ORIGINAL COMMUNICATION

Coexistence of Dense and Sparse Areas in the Longitudinal Smooth Muscle of the Anal Canal:
Anatomical and Histological Analyses Inspired by Magnetic Resonance Images

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Magnetic resonance images of the anal canal show small, circular, low-intensity areas arranged in a row and a high-intensity area surrounding them internally and externally in the longitudinal muscle layer that cannot be explained by current anatomical findings. The purpose of this study was to elucidate the detailed structure of the longitudinal smooth muscle of the anal canal and to interpret the magnetic resonance image of the longitudinal muscle. Specimens for macroscopic anatomy and histology were obtained from six and seven cadavers, respectively. The histological nature of the longitudinal muscle was examined by staining serial transverse and coronal sections of the lateral wall of the anal canal with Masson's trichrome stain and using immunohistochemistry for smooth and skeletal muscle fibers. Dense and sparse areas of smooth muscle fibers coexisted in the longitudinal muscle layer. The dense areas formed columnar muscle bundles approximately 1.0–1.5 mm in diameter, and they continued from the longitudinal muscle bundles of the rectum. The columnar muscle bundles of the longitudinal anal muscle were internally and externally surrounded by sparsely arranged smooth muscle fibers that ran longitudinally. The coexistence of dense and sparse areas of smooth muscle fibers suggests that the structure of the smooth muscle is optimized for its function. This histological nature is probably reflected in the magnetic resonance image of the longitudinal muscle as the coexistence of low- and high-intensity areas. Clin. Anat. 33:619–626, 2020. © 2019 Wiley Periodicals, Inc.

Key words: longitudinal muscle; smooth muscle; magnetic resonance imaging; anal canal; rectum

INTRODUCTION

Magnetic resonance imaging (MRI) is essential for the preoperative evaluation and follow-up of patients with rectal cancer and anal fistula (Stoker, 2009; Bamba et al., 2012; Surabhi et al., 2016). MRI based on the axis along the anal canal enables detailed observation of the muscle layer structure of the anal canal (Fig. 1). The internal and external anal sphincters are depicted as concentric low-intensity areas. The region between the internal and external anal
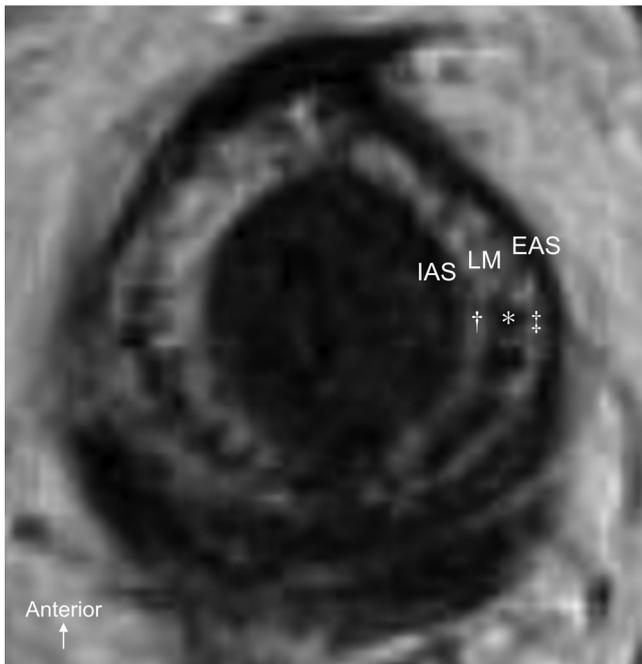
Fig. 1. Magnetic resonance image (T2-weighted image) of the anal canal. This transverse (axial) section perpendicular to the anal canal axis was imaged in the jack-knife position, with 1.5-T magnetic resonance imaging system. In the longitudinal muscle layer, the small circular low-intensity area (asterisk) and the high-intensity areas (obelisk and double obelisk) coexisted. EAS, external anal sphincter; IAS, internal anal sphincter; LM, longitudinal muscle; asterisk (*), low-intensity area; obelisk (†) and double obelisk (‡), high-intensity area.

sphincters is important for diagnosis and treatment of anorectal diseases because this region is the borderline of tumor invasion between T2 and T3 rectal cancer (Bamba et al., 2012), and it also becomes the primary focus of abscess in anal fistula (Parks, 1961). MRI shows small, circular, low-intensity areas with a diameter of approximately 1.0–1.5 mm arranged in a row (indicated by an asterisk in Fig. 1) and high-intensity area surrounding them internally and externally (indicated by an obelisk and a double obelisk in Fig. 1) between the internal and external anal sphincters. The anatomical structure reflected in the coexistence of low- and high-intensity areas in MRI is unknown.

The region between the internal and external anal sphincters contains longitudinal smooth muscle (Lunniss and Phillips, 1992). This muscle directly attaches to the levator ani at the anorectal junction and inferiorly penetrates the external anal sphincter. The importance of this muscle in anal function has been reported (Shafik, 1976; Macchi et al., 2008; Petros and Woodman, 2008; Wagenlehner et al., 2010; Muro et al., 2014; Tsukada et al., 2016). However, only a few reports are available on the histological features of the longitudinal muscle and its interpretation for MRI. Therefore, current anatomical

findings cannot explain the magnetic resonance image of the longitudinal muscle layer.

Recent studies have reported that smooth and skeletal muscles have a structurally close relationship in the anal canal region where the digestive tract and pelvic floor muscle are spatially close (Muro et al., 2014; Tsukada et al., 2016; Nakajima et al., 2017; Muro et al., 2018; Muro et al., 2019). Hence, we hypothesized that the smooth muscle possesses various structures depending on its function and positional relationship with the skeletal muscles and that smooth muscle fibers with different properties and state coexist in the longitudinal muscle layer based on the magnetic resonance image (Fig. 1). The present study aimed to elucidate the detailed structure of longitudinal smooth muscle of the anal canal and to interpret the magnetic resonance image of the longitudinal muscle.

MATERIALS AND METHODS

In this descriptive study, we obtained all cadavers from the Department of Anatomy of our institute for use in clinical studies. They were donated within the guidelines of the Act on Body Donation for Medical and Dental Education law of Japan. The cadavers were fixed by arterial perfusion with 8% formalin and preserved in 30% alcohol to prevent fungal growth and maintain tissue softness. Study approval was obtained from the Board of Ethics of our institute (approval number: M2018-006).

Six cadavers (two males and four females; age at death: range, 49–88 years; mean, 71.3 years) were used for dissection. Each pelvis, including the rectum, anal canal, adjacent muscular tissues, and connective tissues, was obtained en bloc from the cadavers. The pelvises were sectioned in the median plane and dissected from the medial aspect. The anorectal canal was dissected from the luminal side to demonstrate the internal anal sphincter, longitudinal muscle, levator ani, and the external anal sphincter. Tissues constituting the longitudinal muscle were collected during the dissection process and examined histologically.

Histological analysis was performed using 11 hemi-pelvises from seven cadavers (three males and four females; age at death: range, 80–91 years; mean, 84.4 years). In the five hemi-pelvises, the lateral wall tissue of the anorectal canal was obtained to create a histological specimen of the serial transverse (axial) plane to the long axis of the anal canal. The tissue was embedded in paraffin and serially sectioned into 5-µm-thick specimens at 1-mm intervals. In the other six hemi-pelvises, the lateral wall tissue of the anorectal canal was obtained to create a histological specimen of the coronal plane to the long axis of the anal canal. The obtained tissue was embedded in paraffin and sectioned into 5-µm-thick specimens. The histological sections were stained with Masson's trichrome stain. In addition, immunohistological staining was performed with antismooth actin (ready-to-use Actin, Smooth Muscle Ab-1, Clone 1A4; Thermo Fisher Scientific, Fremont, CA) and antiskeletal myosin (ready-to-use Myosin, Skeletal Muscle Ab-2, Clone MYSN02; Thermo Fisher Scientific) to confirm the distribution of the smooth muscle and skeletal muscle fibers. The detailed procedures have been described in previous

reports (Muro et al., 2014; Nakajima et al., 2017; Muro et al., 2018; Muro et al., 2019).

RESULTS

The circular muscle of the rectum and the internal anal sphincter were observed after the removal of the mucosa and submucosal tissue from the luminal side (Fig. 2a). Subsequently, they were also removed to show the longitudinal muscle (Fig. 2b). At the lower rectum level, columnar longitudinal muscle bundles were observed beneath the circular muscle, whereas longitudinal fibers were at the anal canal level. A part of the longitudinal fibers was removed to examine their tissue composition. Because these fibers were histologically confirmed to consist of smooth muscle fibers, they were identified as the internal part of the longitudinal anal muscle (the internal fibers of the longitudinal anal muscle; indicated by an obelisk in Fig. 2b). After the removal of the internal fibers of the longitudinal anal muscle, the columnar bundles of the longitudinal muscle were observed in the anal canal (the columnar bundles of the longitudinal anal muscle; indicated by an asterisk in Fig. 2c). These columnar bundles were formed by the direct continuation of the longitudinal muscle bundles of the rectum (arrows in Fig. 2c). Subsequently, the columnar longitudinal muscle bundles of the anal canal and rectum were continuously removed. The back side of the peritoneum was visible in the upper part of the rectum, whereas the longitudinal fibers were observed in the anal canal (Fig. 3a). A part of the longitudinal fibers was removed to examine their tissue composition. Because these fibers were histologically confirmed to consist of smooth muscle fibers, they were identified as the external part of the longitudinal anal muscle (the external fibers of the longitudinal anal muscle; indicated by a double obelisk in Fig. 3a, b). These external fibers were continuous with the smooth muscle tissue on the surface of the levator ani (indicated by a star in Fig. 3b) and directly attached to the skeletal muscle fibers of the levator ani. After removal of the external fibers of the longitudinal anal muscle, the external anal sphincter was observed (Fig. 3c).

The transverse (axial) section of the upper part of the anal canal showed the coexistence of high- and low-density areas of smooth muscle fibers in the longitudinal muscle layer of the anal canal (Fig. 4). Smooth muscle fibers gathered densely and formed a series of small circles as a cross-section of the columnar bundles (indicated by asterisks in Fig. 4). They were approximately 1.0–1.5 mm in diameter and arranged in a row. Because the small circles were surrounded by other sparsely scattered smooth muscle fibers, the internal and external fibers of the longitudinal anal muscle were confirmed to consist of sparsely arranged smooth muscle
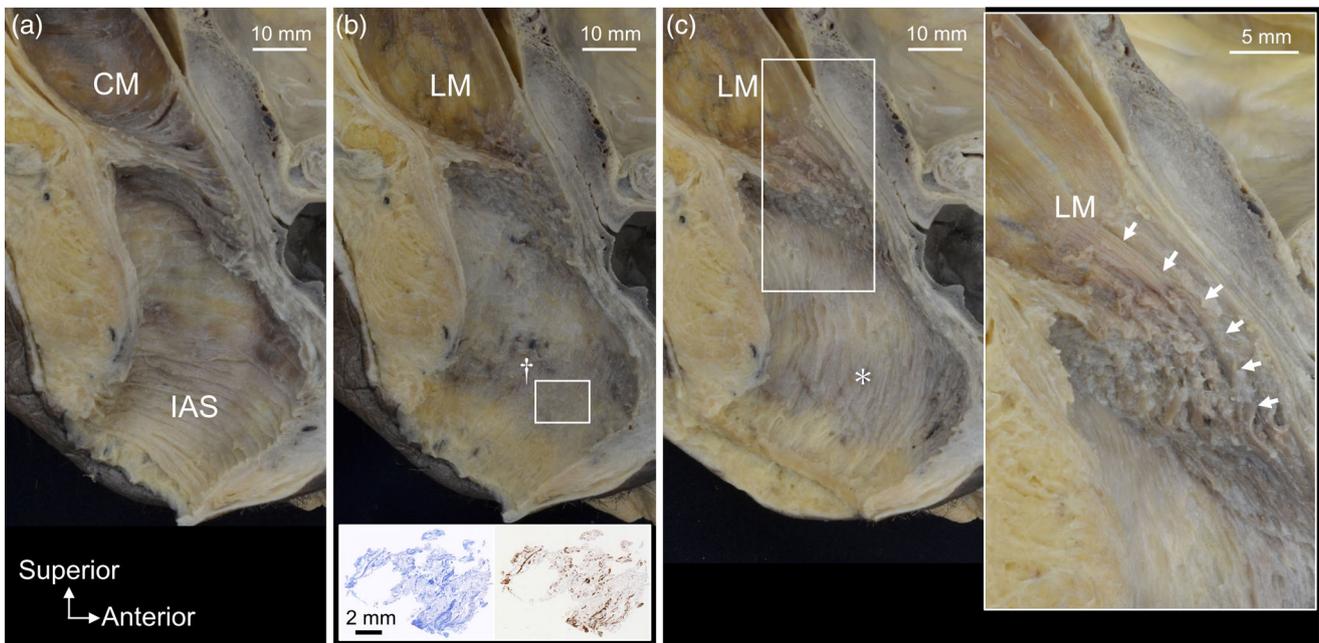

Fig. 2. Medial aspect of the anal canal and rectum. (**a**) Internal anal sphincter. (**b**) Longitudinal fibers (obelisk) after IAS removal. (**c**) Columnar longitudinal muscle bundles (asterisk) continue from the longitudinal muscle bundles of the rectum (arrows) after removal of the internal fibers (obelisk). CM, circular muscle; IAS, internal anal sphincter; LM, longitudinal muscle; obelisk (†), internal fibers of the longitudinal anal muscle; asterisk (*), columnar bundles of the longitudinal anal muscle; arrows, direct continuation between the LM of the rectum and the columnar bundles of the anal canal. [Color figure can be viewed at wileyonlinelibrary.com]
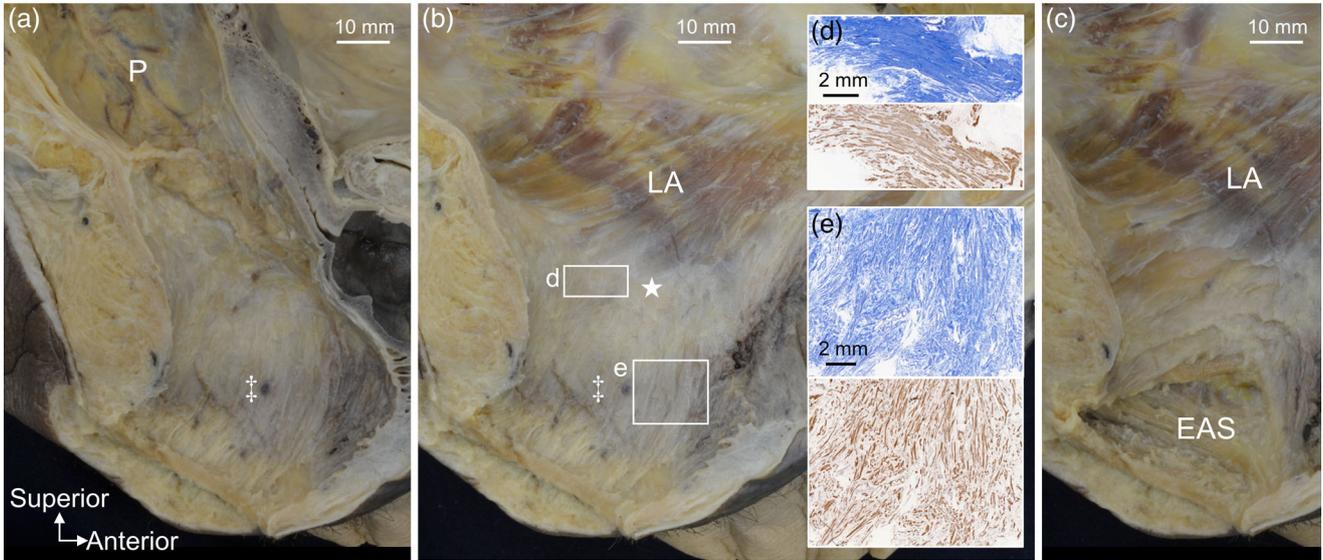
Fig. 3. Medial aspect of the anal canal and the rectum of the same specimen as Figure 2. (**a**) Longitudinal fibers (double obelisk) after removal of the columnar bundles (asterisk in Fig. 2c). (**b**) The tissue on the levator ani (star) and longitudinal fibers (double obelisk) after removal of the pelvic viscera. (**c**) The external anal sphincter after removal of the external longitudinal fibers (double obelisk). EAS, external anal sphincter; LA, levator ani; P, peritoneum; double obelisk (‡), external fibers of the longitudinal anal muscle; star, tissue on the levator ani. [Color figure can be viewed at wileyonlinelibrary.com]
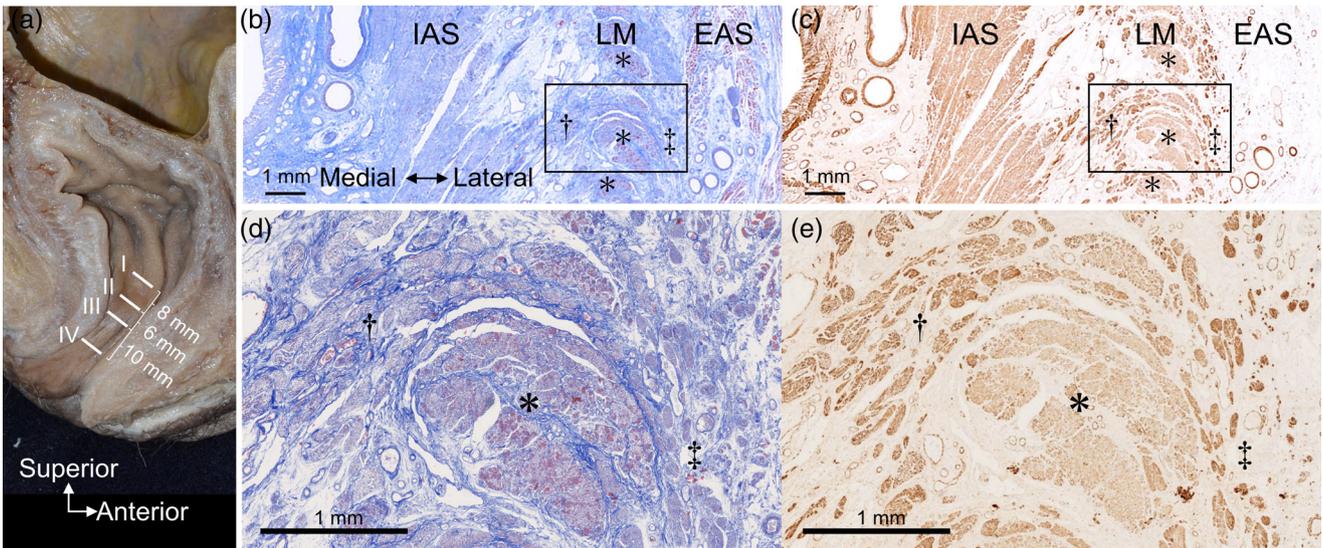

Fig. 4. Transverse (axial) section of the upper part of the anal canal. (**a**) Medial aspect of the anal canal. Sections I, II, III, and IV are equivalent to Figures 4b, 5a,c,e, respectively. (**b**) Masson's trichrome stain of section I. (**c**) Immunostaining with antismooth muscle antibody. (c and **d**) Enlarged view of the rectangular inset in a and b. Densely gathered smooth muscle fibers (asterisk) and sparsely arranged smooth muscle fibers (obelisk and double obelisk) coexisted. EAS, external anal sphincter; IAS, internal anal sphincter; LM, longitudinal muscle; asterisk (*), dense area; obelisk (†) and double obelisk (‡), sparse area. [Color figure can be viewed at wileyonlinelibrary.com]
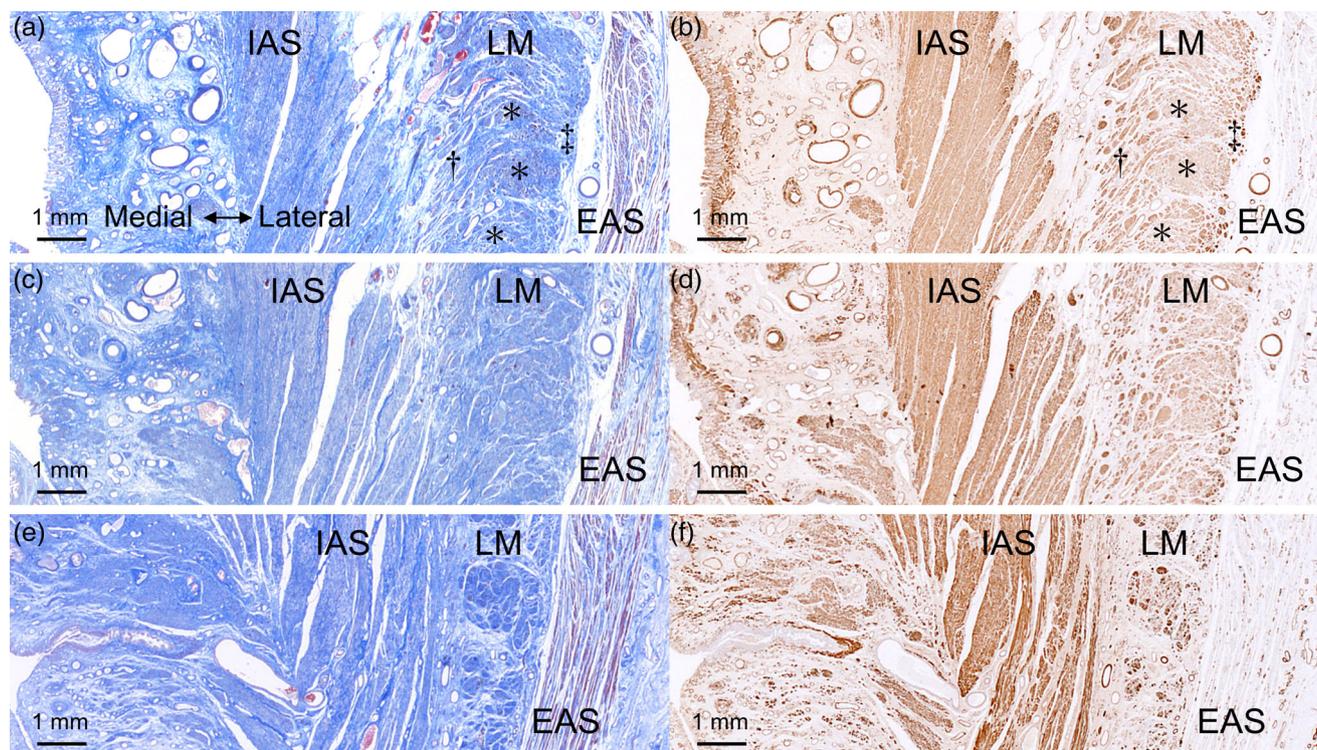
Fig. 5. Transverse (axial) section of the anal canal. Masson's trichrome stain (**a**, **c**, **e**) and immunostaining with antismooth muscle antibody (**b**, **d**, **f**). (a and b) Section II in Figure 4a, 8 mm inferior to Figure 4b,c. The distinction between the dense and sparse areas was relatively obscure. (c and d) Section III in Figure 4a 6 mm inferior to Figure 5A,B. The smooth muscle fibers formed a substantially uniform longitudinal muscle layer. (e and f) Section IV in Figure 4a, 10 mm inferior to Figure 5c,d. The longitudinal muscle was finely dispersed. EAS, external anal sphincter; IAS, internal anal sphincter; LM, longitudinal muscle; asterisk (*), dense area; obelisk (†) and double obelisk (‡), sparse area. [Color figure can be viewed at wileyonlinelibrary.com]

fibers (indicated by an obelisk and a double obelisk in Fig. 4) in the loose connective tissue. In the inferior sections, the distinction between the dense and sparse areas was gradually obscured (Fig. 5a, b), and smooth muscle fibers formed a substantially uniform longitudinal muscle layer (Fig. 5c, d). The longitudinal muscle was finely dispersed in the lower part of the anal canal (Fig. 5e, f).

The coronal section of the lateral wall of the anal canal also showed the coexistence of high- and low-density areas of smooth muscle fibers in the longitudinal muscle layer (Fig. 6). The aggregation of dense smooth muscle fibers in the longitudinal anal muscle (indicated by asterisks in Fig. 6a,b) was similar to that in the longitudinal muscle bundle of the rectum. The dense areas were enclosed internally and externally by other sparsely scattered smooth muscle fibers (indicated by an obelisk and a double obelisk in Fig. 6a,b). The external fibers of the longitudinal muscle directly attached to the skeletal muscle fibers of the levator ani that descended internal to the external anal sphincter (Fig. 6). The difference in the density of smooth muscle fibers was clearly distinguished in

the upper part of the anal canal (arrow I in Fig. 6b); however, the distinction gradually became obscure (arrow II in Fig. 6b), and the smooth muscle tissue was substantially homogenized (arrow III in Fig. 6b) in the inferior region. The longitudinal smooth muscle was branched into narrow bundles (septa) in the lower part of the anal canal (arrow IV in Fig. 6B).

DISCUSSION

The present study clarified that dense and sparse areas of smooth muscle fibers coexisted in the longitudinal muscle layer of the anal canal (Fig. 7). The difference in the density of smooth muscle fibers was dominant in the upper part of the anal canal, but it gradually became obscure in the lower part. The dense areas (indicated by an asterisk in Fig. 7) formed columnar muscle bundles approximately 1.0–1.5 mm in diameter, and they continued from the longitudinal muscle bundles of the rectum. The columnar bundles of the longitudinal anal muscle were internally and externally surrounded by sparsely arranged smooth muscle fibers that ran
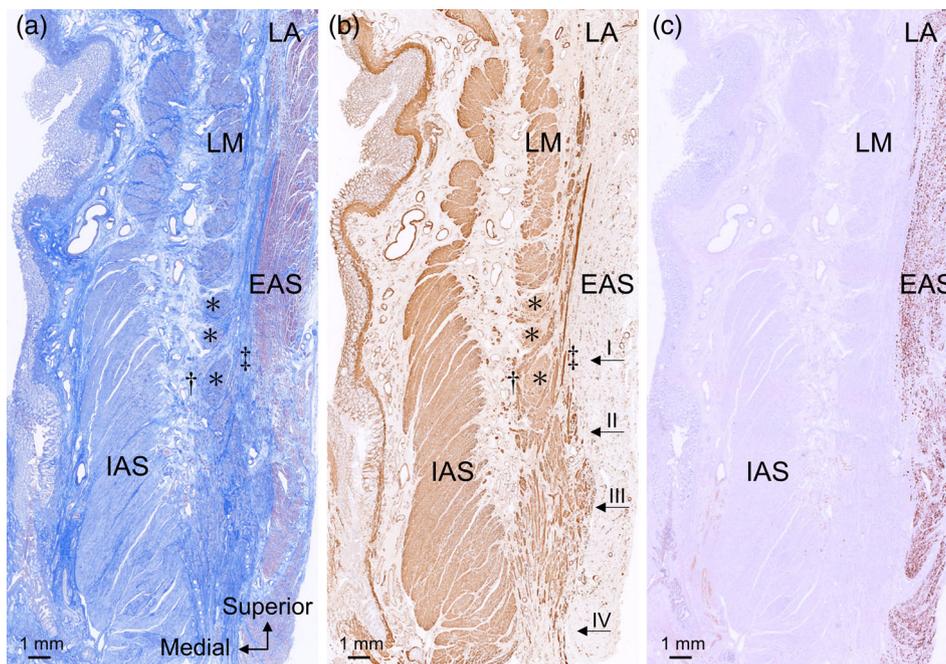
Fig. 6. Coronal section of the anal canal. Masson's trichrome stain (**a**), immuno-staining with antismooth muscle antibody (**b**), and antiskeletal muscle antibody. (**c**) The dense area of the smooth muscle fibers (asterisks) was enclosed internally and externally by sparse area of smooth muscle fibers (obelisk and double obelisk). The external sparse fibers (double obelisk) directly attached to the skeletal muscle fibers of the levator ani. The distinction in density of smooth muscle fibers gradually became obscure (arrows I, II, and III), and the longitudinal smooth muscle was branched into narrow bundles (arrow IV). EAS, external anal sphincter; IAS, internal anal sphincter; LA, levator ani; LM, longitudinal muscle; asterisk (*), dense area; obelisk (†) and double obelisk (‡), sparse area. [Color figure can be viewed at wileyonlinelibrary.com]

longitudinally (indicated by an obelisk and a double obelisk in Fig. 7). The external fibers of the longitudinal anal muscle directly attached to the skeletal muscle fibers of the levator ani in the superior part of the anal canal.

The longitudinal muscle of the anal canal is involved in dynamic pelvic floor function represented by the defecation function (Shafik, 1976; Macchi et al., 2008). In addition, this muscle is also a clini-cally important structure, such as a developmental pathway of anal fistula and a determinant of T-staging for rectal cancer (Parks, 1961; Lunniss and Phillips, 1992; Bamba et al., 2012). The structure of the longi-tudinal muscle has been analyzed focusing on its ori-gin, course, and inferior extension (Milligan and Morgan, 1934; Courtney, 1950; Goligher et al., 1955; Shafik, 1976; Lunniss and Phillips, 1992). A recent study has reported the varied course of the longitudi-nal muscle (e.g., oblique or helical courses) (Macchi et al., 2008). The present study focused on the aggre-gation form or density of smooth muscle fibers in the longitudinal muscle layer of the anal canal. This ana-tomical study was inspired by the magnetic resonance images of the anal canal and revealed the coexistence of dense and sparse areas of the smooth muscle fibers in the longitudinal muscle. Furthermore, this

study also showed the three-dimensional structure of smooth muscle tissue constituting the longitudinal muscle and the relationship with the surrounding structures. The most well-known review paper on the longitudinal anal muscle pointed out that "descrip-tions in one plane cannot explain the anatomy of a complex three-dimensional organ" (Lunniss and Phil-lips, 1992). The present study seems to have over-come this problem by combining macroscopic and microscopic research methods.

The longitudinal muscle of the anal canal is struc-turally close to the surrounding skeletal muscles and is speculated to have functions such as fixing the anorectum to the pelvis and everting the anus during defecation (Shafik, 1976; Lunniss and Phillips, 1992; Macchi et al., 2008;Muro et al., 2014 ; Nakajima et al., 2017 ; Muro et al., 2018 ; Muro et al., 2019). Our previous anatomical studies clarified the suspen-sion structure of the external anal sphincter by the longitudinal muscle and the smooth muscle extension structure of the longitudinal muscle (Muro et al., 2014; Nakajima et al., 2017; Muro et al., 2018; Muro et al., 2019). Based on these research findings, we hypothesized that the smooth muscle may possess various structures depending on its function and the
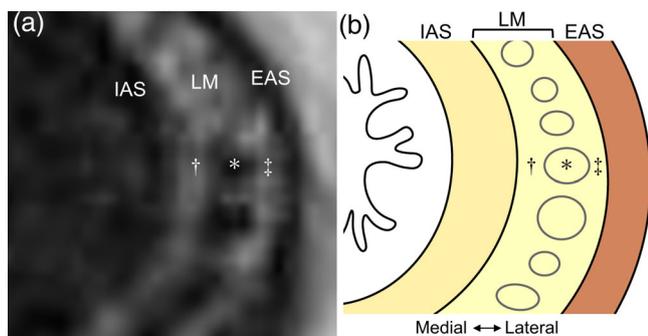
Fig. 7. Illustration and magnetic resonance image of the transverse (axial) section of the anal canal. Magnetic resonance image of the longitudinal muscle probably reflects the coexistence of dense area (asterisk) and sparse area (obelisk and double obelisk) of smooth muscle fibers. The dense areas form columnar muscle bundles approximately 1.0–1.5 mm in diameter, and they are internally and externally surrounded by sparsely arranged smooth muscle fibers. The dense areas (columnar bundles) are probably depicted as low intensity, and the sparse areas in the loose connective tissue as high intensity. EAS, external anal sphincter; IAS, internal anal sphincter; LM, longitudinal muscle; asterisk (*), dense area; obelisk (†) and double obelisk (‡), sparse area. [Color figure can be viewed at wileyonlinelibrary.com]

positional relationship with the skeletal muscles in the anal canal region where the digestive tract and pelvic floor muscle are spatially close. The present study clarified the diverse smooth muscle fibers in the longitudinal muscle of the anal canal: the coexistence of the densely gathered smooth muscle fibers and sparsely arranged ones. The dense area was continuous with the longitudinal muscle bundles of the rectum, whereas the sparse area directly attached to the skeletal muscle fibers of the levator ani. These findings suggest that smooth muscle fibers with different properties and state coexist in the longitudinal muscle layer based on the relationship with the skeletal muscle, and their structures are optimized for the function of each smooth muscle fiber. Dense smooth muscle fibers can be interpreted as gut-specific longitudinal muscle, and sparse smooth muscle fibers as smooth muscle tissue responsible for adhesion to levator ani. We speculate that dense smooth muscle fibers are optimized for shortening the anal canal by forming dense bundles, and sparse smooth muscle fibers are optimized for fixation with skeletal muscles by forming flexible shapes.

A histological study involving fetuses at 15 to 30 weeks of gestation reported that smooth muscle fiber aggregates form small circles and are arranged in a row in the axial plane of the anal canal, described as having "rosette-like appearance," and these aggregates were found regardless of sex and developmental stage (Arakawa et al., 2010). The present study also found this structure (dense area: columnar bundles) in adults, which was surrounded by sparsely arranged smooth muscle fibers. Therefore, in the developmental process, the sparse area is assumed to be formed later than the

dense area. Such differences in formation time may lead to differences in the properties and state of smooth muscle fibers and to the coexistence of different densities, that is, diversification and optimization of smooth muscle fiber morphology. Further consideration is suggested from the characteristics of the sparse area, that is, adhesion to the levator ani. During the developmental process, the levator ani probably attached to the precursor tissue of the longitudinal muscle. The middle part of the precursor tissue may differentiate into the dense area, and the tissue pulled by adhesion with the levator ani may expand and differentiate into the sparse area.

The coexistence of different densities of smooth muscle fibers may be reflected in the magnetic resonance images of the longitudinal muscle. MRI is useful for assessing the morphology of anorectal diseases, such as invasion of rectal cancer and extent of abscess of anal fistula (Stoker, 2009; Bamba et al., 2012; Surabhi et al., 2016).

For example, when MRI is used for preoperative evaluation of intersphincteric resection for rectal cancer, a retrospective study reported that MRI evaluation of the longitudinal muscle layer is probably useful for T-staging of cancer (Bamba et al., 2012). The conventional MRI method depicts the longitudinal muscle layer of the anal canal only as high intensity (Surabhi et al., 2016). However, MRI along the anal axis imaged in the jack-knife position can show the muscle layer structure of the anal wall in detail; the image of the coexistence of low- and high-intensity areas is consistently depicted in the longitudinal muscle layer (Fig. 1). Based on the present findings, the differences in the density of smooth muscle fibers may appear as differences in the signal strength of MRI. The dense areas (columnar bundles) are probably depicted as low intensity, and the sparse areas in the loose connective tissue as high intensity (Fig. 7). Thus, we believe that our new findings on the longitudinal anal muscle are potentially useful for the establishment of anatomical basis of the anal canal, which is necessary for improving the accuracy of cutting-edge imaging and endoscopic surgery. However, an important limitation of this study is the age bias of the cadavers. Only elderly cadavers were observed because of the rarity of young cadavers. In addition, the sample size of the present study may be small. However, this cannot be verified as there is no established standard sample size for anatomical research (Tomaszewski et al., 2017).

In conclusion, the longitudinal muscle of the anal canal has dense and sparse areas of smooth muscle fibers. In the anal canal region where the digestive tract and the pelvic floor muscle are spatially close, smooth muscle fibers with different properties and state coexist in the longitudinal anal muscle based on the relationship with the skeletal muscles. This suggests that the smooth muscle structure is optimized for its function. In addition, the coexistence of dense and sparse areas of smooth muscle fibers is probably reflected in the magnetic resonance image of the longitudinal muscle.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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