAM was *colored dark violet* due to the presence of an abundant number of elastic fibers. Presence of the LAM in the intersphincteric space, as shown by incubation with antibodies against **d** striated and **e** smooth muscle fibers. **d**, **e** Corresponding to the *squares labeled D and E* in **b** and **c**, and were prepared at the same magnification (*scale bar* in **d**, 1 mm). The LAM contained no striated muscles (**d**). *Asterisks* indicate artifactual spaces caused during the histological procedures. *ICR* internal circular muscle layer of the rectum

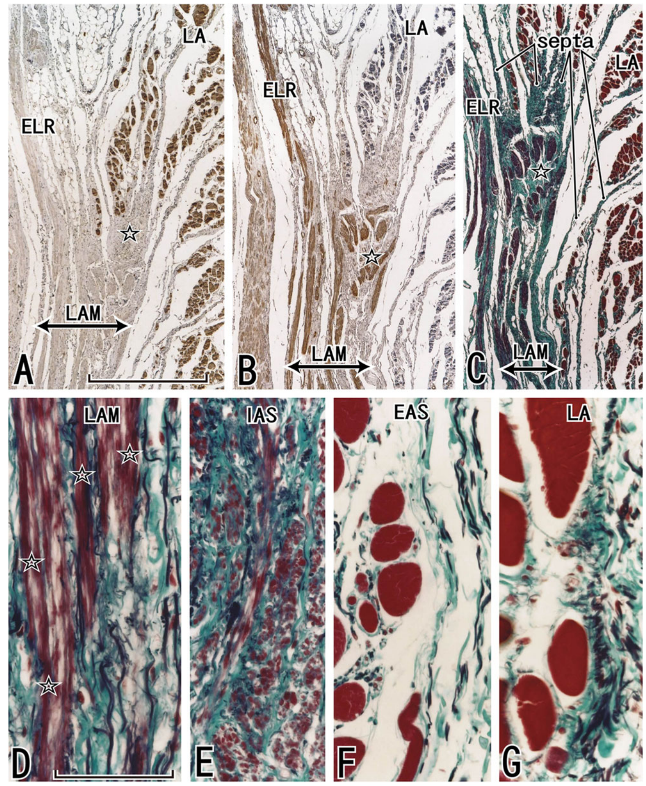

Fig. 4 The meeting area between the multiple intramuscular septa of the levator ani and the external longitudinal muscle layer of the rectum. The specimen was the same as that shown in Fig. 3. This specimen was incubated with antibodies against **a** striated and **b** smooth muscle fibers, and with **c** Elastica-Masson stain, were prepared at the same magnification (*scale bar* in **a**, 1 mm), and exhibited a smooth muscle-rich connective tissue mass (*star*) receiving the multiple intramuscular septa (septa in **c**) of the levator ani (LA). These smooth muscles join the external longitudinal muscle layer of the rectum (ELR) to provide the longitudinal anal muscle (LAM). **d**, **e**, **f**, **g** Higher magnification (*scale bar* in **d**, 0.1 mm) of the LAM, the internal anal sphincter (IAS), the external anal sphincter (EAS), and the levator ani, after Elastica-Masson staining. **d**–**f** Elastic fibers were *colored black*, with smooth muscle fibers in the LAM carrying no striations (**d**)

meeting site. The fascia of females tended to contain greater numbers of smooth muscles fibers than did the fascia of males. In seven type 1 and four type 2 specimens, the numbers of smooth muscles increased at the interface, providing a bulky mass to receive almost all the muscle fibers of the levator ani (Fig. 5b). In these specimens, it was difficult to determine whether the smooth muscles originated from the fascia or septa. Conversely, in six type 1 and 15 type 2 specimens, the number of smooth muscles at the interface was small, with all smooth muscle fibers per section being <2 mm in length. At lower magnification, the LAM appeared to be a simple continuation of the ELR (Figs. 3a and 5h). For example, no or few smooth muscles were present in the intramuscular septa of the levator ani in one specimen (Fig. 4b), whereas smooth muscles were evident in another specimen (Fig. 5d).

When smooth muscles were not evident at the interface between the levator and ELR, multiple thin collagen fiber bundles were found to connect the striated muscle fibers and ELR. However, we did not find any specific connective tissue such as the hiatal ligament [20] although this term has recently been used to describe anococcygeal connective tissue [21].

Other observations

The external anal sphincter was separated from the inferior end of the levator ani by a loose space containing vessels (Figs. 1a, 2a, and 5). In contrast, the internal and external anal sphincters were attached to each other. Almost all muscle fibers in these sections were cut transversely in the anal

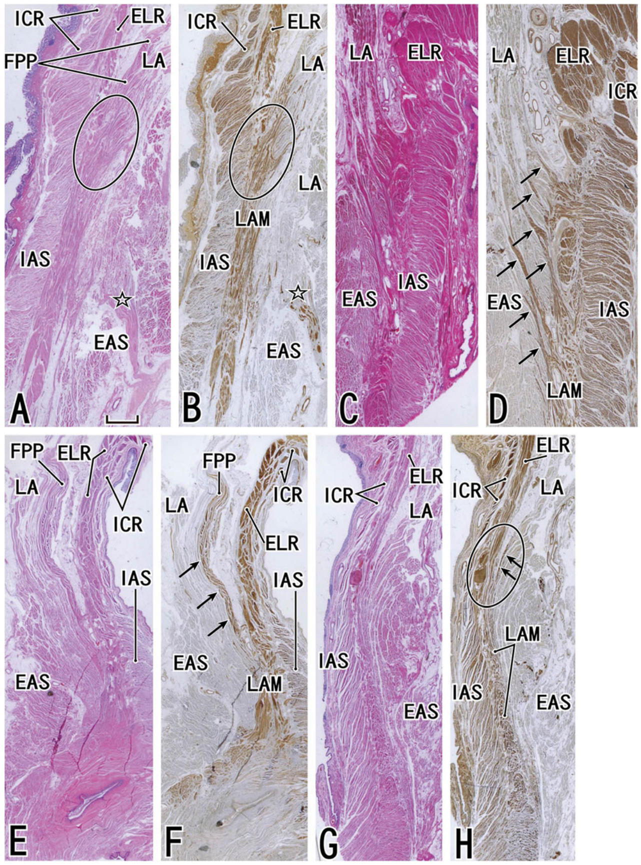

Fig. 5 Variations in morphologies at the levator-rectum interface. All specimens in this figure differed from those in Figs. 1, 2, 3, and 4. All panels were prepared at the same magnification (*scale bar* in **a**, 5 mm). **a**, **c**, **e**, **g** HE staining; **b**, **d**, **f**, **h** antibody against smooth muscle fibers. **a**, **b** Taken from an 82-year-old man, almost all striated muscle fibers of the levator ani (LA) were attached to smooth muscle mass (*encircled*), with the latter joining the external longitudinal muscle layer of the rectum (ELR). In addition, a thick fascia, rich in smooth muscles, was found to separate the levator ani from the external anal sphincter (EAS). **c**, **d** Taken from an 80-year-old man, the multiple intermuscular septa of the levator ani (*arrows*), containing abundant smooth muscles, joined the external longitudinal muscle layer of the rectum. **e**, **f** Taken from a 79-year-old woman, the smooth muscle-rich fascia pelvis parietalis (FPP) lining the levator ani (*arrows*) joined the external longitudinal muscle layer of the rectum. **g**, **h** Taken from a 75-year-old man, few smooth muscles (arrows) is seen at the interface between the levator ani and rectum. Thus, the longitudinal anal muscle (LAM) appeared to be a direct continuation of the longitudinal muscle layer of the rectum

sphincters. Elastic fibers bundled striated and smooth muscle fibers in the external and internal anal sphincters, respectively (Figs. 2e, f and 4e, f). Likewise, the perimysium of the levator ani contained elastic fibers (Fig. 4g), which were apparently connected with the fascia pelvis parietalis. The submucosal muscle of the anus, which has been shown to be a smooth muscle layer [22], was sometimes as thick as the internal anal sphincter (Fig. 1c).

Discussion

Assessment of 50 cadaveric specimens indicated that striated muscle fibers of the levator ani were unlikely to join the LAM in elderly Japanese men and women. None of the samples showed evidence of any striated muscle in the LAM. Although the levator ani was reported to send striated muscle fibers to the LAM, no photographic or histological evidence was included [23]. Striated muscles in the LAM were reported to be gradually replaced by elastic fibers with age [24, 25], suggesting that the absence of striated muscle in the LAM of elderly Japanese individuals may have been the result of age-related degeneration. These striated muscle fibers have been described as coming from the puboanalis, puborectalis, and perirectal parts of the levator ani muscle, and joining the ELR [8]. However, we observed a striated muscle bundle, part of the levator ani, originating from the fascia pelvis parietalis and extending inferiorly along the LAM. This muscle bundle may be identical to previously described outer striated muscle fibers of the LAM [6], but the variant muscle ended on the superior side of the intersphincteric space. This variant striated muscle bundle may depend on ethnicity, being absent from Japanese individuals but present in other human populations.

The integrated pelvic floor theory [12, 13] predicts that the LAM and levator plate (the posterior part of the levator ani inserting into the coccyx) should provide a postero-inferior traction force to the bladder neck and vagina. This force is required to close the proximal urethra against intra-abdominal pressure, as well as to support the vagina in a suitable position.

Table 1 Patterns in the meeting site between the levator ani and the rectum

Types	Origin of smooth muscle fibers	Configuration of smooth muscle fibers to the rectum			
		Bulky[a]	Long and fibrous	Few short fibers[b]	Total
Type 1	Fascia pelvis parietalis	7 (2 M, 2 F)	13 (6 M, 10 F)	6 (2 M, 4 F)	26 (10 M, 16 F)
Type 2	Multiple intramuscular septa	4 (4 M)	5 (2 M, 3 F)	15 (9 M, 6 F)	24 (15 M, 9 F)

M male cadavers, *F* female cadavers

[a] Smooth muscle fibers formed a bulky mass that received almost all the striated muscle fibers of the levator ani

[b] Few smooth muscle fibers, less than 2 mm in length, were seen in 15 cadavers (nine males and six females)

We found that the LAM consisted of multiple longitudinal bundles of both smooth muscles and elastic fibers, a structure that was likely highly resistant to tension along the supero-inferior axis and able to recover its original length. Since the LAM apparently does not contain a myenteric nerve plexus [15], it is unlikely to participate in organized contractions or peristalsis under the control of nerves. Being different from striated muscles, LAM composed of smooth muscle is unlikely to provide strong and long-termed traction power. In addition, the integrated theory expands an original concept of the levator plate [26], with the latter expected to be a strong posterior traction force. However, the attachment of the levator ani to the coccyx seems to be highly elastic in elderly Japanese individuals [21, 27]. In place of the LAM and levator plate, the external anal sphincter in elderly Japanese women seemed to provide postero-inferior traction for the vagina because this sphincter is thick and extends anterolaterally [28].

It is not clear whether smooth muscle fibers of the LAM synchronize with the levator ani under the control of nerve impulses. The LAM and the puborectalis, a perirectal part of the levator ani, was reported to correspond to the detrusor of the anal canal, in contrast to the anal sphincters [26]. Although our findings were similar, the LAM acted as an elastic skeleton to maintain the original shape and length of the anal canal, rather than acting as a detrusor. Smooth muscles and elastic fibers usually coexist in arterial walls because elastic fibers are necessary to maintain the three-dimensional configuration of smooth muscle fibers [29]. Likewise, in the LAM, elastic fibers seemed to maintain a smooth muscle configuration. According to Bayliss' rule, smooth muscle cells in the arterial wall can counteract against the effects blood pressure without nerve or hormonal control [30, 31]. Applying this rule to smooth muscle-containing connective tissue on the pelvic floor showed that, following the release of mechanical stress (e.g., removal of a fecal mass), the smooth muscles in the LAM seemed to strongly recover the anal canal length [32].

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Conflict of interest The authors declare that they have no conflict of interest.

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