# MRI of anorectal malformations and relationship of the developmental state of the spincter muscle complex with fecal continence

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***Background:*** **Accurate evaluation of anorectal malformation (ARM) has long been considered difficult, because no physical findings or diagnostic modalities are available for a correct assessment of the level and type of this malformation, the developmental state of the sphincter muscle complex (SMC), and the presence of associated anomalies. This study was undertaken to evaluate the developmental state of the caudal region and SMC in ARM and its relationship with fecal continence by magnetic resonance imaging (MRI).**

***Methods:*** **We investigated 39 patients with ARM by MRI (28 boys and 11 girls), aged from 3 days to 12 years. Imaging studies involved the level and type of ARM, the associated anomalies of the spinal cord, spine, urogenital system, and the relationship of the developmental state of the SMC and fecal continence.**

***Results:*** **ARM was detected by MRI in all the 39 patients, and clinically proven fistulae were found in 97% (31/32) with T2-weighted images (T2WI). Associated anomalies of the spinal cord and spine were found in 41% patients, and those of the urogenital system in 21%. Incontinence was found in 71% patients when the relative width of the puborectalis muscle (PRWR) was <0.18 and the relative width of the external anal sphincter (EASWR) <0.15, and normal continence in 91% when PRWR was >0.18 and EASWR >0.15.**

***Conclusions:*** **MRI proves to be the best modality to accurately determine the level and type of ARM, the type of fistula, the developmental state of the SMC, and the associated anomalies of the spinal cord, spine, and urogenital system. MRI may demonstrate maldevelopmental indexes of the SMC, which are significantly important in planning the treatment of ARM and predicting the outcome after reconstructive surgery.**

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***Key words:*** **anorectal malformation; sphincter muscle complex; continence; MRI**

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## Introduction

Operative strategies for anorectal malformation (ARM) vary with its level, type and associated anomalies. For optimal operative strategies and better therapeutic effects, it is important to assess the level of neonatal atresia, the developmental state of the sphincter muscle complex (SMC) and the presence of associated anomalies. The SMC is recognized to play a key role in postoperative fecal continence.[1] The traditional assessment of SMC relies on plain radiography or invertography, which is indirect and inaccurate for intermediate and high ARM. It is also difficult for clinicians to understand the anatomic structure of the pelvic floor and predict postoperative fecal continence. Although gross maldevelopment of the SMC is easily depicted in ARM, objective criteria for maldevelopment of the SMC are still not available. In this study, high spatial-resolution body phased-array (or head coil) magnetic resonance imaging (MRI) was used to identify the level of ARM, the presence of fistulae and associated abnormalities, and the developmental state of the SMC. The image variance of the SMC and objective anorectal function were taken to screen out objective criteria for the maldevelopment of the SMC.

## Methods

### Patients

Thirty-nine patients with ARM (28 boys and 11 girls) were enrolled in this study. Their age ranged from 3 days to 12 years (average 2.7 years). High ARM developed in 12 patients (including 1 patient with cloacal malformation), intermediate ARM in 11, and low ARM in 16 (including 1 patient with Currarino triad). All patients were subjected to preoperative MRI of the abdomen and pelvis. In 30 patients aged more than 5 years who received postoperative MRI, 9 had high ARM, 8 intermediate ARM, and 13 low ARM. The patients with preoperative SMC dysplasia detected by MRI and postoperative fecal continence were followed up.

### MRI studies

#### *Scanning method and parameters*

MRI was done with a 1.5T magnet (Semens). All patients were placed in a supine position with the pelvis concentrated on the coil. The head coil was applied in babies, and the body-phased array was suitable for elder children. Chloral hydrate (100 mg/kg) was used for newborns and infants, and thiopental (30 mg/kg) per rectum for elder children. In patients who had not received bowel preparation, a catheter was deposited to empty the bladder before the study.

Sagittal, coronal, transverse tubo spin-echo (SE) T1-weighted and fast spin-echo (FSE) T2-weighted images of the abdominal and pelvic region were obtained in all patients, and short T1 inversion recovery (STIR) T1-weighted images were obtained in babies. First, the T1-weighted sequences were performed in the transverse plane from the superior border of the pubic symphysis to the anus, and then the T2-weighted sequences were performed in the sagittal, coronal, and the transverse planes. The sagittal and coronal planes included the pelvis, lumbosacral spinal cord, spine and kidneys. The transverse T1-weighted, sagittal and coronal T2-weighted sequences served for accurate planning of the transverse T2-weighted sequences, because they were important to angle these planes exactly perpendicular to the long axis of the anal canal. The thickness of the slice was kept as thin as possible (3 to 5 mm) with a small interslice gap (usually 0-0.25 mm). The total time for MRI was about 20 minutes.

#### *Image analysis*

The level of atresia, the developmental state of the SMC, the spinal cord and spine, and the length of the puborectalis and external anal sphincter were evaluated on the sagittal plane. The relationship of the level of atresia to the SMC, and the thickness of puborectalis and external anal sphincter were evaluated on the coronal plane. Transverse position on the pubococcygeal plane and I plane mainly displayed the width of puborectalis and external anal sphincter, and the direct sign of the level of anorectal atresia. The width was measured at 3 and 9 o'clock at the midanal level of the anal canal. The measurements used softwere callipers on a 0.01-mm scale. Values were expressed in millimeters. MR images were evaluated independently by an experienced radiologist or pediatric surgeon.

#### *Diameter line measurement and muscular development index*

The half distance of ischial tuberosities was defined as half of distance between the inner border of two ischial tuberosities. The length of pubococcygeal line was the distance from the inferior border of the pubic symphysis to the sacrococcygeal joint. The muscle length was measured along the pubococcygeal line on the sagittal plane, whereas the muscle width was measured along the lateral axis on the transverse plane. The total width of muscle was defined as the sum of both left and right muscle width of the rectum or anal canal.

The relative width of puborectal muscle (RWPR) = the total width of puborectal muscle / the half distance of ischial tuberosities, whereas the relative width of external anal sphincter (RWEAS) = the total width of external anal sphincter / the half distance of ischial tuberosities. The relative length of puborectal muscle (RLPR) = the length of puborectal muscle / the length of the pubococcygeal line. The relative length of external anal sphincter (RLEAS) = the length of posterior external anal sphincter / the length of the pubococcygeal line.

According to the different characteristics of imaging of the SMC in ARM, the developmental state of the SMC was divided into three degrees as excellent, good, and poor development.

### Evaluation of the objective anorectal function

#### *Manometry*

Synectics (Polygraf) dynamic manometry was performed in all patients. A prototype perfusion catheter was placed in the rectum and anus to detect the length of anorectal high pressure region, anorectal contraction pressure difference, as well as the compliance and sensibility of the rectum.

#### *Defecography*

Twenty-five to eighty ml contrast medium was infused into the rectum through a Foly's catheter.

Barium spillage and retention, which reflected the controllability of the anorectum, were observed.

Anorectal angulation was measured by MRI: the included angle of the rectal approximal axial line (the posterior wall of the rectum) and anal axial line.

### Statistical analysis

The data were expressed as mean ± SD. Statistical analysis was made using Fisher's exact test. The relationships between the levels of ARM and the developmental state of the muscles were analyzed by linear correlation analysis. Ninety-five percent confidence intervals for the difference between medians were calculated. The statistical analyses were made by a software package (SPSS for Windows, release 10.0). A *P* value of less than 0.05 was considered statistically significant.

## Results

### MRI classification of ARM

Thirty-nine patients with ARM were investigated with MRI. Eleven patients had high ARM (Fig. 1), 11 intermediate ARM, 15 low ARM (Fig. 2), 1 low ARM with Currarino syndrome (Fig. 2), and 1 high cloacal malformation (rectovesical fistula, Fig. 3). All patients were confirmed operatively. In 32 patients with fistulae, only 16 patients (50%) were detected correctly with T1-weighted images (T1WI). While with T2-weighted images (T2WI), the fistulae were depicted correctly in all but one patient who had a rectovesical fistula proved surgically (97%) (Fig. 1). The remaining 7 patients had no fistula: high ARM (1 patient), intermediate ARM (2), and low ARM (4). At the low level ARM patients, the position of the anal canal and its relationship with the superficial transverse muscle of the perineum were shown in Fig. 4.

### MRI index of SMC development

The development of the puborectalis and external anal sphincter in different types of ARM on traverse and sagittal planes was determined (Tables 1 and 2). The more plentiful layers were displayed in T2WI, the character of muscle shape was clearly visible on the STIR sequence (Fig. 5).

The 95% confidence intervals for the difference between medians of the relative width of SMC were calculated as mean±1.96SD. The 95% confidence intervals of medians for poor development of the puborectalis and external anal sphincter were 0.15±0.03 and 0.13±0.02, respectively (Table 3).

The relative widths of puborectalis and external anal sphincter were compared in normal children and those with ARM of different types. There was a significant difference between the relative width in patients with high ARM and intermediate ARM (PRWR $q$=11.327, $P$<0.01; EASWR $q$=11.643, $P$<0.01), and the relative width in patients with high ARM and low ARM (PRWR $q$=13.313, $P$<0.01; EASWR $q$=11.787, $P$<0.01). There was no significant difference in other groups, including those with the relative width with intermediate and low ARM (PRWR $q$=0.908, $P$>0.05; EASWR $q$=0.442,





**Table 1.** Developmental state of the puborectalis in patients with ARM on different planes

| SMC | Traverse plane (width) | | | Sagittal plane (length) | | |
|---|---|---|---|---|---|---|
| | Excellent | Good | Poor | Excellent | Good | Poor |
| Low (16) | 14 | 2 | 0 | 14 | 2 | 0 |
| Intermediate (11) | 4 | 6 | 1 | 5 | 5 | 1 |
| High (12) | 1 | 5 | 6 | 1 | 4 | 7 |
| Total (39) | 19 | 13 | 7 | 20 | 11 | 8 |

The developmental state of width and length of the puborectalis is positively correlated ($R$=0.987, $P$<0.01) in different ARM patients. The development of width is highly correlated with that of length in the same atresia level of ARM.

**Table 2.** Developmental state of external sphincter in patients with ARM on different planes

| SMC | Traverse plane (width) | | | Sagittal plane (length) | | |
|---|---|---|---|---|---|---|
| | Excellent | Good | Poor | Excellent | Good | Poor |
| Low (16) | 14 | 2 | 0 | 14 | 2 | 0 |
| Intermediate (11) | 4 | 6 | 1 | 4 | 6 | 1 |
| High (12) | 1 | 3 | 8 | 1 | 4 | 7 |
| Total (39) | 19 | 11 | 9 | 19 | 12 | 8 |

The developmental state of width and length of external sphincter is positively correlated ($R$=0.995, $P$<0.01) in different ARM patients. The development of width is highly correlated with that of length in the same atresia level of ARM.

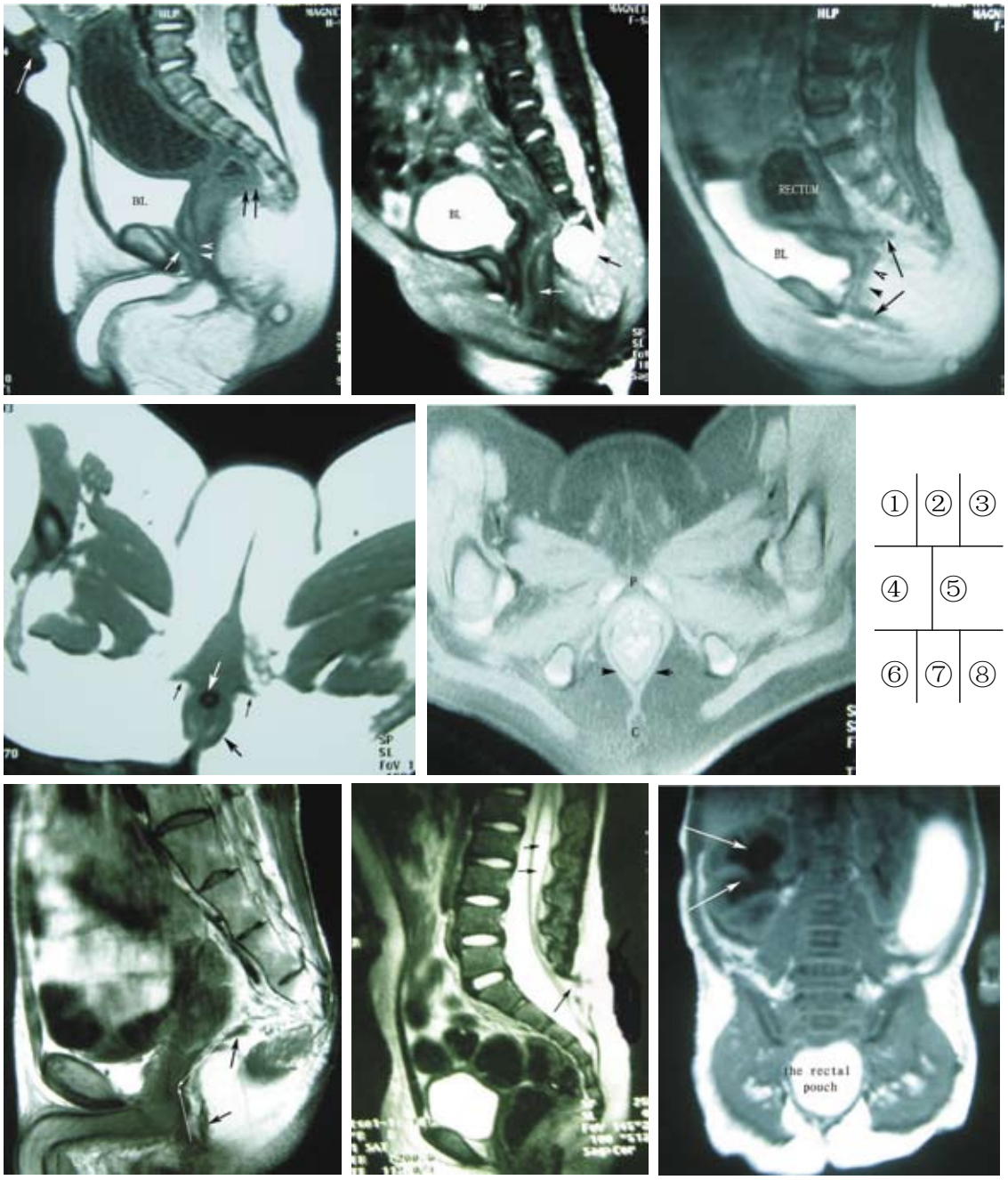


**Fig. 1.** Sagittal FSE T2-weightted image in a 9-month-old boy with a transverse colon stoma (long white arrow), a high ARM, and a rectourethral fistula. The rectal pouch ends above the levator ani muscle (black arrow), and the fistula (white arrowhead) extends anteriorly to the urethra (short white arrow), BL, and bladder.
**Fig. 2.** Sagittal FSE T2-weighted image in a 1-year-old girl with low ARM and rectovaginal fistula (white arrow), who had Currarino triad including three correlative processes of anorectal malformation, sacral bony defect, and presacral mass (black arrow). The rectal pouch ends below the levator ani muscle, BL, and bladder.
**Fig. 3.** Sagittal FSE T2-weighted image in a 2-year-old girl with cloacal deformity (high type) showing a 3-cm length co-tube (arrowhead), the maldeveloped puborectalis muscle and external anal sphincter (arrow), BL, and bladder (scaphoid).
**Fig. 4.** Traverse SE T1-weighted image in a 3-month-old girl. The anal canal at the level of the superficial transverse perineal muscle (small dark arrow) showed an ectopic anterior location (white arrow), ventral to the external sphincter (large dark arrow). The external anal sphincter depicted as normal development (EASWR=2.2/5.6=0.39).
**Fig. 5.** Traverse STIR T1-weighted image in a 5-month-old boy with high ARM failed to show rectal lumen structures on the PC plane. The puborectalis muscle was easily depicted as maldevelopment (PRWR=0.3/2.1=0.14).
**Fig. 6.** Sagittal SE T1-weighted image in a 13-year-old boy with complete incontinenece after reconstructive surgery for a high imperforate anus. PR and EASTR cloudiness indicate the maldevelopment (black arrow), and the anorectal angle of 118º.
**Fig. 7.** Sagittal FSE T2-weighted image in a 5-year-old girl with a high ARM and rectovaginal fistulae, who has incontinence after reconstructive surgery. A tethered spinal cord (long arrow) and a syringomyelia on T12-L4 (short arrow). The vertebral column terminated at L5-S1.
**Fig. 8.** Coronal FSE T2-weighted image in an 8-month-old boy with high ARM. Arrows indicate right hydronephrosis.

**Table 3.** Developmental quantization indexes for patients with SMC in different imaging

| Degree | Puborectalis | | External sphincter | |
|---|---|---|---|---|
| | *n* | Relative magnitude of width (PRWR) | *n* | Relative magnitude of width (EASWR) |
| Excellent (I) | 19 | 0.51±0.04 | 19 | 0.45±0.03 |
| Good (II) | 13 | 0.48±0.03 | 11 | 0.42±0.02 |
| Poor (III) | 7 | 0.15±0.03 | 9 | 0.13±0.02 |

I and II: $q$=1.641, $P$>0.05; I and III: $q$=26.247, $P$<0.01; II and III: $q$=21.191, $P$<0.01.

**Table 4.** Different types of ARM muscular developmental quantization indexes

| Classification | *n* | Relative magnitude of width | |
|---|---|---|---|
| | | Puborectalis | External sphincter |
| Normal | 15 | 0.51±0.02 | 0.45±0.02 |
| Low | 16 | 0.49±0.04 | 0.44±0.03 |
| Intermediate | 11 | 0.47±0.05 | 0.43±0.04 |
| High | 12 | 0.28±0.06* | 0.25±0.07* |

*: $P$<0.01.

**Table 5.** SMC developmental indexes and postoperative anorectal function

| Developmental indexes | *n* | Anorectal clinical score | | | Fecal incontinence | Constipation |
|---|---|---|---|---|---|---|
| | | Excellent | Good | Poor | | |
| PRWR <0.18 EASWR <0.15 | 7 | 0 | 2 (29%) | 5 (71%) | 5 | 0 |
| PRWR >0.18 EASWR >0.15 | 23 | 16 (70%) | 5 (21%) | 2 (9%) | 1 | 1 |

**Table 6.** SMC developmental indexes and postoperative objective examinations

| Developmental indexes | *n* | Manometry | | MRI | Barium enema examination | |
|---|---|---|---|---|---|---|
| | | Length of high-pressure (mm) | Contraction pressure difference (kPa) | Anorectal angulation (degree) | Spillage (*n*) | Retention (*n*) |
| PRWR <0.18 EASWR <0.15 | 7 | 10.88±3.64 | 0.85±0.20* | 126.0±15.3* | 5 | 0 |
| PRWR >0.18 EASWR >0.15 | 23 | 20.26±4.34 | 2.24±1.02* | 82.0±11.8* | 1 | 1 |

*: $P$<0.01.

$P$>0.05), with intermediate and normal ARM (PRWR $q$=1.344, $P$>0.05; EASWR $q$=1.787, $P$>0.05), and with normal and low ARM (PRWR $q$=0.495, $P$>0.05; EASWR $q$=1.493, $P$>0.05) (Table 4).

### Postoperative follow-up

Thirty patients with ARM were followed up postoperatively for 1-6 years. When PRWR was <0.18 and EASWR <0.15. clinical score (Clinical Score Criteria of Chinese Medical University, 1983) for 71% of the patients was less than 2 and they suffered from fecal incontinence. When PRWR was >0.18 and EASWR >0.15, clinical score for 70% of the patients was excellent, 21% good, and 9% poor in fecal continence (Table 5).

### Developmental index of the SMC, anorectal manometry and barium enema

Anorectal contraction pressure in 7 patients with poor development of the SMC (PRWR <0.18 and EASWR <0.15) was significantly lower ($q$=25.147, $P$<0.01). The length of the anal canal high-pressure region in patients with poor development of the SMC was shorter than that in patients with good or excellent development of the SMC ($q$=11.1617, $P$<0.01), and anorectal angle was more than 100° (Fig. 6), and barium spillage was found in 5 of the 7 patients. In 23 patients with excellent or good development of the SMC (PRWR >0.18 and EASWR >0.15), one patient had barium spillage and another one had barium retention, with an anorectal angle of less than 90° (Table 6).

### Associated anomalies

An additional advantage of MRI over other imaging modalities is to detect associated anomalies especially of the spinal cord, spine and urogenital system. In this study, associated anomalies of the spinal cord or the spine were found in 16 (41%) of the 39 patients, 14 cases of high or intermediate ARM and 2 of low ARM. In these patients, 7 demonstrated anomalies of sacrococcygeal vertebrae including the absence, agenesis, flat and reverse curvature of sacral vertebrae, and 9 anomalies of both sacrococcygeal vertebrae and sacral cord including wedge-shaped or blunted conus (2 patients), tethered spinal cord (3), syringomyelia (1), and myelocystocele and lipoma (3) (Figs. 2 and 7). Anomalies

of the genitourinary system were found in 8 of the 39 patients including unilateral renal hypoplasia (2 patients), renal ectopia (1), renal cyst (2), hydronephrosis (1) (Fig. 8), cystic agenesis (1), and uterovaginal agenesis (1). Two of these 8 patients demonstrated multiple associated anomalies of the genitourinary system.

## Discussion

Traditional X-ray film and retrograde contrast examination fail to directly display the relationship between the blind end of the rectum and the SMC. Ultrasonography can detect the distance between the blind end of the rectum and anal pit, but can not depict distinct image of the SMC.[2,3] CT can directly reveal the developmental state of the SMC, but is limited to the transverse plane and difficult to demonstrate atresia level and fistulae.[4-7] Endoanal MRI possesses a higher resolution on muscles, but the signal intensity would decrease rapidly in the region beyond 3 cm from the coil, making it impossible to display other anomalies in patients with ARM. Meanwhile, infants may feel uncomfortable to some extent using endoanal coil, which is not suitable for the inspection of infants and patients with ARM.[8,9] Phased-array MRI (head coil in babies) however can perspicuously display all anomalies of ARM.





### Application of MRI techniques

At present, no defined sequences or parameters on MRI are available for patients with ARM. Recent reports[4,9-12] have indicated that MRI of ARM can be done in spin-echo T1-weighted, fast spin-echo T2-weighted, and short T1 inversion recovery T1-weighted sequences. The sagittal plane includes the pelvis, lumbosacral vertebrae, and kidneys, whereas the axial and coronal planes are initially limited to the pelvis angulated parallelly and perpendicularly to the pelvic floor, respectively. When malformations of the spinal cord, spine, the high level of ARM, and/or kidneys are detected on the sagittal images, additional axial and/or coronal images in the region of interest were obtained. In this study, the level of ARM was depicted correctly in all patients with T1WI and T2WI. The detection rate for fistula was 50% for T1-weighted images, but the higher signal intensity of fistular mucosa on FSE T2-weighted sagittal and axial images could increase the detection rate, despite the difficulty in delineating the exact site of entrance in the genitourinary system. The fistulae were depicted correctly in all patients but one who had a minimal rectovesical fistula, giving a diagnostic rate of 97%. On muscular images, however, the sphincters were clearly demonstrated by routine T1WI and T2WI sequences. The anatomic characteristics of the SMC were depicted more affluently on T2-weighted images. Hence, if the sphincters cannot be demonstrated clearly with routine MRI in newborns or infants with the poor developmental state of the sphincters, short T1 inversion recovery T1-weighted sequences can be applied. As a result, fat signal is restrained and the shape and border of muscles can be detected more distinctly.

### Relationship between the blind end of the rectum and SMC

In the blind end of the rectum in newborns, meconium containing mixture of mucus and lipid has a higher intensity on both T1WI and T2WI sequences in sharp contrast to the surrounding tissues as a good contrast medium of MRI.[10,13] T2WI image is particularly helpful in the evaluation of low and intermediate ARM, because the high signal intensity of anorectal mucosa allows better delineation of the anorectum, SMC and perineum. The level of ARM can be defined as two classical planes, PC plane and I plane, in transverse sequences. It is a low level of ARM if the rectum can be seen from the two planes. If the rectum can be seen on PC plane but not on I plane, the level of ARM is intermediate. It is a high level of ARM if the rectum can not be seen on both planes. The position of the anal canal and its relationship with SMC can be detected correctly at the low level ARM patients, with the superficial transverse muscle of the perineum as a mark for defining the dorsal surface of the genitourinary diaphragm. Normally the anal canal is located behind this muscle. When it is at the level of or ventral to this muscle, the anal canal has an ectopic anterior location outside of the SMC. On the sagittal plane, it is high, intermediate, and low ARM if the atresia of the distal rectum is above, through, and below the SMC, respectively. On the coronal plane, the relationship between the levator ani muscle, the puborectalis, external anal sphincter, and anorectum can be demonstrated clearly. These findings suggest that the level of anal atresia can be depicted more accurately on sagittal and coronal planes.

### Muscular developmental state and developmental index

Our previous MRI study[14] showed that the absolute length and width of the puborectalis and external anal sphincter significantly increased with age, but

the relative length and width of the muscles were not correlated with age below 14 years. Therefore, this relative values might be taken as objective criteria for the measurement of the SMC in children during the development period. In this study, excellent or good developmental state of muscles was found in all patients with low ARM except one patient with intermediate ARM, and in some patients with high ARM. Furthermore, the width and length of the two muscles developed uniformly and were positively correlated ($r$=0.987, 0.995, $P$<0.01). Hence, the relative width of puborectalis and external anal sphincter measured on transverse planes was chosen as the developmental index of muscles. To describe the developmental state of muscles quantitatively, we divided MRI of the SMC into three degrees based on the demonstration of images: excellent development, good development, and poor development. The most significant values in the changing process were analyzed, and a 95% confidence interval was selected to demonstrate the developmental state or index of muscles. The diagnostic criteria for the poor developmental state of muscles were defined as PRWR <0.18 and EASWR <0.15. According to these criteria, the good developmental state of the two muscles was found in low and intermediate ARM, and the developmental index in patients with high ARM was significantly lower than that in the other two groups, which coincided with the literature report.[4,15,16] When PRWR was <0.18 and EASWR <0.15, 71% of the patients with ARM suffered from anal incontinence postoperatively. When PRWR was >0.18 and EASWR >0.15, 91% of the patients with ARM had good continence, and the poor continence in the remaining patients was mainly due to constipation. Thus, this developmental index can be taken as quantitative criterion for poor developmental state of puborectalis and external anal sphincter.

### Relationship between developmental index of muscles, objective anal function, and clinical score

In this study, anal function was examined in 30 patients of more than 5 years old. The length of high-pressure region of the anal canal was significantly shorter in the poor SMC group than in the good SMC group. The length of high-pressure region of the anal canal represents the functional distribution of the internal and external sphincters, which becomes shorter when the development of external sphincter is poor.

Anorectal contraction pressure in the poor SMC group was significantly lower than that in the good SMC group. The angle of the anal canal represents the status of the puborectalis. The angle was found more than 100º in 5 of 7 patients with poor muscular development by barium spillage, and less than 90º in patients with good muscular development. These findings suggested that the developmental index of muscles is consistent with the objective examination results of anal function.

### MRI of associated anomalies

The occurrence of severe deformities of the genitourinary system is associated with a high level of anal atresia.[17-21] In our study, the deformities were found in 13 (33%) of 39 patients with anorectal malformation, including 10 (26%) patients with high or intermediate ARM, and 3 (7%) patients with low ARM. MRI was shown to be able to delineate the different aspects of cloacal malformation, including the uterus, vagina, maldeveloped puborectalis, and external anal sphincter. We consider the anomalies of the lumbosacral vertebrae and spinal cord are related to the level of ARM. Although the anomalies are not surgically correctable, early detection is required for a better prognosis and management of patients. Besides, MRI is also able to accurately detect all different aspects of the Currarino triad including three correlative process of congenital anorectal malformation, sacral agenesis, and presacral mass.[22-24] In this study, one patient was detected this way and subsequently subjected to posterior sagittal anorectoplasty, showing a pathologically confirmed presacral mass, a teratoma. The anal function of this patient was good during a postoperative follow-up. Hence, MRI should be given preoperatively to optimize the therapeutic regimen.

### Clinical significance

MRI with a body-phased array coil or head coil is a non-invasive diagnostic method for different aspects of ARM including associated anomalies. The objective criteria for maldevelopmental width of the puborectalis and external anal sphincters found in this study provide a direct imaging reference to help pediatric surgeons in planning operative strategies and predicting long-term prognosis. It is our opinion that MRI should be performed in all children with ARM.

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**Competing interest:** None declared.
**Contributors:** TST proposed the study and wrote the first draft. All authors contributed to the intellectual content and approved

the final version. RQL is the guarantor.

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