# Topographic Anatomy of the Anal Sphincter Complex and Levator Ani Muscle as It Relates to Intersphincteric Resection for Very Low Rectal Disease

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**BACKGROUND:** Intersphincteric resection has become a widely used treatment for patients with rectal cancer. However, the detailed anatomy of the anal canal related to this procedure has remained unclear.

**OBJECTIVE:** The purpose of this study was to clarify the detailed anatomy of the anal canal.

**DESIGN:** This is a descriptive study.

**SETTINGS:** Histologic evaluations of paraffin-embedded tissue specimens were conducted at a tertiary referral hospital.

**PATIENTS:** Tissue specimens were obtained from cadavers of 5 adults and from 13 patients who underwent abdominoperineal resection for rectal cancer.

**MAIN OUTCOME MEASURES:** Sagittal sections from 9 circumferential portions of the cadaveric anal canal (histologic staining) and 3 circumferential portions from patients were studied (immunohistochemistry for smooth and skeletal muscle fibers).

**RESULTS:** Longitudinal fibers between the internal and external anal sphincters consisted primarily of smooth

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muscle fibers that continued from the longitudinal muscle of the rectum. The levator ani muscle attached directly to the lateral surface of the longitudinal smooth muscle of the rectum. The length of the attachment was longer in the anterolateral portion and shorter in the posterior portion of the anal canal. In the lateral and posterior portions, the levator ani muscle partially overlapped the external anal sphincter; however, there was less overlap in the anterolateral portion. In the posterior portion, thick smooth muscle was present on the surface of the levator ani muscle and it continued to the longitudinal muscle of the rectum.

*LIMITATIONS:* We observed only limited portions in some surgical specimens because of obstruction by tumors.

**CONCLUSIONS:** The levator ani muscle attaches directly to the longitudinal muscle of the rectum. The spatial relationship between the smooth and skeletal muscles differed in different portions of the anal canal. For intersphincteric resection, dissection must be performed between the longitudinal muscle of the rectum and the levator ani muscle/external anal sphincter, and the appropriate surgical lines must be selected based on the specific structural characteristics of each portion.

*KEY WORDS:* Anal canal; Clinical anatomy; Intersphincteric resection; Levator ani muscle; Longitudinal muscle; Low rectal cancer.

Thanks to advances in surgical techniques, intersphincteric resection (ISR) is now widely used as an alternative treatment option that preserves the anus and, thus, anal continence for patients with rectal

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cancer.<sup>1-4</sup> To preserve the anus with ISR, the rectum is divided from the levator ani muscle (LAM) using an abdominal approach, followed by dissection between the internal anal sphincter (IAS) and the external anal sphincter (EAS) using both abdominal and perianal approaches. For this procedure, it is essential that rectal surgeons have detailed knowledge of the anatomic relationship between the rectum and LAM and between the IAS and EAS.

It has been reported that fibers of the longitudinal muscle (LM) of the rectum blend with fibers of the LAM, thereby producing a conjoined,<sup>5–7</sup> combined,<sup>8</sup> or conjoint<sup>9</sup> LM layer between the IAS and EAS. However, the detailed anatomy of the region between the IAS and EAS, the so-called intersphincteric space, and the precise surgical line within this space have not yet been clarified.

Furthermore, with the progress of laparoscopic techniques, some surgeons now perform ISR endoscopically.<sup>10–13</sup> During endoscopic surgery, structures in the deep pelvic region can be observed more clearly and at greater magnification than they can during open surgery. During endoscopic ISR, the attachment of the LAM to the rectum and the intersphincteric space can be seen very clearly from the abdominal approach, and it becomes possible to more precisely select a surgical line; however, the optimal surgical line in this area remains undefined.

The purpose of this study was to describe in detail the anatomy of the LAM, EAS, IAS and rectum so as to enable improved radial margins while reducing the incidence of postoperative incontinence in patients with rectal cancer. We used cadaveric specimens to study specifically intended sections, as well as surgical specimens from patients who underwent abdominoperineal resections (APRs), which offer better consistency and morphologic quality than muscle tissue from formalin-fixed postmortem material.

# **MATERIALS AND METHODS**

#### **Cadaveric Specimens**

Five pelvises from 5 Japanese cadavers (1 man and 4 women; age range, 84.0–100.0 years; mean age, 90.4 years) obtained from the dissecting room of the School of Medicine, Tokyo Medical and Dental University, were used for cadaveric studies. The cadavers were donated to the Tokyo Medical and Dental University Department of Anatomy for use in clinical studies, within the guidelines of the Act on Body Donation for Medical and Dental Education law of Japan. Cadavers were fixed by arterial perfusion with 8% formalin and preserved in 30% alcohol to resist fungus and to maintain the softness of the tissues. The rectum, anus, and circumjacent muscles and connective tissue were removed en bloc, hewn out radially into blocks, fixed in 10% formalin, dehydrated, and embedded in paraffin. A total of 9 segments of the anal canal, from anterolateral to posterior, were sectioned in the sagittal plane. The cutting

lines of the 9 segments are indicated in the bottom right key in Figure 1.

#### **Surgical Specimens**

Thirteen specimens from 13 Japanese patients (9 men and 4 women; age range, 41.0–84.0 years; mean age, 68.6 years) who underwent APR for rectal cancer in the National Cancer Center Hospital East without neoadjuvant chemoradiation were used. The specimens were fixed in 10% formalin  $\approx$ 2 hours after they were resected. They were then dehydrated and embedded in paraffin, after which 4-µm–thick sagittal sections were prepared. Sections that were not affected by the tumors were observed. This pathological study was performed in accordance with the provisions of the Declaration of Helsinki, and the protocol for this study was approved by the ethics committees of the National Cancer Center Hospital East (2013–143).

#### Histological and Immunohistochemical Staining

Sections were stained with hematoxylin and eosin and Elastica van Gieson. The distributions of smooth and skeletal muscle were confirmed through immunohistochemical staining of surgical specimens. For antigen retrieval, the slides were microwaved in 10 mM sodium citrate buffer (pH 6.0). Endogenous peroxidase activity was inactivated by incubating the tissues for 30 minutes in methanol containing 0.3% H<sub>2</sub>O<sub>2</sub>. Nonspecific binding was blocked by incubating the tissues for 30 minutes at room temperature in phosphate-buffered saline containing 0.05% Tween 20 and 5% goat serum. Once blocked, the sections were incubated overnight at room temperature with antismooth muscle actin (ready-to-use, Actin, Smooth Muscle Ab-1, Clone 1A4; Thermo Fisher Scientific, Fremont, CA) and antiskeletal muscle myosin (ready-to-use, Myosin, Skeletal Muscle Ab-2, Clone MYSN02, Thermo Fisher Scientific) as primary antibodies. The sections were then washed and incubated for 30 minutes at room temperature with biotinylated goat antimouse IgG (1:200 dilution, Vector Laboratories, Burlingame, CA) as the secondary antibody. The signal was amplified using a VECTASTAIN ABC kit (Vector Laboratories) and TSA Biotin System (PerkinElmer, Tokyo, Japan) according to the manufacturer instructions, and the immunocomplexes were detected using 3,3'-diaminobenzidine (Wako, Osaka, Japan) as the chromogen. Finally, the sections were counterstained using hematoxylin.

#### **Statistical Analysis**

We used the Wilcoxon test with the Bonferroni method to compare the continuous variables of 3 cohorts (lateral, anterolateral, and posterior). The level of significance was set at p < 0.017 by dividing 0.05 by 3. All of the statistical analyses were performed using JMP version 8.0 (SAS Institute Inc, Cary, NC). All of the measurements were made by



**FIGURE 1.** Cadaveric specimens. Sections were stained with Elastica van Gieson. The sections are labeled as shown in the bottom right key. The longitudinal fibers that continued from the LM are indicated by the asterisks in A–I. The longitudinal fibers that ran between the skeletal muscles of the inferior part of the EAS are indicated by arrowheads in A, B, and E–I. (In C and D, these fibers are not observed because of the condition of the sections.) The borders of the CM and IAS are indicated by solid black lines in A–I. The borders of the EAS, LM, and LAM are indicated by dotted black lines in A–I. CM = circular muscle of the rectum; LM = longitudinal muscle of the rectum; IAS = internal anal sphincter; EAS = external anal sphincter; LAM = levator ani muscle.

2 of the authors (Y.T. and K.A.), and the mean value was used for statistical analysis.

# **RESULTS**

## **Findings in Cadaveric Specimens**

The lateral portion of the anal canal consisted of several muscular layers. The inner smooth muscular layer, which continued from the circular muscle of the rectum, was the IAS, and the outer skeletal muscular layer surrounded by the fat tissue of the ischiorectal fossa was the EAS. The skeletal muscle that continued from the pelvic wall could be identified as the LAM. It is generally accepted that the LAM is composed of 3 muscles, the puborectalis, pubococcygeus, and iliococcygeus; however, these muscles could not be distinguished histologically. Between the IAS and EAS, longitudinal fibers were observed. These longitudinal fibers continued from the LM of the rectum and

ran between the skeletal muscles of the inferior portion of the EAS (Fig. 1A).

We then studied the morphological differences in the muscles composing the anal canal after dividing the canal into 9 segments. Observation of the 9 segments revealed that the shape and thickness of these muscles differed according to portion. The LAM was thick and straight in the anterolateral portion (Figs. 1C–E) but gradually became curved in the lateral and posterolateral portions (Figs. 1A, B, F, and G). In the posterior portion, the LAM was more curved and thinner (Figs. 1H and I). The shape of the EAS also differed according to portion. From the anterolateral to the posterolateral portion, the EAS was folded inward (Figs. 1A–G). In the posterior portion, the EAS was folded partially inward and partially outward (Figs. 1H and I).

When these 9 segments were compared, the anal canal could be divided into 3 portions based on the morphological differences in the LAM and EAS. In the anterolateral portion, the LAM was thick and straight, whereas the EAS was folded inward. In the lateral portion, the LAM was relatively thick and curved, and the EAS was folded inward. In the posterior portion, the LAM was thin and curved, and the EAS folded partly inward and partly outward. The morphology of the posterolateral portion was nearly the same as the lateral portion.

## Findings in Surgical Specimens After APR

Based on the serial observations of cadaveric specimens, we studied 3 portions of the anal canal, anterolateral, lateral, and posterior, in more detail using surgical specimens after APR. These surgical specimens offered better consistency with higher morphologic quality of the muscle tissue than was seen with muscle tissue from cadaveric specimens.

## Lateral Portion of the Anal Canal

The lateral portion of the anal canal could be divided into several muscular layers: the IAS, EAS, LAM, and longitudinal fibers between the IAS and EAS. Immunohistochemical staining revealed that these longitudinal fibers consisted primarily of smooth muscle fibers that continued from the LM of the rectum. The LAM could be distinguished from the EAS because the skeletal muscle that had been resected during APR and was separated from the EAS by a small gap of connective tissue. A difference in fiber direction also facilitated differentiation of these 2 muscles. However, because it was difficult to discern the puborectalis muscle from the deep part of the EAS, the puborectalis muscle is not indicated separately in the figures.

We confirmed that the LAM is attached to the lateral surface of the LM (Figs. 2A–C). Detailed observation revealed several features of the relationship between the LAM and LM and between the LAM and EAS. We observed that the LAM and EAS partially overlapped within the anal canal (LAM inside and EAS outside; Fig. 2C). The overlapping fraction (calculated as "a/b × 100 (%)" in Fig. 2A) was 30.9% of the total height of the EAS in the mean (range, 21%–44%).

Furthermore, at the site of attachment of the LAM to the LM, the shape of the attachment was indented (Figs. 2D and E). Under high magnification, we could see that the smooth muscle fibers of the LM attached directly to the skeletal muscle fibers of the LAM and formed the



**FIGURE 2.** Surgical specimens from patients who underwent APR. The sections were from the lateral portion of the anal canal. The overlapping portion between LAM and EAS is labeled "a." The total height of EAS is labeled "b." The length of attachment of the LAM to the LM is labeled "c." D and E, Enlarged views of the blue square in A. F and G, Enlarged views of the dotted blue square area in D. H and I, Enlarged views of the red square area in A. Smooth muscle fibers of the LM that directly attach to the skeletal muscle fibers of the LAM are indicated by black arrows in F and G. The thin smooth muscular structure present on the surface of the LAM is indicated by the red arrow in D. The smooth muscle fibers of the IAM running through the inferior part of the EAS are indicated by black arrowheads in H. APR = abdominoperineal resection; CM = circular muscle of the rectum; LM = longitudinal muscle of the rectum; IAS = internal anal sphincter; EAS = external anal sphincter; EAM = levator ani muscle; ME = hematoxylin and eosin.

indented attachment (Figs. 2F and G). The mean length of the attachment (shown as "c" in Fig. 2D) was 5.0 mm (range, 1.7–8.8 mm). Inferior to the LAM-LM attachment site, we found that the LAM did not attach tightly to the LM or EAS (Fig. 2A). On the surface of the LAM, a thin smooth muscular structure was detected, and it is this smooth muscular structure that continued to the LM (Fig. 2D).

The EAS was folded inward in the lateral portion. We also observed that fibers from the LM, which we identified immunohistochemically to be smooth muscle, ran through the skeletal muscles in the inferior part of the EAS (Figs. 2A, H, and I).

#### Anterolateral Portion of the Anal Canal

In the anterolateral portion of the anal canal, the IAS, EAS, LAM, and LM were identified in the same manner as in the lateral portion (Figs. 3A–C). Just as in the lateral portion, the LAM attached directly to the LM (Figs. 3A–C), although the shape of the attachment was slightly different in the anterolateral portion. The overlapping fraction in the anterolateral portion was 18.2% of the total height of the EAS in the mean (range, 13%–25%), smaller than in the lateral portion (Fig. 3C).

Just as in the lateral portion, the site of LAM-LM attachment was indented (Figs. 3D and E). High magnification revealed that smooth muscle fibers of the LM attached directly to the skeletal muscle fibers of the LAM and formed the indented attachment (Figs. 3F and G). However, the length of the attachment was longer than in the lateral portion (mean, 9.2 mm; range, 7.0–11.5 mm). The thin smooth muscular structure on the surface of LAM, which continued to the LM, was also observed at this portion (Fig. 3D). The EAS was folded inward, and several smooth muscle fibers of the LM ran through the skeletal muscles in the inferior portion of the EAS (Figs. 3A, H, and I).

## **Posterior Portion of the Anal Canal**

In the posterior portion of the anal canal, a layered structure consisting of smooth and skeletal muscles was observed, just as in the other 2 portions (Fig. 4). The distinguishing characteristic of the posterior portion was the presence of thick tissue on the superior surface of the LAM (Fig. 4A), which immunohistochemical analysis revealed to consist mainly of smooth muscle fibers that continued to the LM (Fig. 4B).

As in the lateral and anterolateral portions, at the site of LAM-LM attachment, smooth muscle fibers of the LM attached directly to the skeletal muscle fibers of the LAM.



**FIGURE 3.** Surgical specimens from patients who underwent APR. The sections were from the anterolateral portion of the anal canal. D and E, Enlarged views of the blue square are in A. F and G, Enlarged views of the dotted blue square area in D. H and I, Enlarged views of the red square area in A. The smooth muscle fibers that directly attached to the skeletal muscle fibers of LAM are shown by the arrows in D–G. The thin smooth muscular structure present on the surface of the LAM is indicated by the red arrow in D. The smooth muscle fibers of the LM running through the inferior part of the EAS are indicated by arrowheads in H. APR = abdominoperineal resection; CM = circular muscle of the rectum, LM = longitudinal muscle of the rectum, IAS = internal anal sphincter, EAS = external anal sphincter, LAM = levator ani muscle; m = muscle; HE = hematoxylin and eosin.



**FIGURE 4.** Surgical specimens from patients who underwent APR. The sections were from the posterior portion of the anal canal. Thick smooth muscle present on the surface of the LAM is indicated by the red arrow in B. Smooth muscle fibers of the LM running through the inferior part of the EAS are indicated by arrowheads in B, and the anococcygeal ligament (which was cut during APR) is indicated by the black arrow in B. APR = abdominoperineal resection; CM = circular muscle of the rectum, LM = longitudinal muscle of the rectum, IAS = internal anal sphincter, EAS = external anal sphincter, LAM = levator ani muscle; m = muscle; HE = hematoxylin and eosin.

However, because the length of the attachment was shorter than in the other portions, the indented shape of the attachment was not clear in the posterior portion. The mean length of the LAM-LM attachment was 2.4 mm (range, 0–4.4 mm) in the posterior portion, and the mean fraction of LAM overlap on the EAS was 35.3% of the total height of the EAS (range, 26%–52%).

The EAS in the posterior portion was folded partly inward and partly outward (Fig. 4C). Several smooth muscle fibers of the LM ran through the skeletal muscles of the inferior part of the EAS and connected with the fibers of the anococcygeal ligament, which was attached to the dorsal surface of the coccyx (Figs. 4B and C).

#### Statistical Analysis of Quantitative Measurements

The quantitative measurements (overlap of LAM and EAS and attachment of LAM and LM) made in the 3 portions of the anal canal were analyzed statistically. The fraction of

LAM overlap on the EAS in the anterolateral portion was significantly smaller than in the lateral or posterior portion (p < 0.017; Fig. 5A). The length of the attachment of the LAM to the LM was significantly longer in the anterolateral portion and significantly shorter in the posterior portion than in the lateral portion (p < 0.017; Fig. 5B).

# DISCUSSION

The purpose of this study was to determine the relationship between the rectum and muscles composing the anal canal. We therefore examined the anal canal from the anterolateral portion to the posterior portion. Within the anterior portion, the rectum and muscles that compose the anal canal show a strong association with the bulbospongiosus muscles, perineal muscles, rectoure thral muscle, and urogenital organs. Consequently, the anatomy of the anterior portion could not be simply compared with other portions (anterolateral, lat-



**FIGURE 5.** Boxplots of the overlap fraction between LAM and EAS in the 3 portions in the anal canal (A) and the length of attachment of the LAM to the LM (B). LAM = levator ani muscle, EAS = external anal sphincter, LM = longitudinal muscle of the rectum. \*p < 0.017.

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eral, and posterior). Moreover, the data collected on the anterior portion were extensive and may be too comprehensive for this article. For these reasons, although we know that the anatomy of the anterior portion is very important for ISR, the anterior portion was excluded from this study.

Using immunohistochemistry to analyze the surgical specimens, we found that the structural differences among the 3 examined portions of the anal canal were caused not only by morphological differences in the muscles but also by the differences in the spatial relationship between smooth and skeletal muscles. In the anterolateral portion, the LAM was thick, and the length of the attachment of the LAM to the LM was long. In the posterior portion, by contrast, the LAM was thin, the length of the LAM-LM attachment was short, and a thick smooth muscle layer was present on the surface of the LAM. This smooth muscle filled the gap (hiatus) between the LAM and LM and was situated in the same space as the hiatal ligament reported by Shafik.14 Using immunohistochemistry, we clarified that this structure consisted mainly of smooth muscle, which is thought to complement or facilitate the attachment of the LAM to the LM. This smooth muscle was thick in the posterior portion, whereas it was thin in the lateral and anterolateral portions. When we examined the relationship between the smooth and skeletal muscles, we found that the LAM could be divided into 2 parts, an attached part and an unattached part. The LAM was partially attached to the LM, mediated by smooth muscle, and partially overlapped EAS and ran along the LM without attachment. In the anterolateral portion, the LAM mainly attached to the LM, and the unattached part was short. In the lateral portion, the LAM was partially attached to the LM, and part of it ran unattached along the LM. In the posterior portion, only a short segment of the LAM attached to the LM; it mainly ran along the LM without attachment. Based on these findings, we made schemata

of the structures of the 3 portions of the anal canal (Fig. 6). The specimens were studied using both high and low magnification, enabling gross morphological differences to also be depicted from this mainly histological study.

It was reported previously that the LAM is conjoined or mixed with the LM of the rectum and forms a conjoined,<sup>5-7</sup> combined,8 or conjoint9 LM layer within the intersphincteric space, between the IAS and EAS. However, those reports were based on hematoxylin and eosin-stained preparations, and thus the distributions of smooth and skeletal muscle in the anal canal may be unclear. The detailed anatomy of the anal canal was described recently in 3 reports. Arakawa et al<sup>15</sup> reported a septum (not muscle) between the IAS and EAS. However, in the present study it was obvious that the longitudinal layer between the IAS and EAS consisted mainly of smooth muscle. Shafik<sup>14</sup> described 3 layered structures between the IAS and EAS: a direct continuation of the outer muscle coat of the rectum, a direct prolongation of the pubococcygeus, and an extension of the top loop of the external sphincter. Finally, Macchi et al<sup>16</sup> reported that the outer layer of the conjoint LM was skeletal muscle and the inner layer was smooth muscle. These findings are similar to ours for the lateral portion (the LAM overlapped the EAS), but, as we discussed above, our findings differed in the other portions.

Considering the posterior portion of the anal canal macroscopically, it is generally accepted that there is a thick white bundle on the superior surface of the LAM. Shafik<sup>14</sup> described this structure as the "hiatal ligament," whereas Kinugasa et al<sup>17</sup> reported it as the "ventral layer of the anococcygeal ligament," and Muro et al<sup>18</sup> reported that it consists of smooth muscle. Here, we observed that this is a smooth muscular structure present not only in the posterior portion but also in the lateral and anterolateral portions. These smooth muscles are considered to fill the levator hiatus and the gap between the LAM and rectum.



FIGURE 6. Schema of the spatial relationships among the 3 portions in the anal canal. Surgical lines from the abdominal approach (solid line) and perianal approach (dotted line) are also shown. The white spaces between the muscles indicate that the muscles do not attach tightly. CM = circular muscle of the rectum; LM = longitudinal muscle of the rectum; IAS = internal anal sphincter; EAS = external anal sphincter; LAM = levator ani muscle.

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When ISR is performed, the surgical procedure must be varied according to the portion of the anal canal. In the anterolateral portion, the length of the attachment of the LAM to the LM is relatively long, and, after their detachment, it is considered to be easy to reach the layer between the LM and EAS. Because of the overlap of the LAM and EAS in the lateral portion, the surgical line from the abdominal approach tends to be ventral to the LAM, and the surgical line from the perianal approach tends to be dorsal to the LAM. Consequently, there may be a gap in the surgical planes. In the posterior portion, it is necessary to cut the smooth muscular structure ventral to the LAM to approach the area between the LM and LAM. The surgical lines for the 3 portions are shown in Fig. 6. The important point is not which anatomical planes should be followed for ISR but to know the likelihood of a gap in the surgical planes during ISR. This gap is created by the (overlapped) LAM. When the surgical planes from the abdominal and perianal approaches are not matched, surgeons should cut the LAM between the abdominal and perianal planes to complete the intersphincteric dissection. Moreover, the gap in the surgical plane is characteristic in the lateral portion. In the anterolateral portion, the LAM slightly overlaps the EAS; thus, the surgeon may not dissect between the LAM and EAS from the perianal approach. In the posterior portion, the overlapped LAM is not tightly attached to the EAS; however, the LAM rarely becomes the source of a gap in the surgical planes because the LAM itself is thin. These observations prompted us to hypothesize that, because fibers from the LM run between the skeletal muscles of the inferior part of the EAS, the LM and EAS are closely involved in anal function. Therefore, careful consideration is necessary, because cutting the LM itself may lead to anal dysfunction.

A limitation of this study is that, although observation of the surgical specimens revealed the detailed relationship between smooth and skeletal muscles, we were able to observe only limited parts in some specimens because of obstruction by tumors. In the future, we would like to apply the findings of this study to a surgical procedure. To achieve an accurate and reproducible surgical procedure, it will be necessary to modify the dissection procedure according to the specific portion of the anal canal. In addition, we will extend this study to clarify the anatomic basis for the functional movement of the anal canal.

## CONCLUSION

The LAM attaches directly to the LM, and a mixed layer of smooth and skeletal muscle fibers does not exist between the IAS and EAS. The spatial relationship between the smooth and skeletal muscles differs in different portions of the anal canal. When performing ISR, after identifying the LAM/ EAS based on the muscle fiber direction and the muscular contraction with a monopolar cautery knife, it is necessary to dissect between the LM and LAM/EAS, and appropriate surgical lines must be selected based on the specific anatomic characteristics of each portion in the anal canal.

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