Hirschsprung's disease: early diagnosis and long-term outcomes

Meinds, Rob Jelle

CHAPTER 1

General introduction and aims

Hirschsprung's disease (HD) is a congenital defect of the intestines and is named after its discoverer, Harald Hirschsprung. Hirschsprung's report dates from 1887 and describes the medical cases of two infants who had died from constipation in association with extreme dilatation and hypertrophy of the colon.[1] At the time, Hirschsprung mistakenly believed that it was the dilated segment of the colon that was pathological. In 1949, more than half a century later, Swenson and colleagues came to the conclusion that not the dilated part of the colon was pathological, but the more distal, narrow part of the colon (Figure 1).[2] They reasoned that the extreme dilatation of the colon was caused by an inborn obstruction at the distal end of the intestines that was blocking the passage of feces. Backed by this theory, Swenson and Bill proceeded to remove the obstructing segment of the intestines and achieved surprisingly good results.[3] At around the same time as Swenson and colleagues were undertaking their surgical experiments, the underlying cause of HD was discovered by Whitehouse and Kernohan by comparing colon specimens of HD patients with colon specimens of non-HD controls.[4] In the former, they discovered a complete absence of ganglion cells in both plexuses of the enteric nervous system, as well as a profound hypertrophy of nerve bundles. These two discoveries revolutionized the diagnosis and treatment of HD and greatly improved the morbidity and mortality of patients who suffer from this previously fatal disease. Shortly after Swenson and Bill reported on their surgical technique, other techniques were developed, including those of Rehbein, Duhamel, and Soave.[5–7]

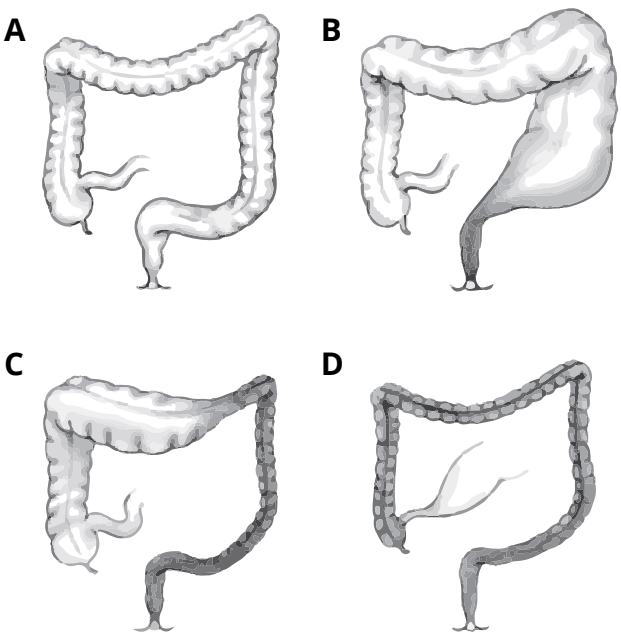

Figure 1
Different types of Hirschsprung's disease based on the length of the aganglionic intestines.
A: Normal bowel
B: Short-segment variant (up to the sigmoid)
C: Long-segment variant
D: Total colonic aganglionosis

Even today new discoveries are being made that help us to better understand the pathophysiology and improve the treatment of HD. An overview of these discoveries is given in this chapter, followed by the content and aims of this thesis.

ETIOLOGY

The main feature of HD is the absence of ganglion cells in the distal intestines. This was first discovered by Whitehouse and Kernohan in 1948.[4] The absence of ganglion cells, known as aganglionosis, is thought to be the result of faulty migration of neural crest cells during the embryonic development of the enteric nervous system.[8] In normal fetal development the neural crest cells migrate in a cranial-to-caudal direction between the 4th and 7th week of gestation, starting out from the esophagus and ending in the anal canal.[9] Currently, two theories exist that explain why these neural crest cells fail to reach the distal intestines. The first theory proposes that the cells mature or differentiate into ganglion cells too early during their migration.[10] The second theory proposes that they do reach their destination, but fail to differentiate, proliferate, or survive.[11,12] No hard evidence is available to confirm or refute either theory. The most likely explanation is that faulty migration is the result of a combination of factors, which can differ between individual patients.[13] In between the aganglionic and ganglionic intestines is a segment known as the transition zone. This zone contains a reduced number of ganglion cells and marks the transition between the healthy and HD-affected intestines. It is considered dysfunctional, similar to the aganglionic segment, because of its reduced number of ganglion cells and decreased peristalsis.

PATHOPHYSIOLOGY

In HD the affected intestines are characterized by a constant increased tonus of the smooth bowel muscles that blocks the passage of stool. In healthy intestines, smooth muscles cells are innervated by sympathic (inhibitory) neurons and parasympathic (excitatory) neurons. Jointly, these neurons are responsible for the motility of the gut in conjunction with the complex architecture of the enteric nervous system (Figure 2). The most important neurotransmitter responsible for inhibition is nitric oxide (NO). It mediates relaxation of smooth muscle cells along with other inhibitors, such as vasoactive intestinal polypeptide, and carbon monoxide.[14] Additionally, excitatory neurons produce neurotransmitters that mediate the contraction of smooth muscle cells, most importantly, acetylcholine (ACh).[14]

While HD is characterized by the absence of intrinsic ganglion cells in both plexuses, there are still nerve fibers that innervate the smooth muscle cells of the affected intestines (Figure 2). The exact origin of these nerve fibers is unknown. It is thought that they have an extrinsic origin, such as the pelvic nerve plexus, and proliferate into the bowel wall because of their failure to connect with the absent, intrinsic ganglion cells of the enteric nervous system.[15] For unknown reasons, the release by these nerve fibers of inhibitory neurotransmitters, such as NO, is decreased, while there is an increased release of excitatory neurotransmitters, such as ACh.[16,17] The increased excitation of the smooth muscle fibers by ACh and the absence of inhibition by NO, are thought to be responsible for the constant increased tonus of the intestines and the lack of propagation of peristaltic waves in HD.[18]

Another consequence of enteric nervous system abnormalities in patients with HD is the absence of the rectoanal inhibitory reflex.[19] In healthy bowels this reflex is responsible for the relaxation of the internal anal sphincter upon rectal distension and stimulation (Figure 3A). Relaxation of the internal anal sphincter is vital for the smooth passage of stool.

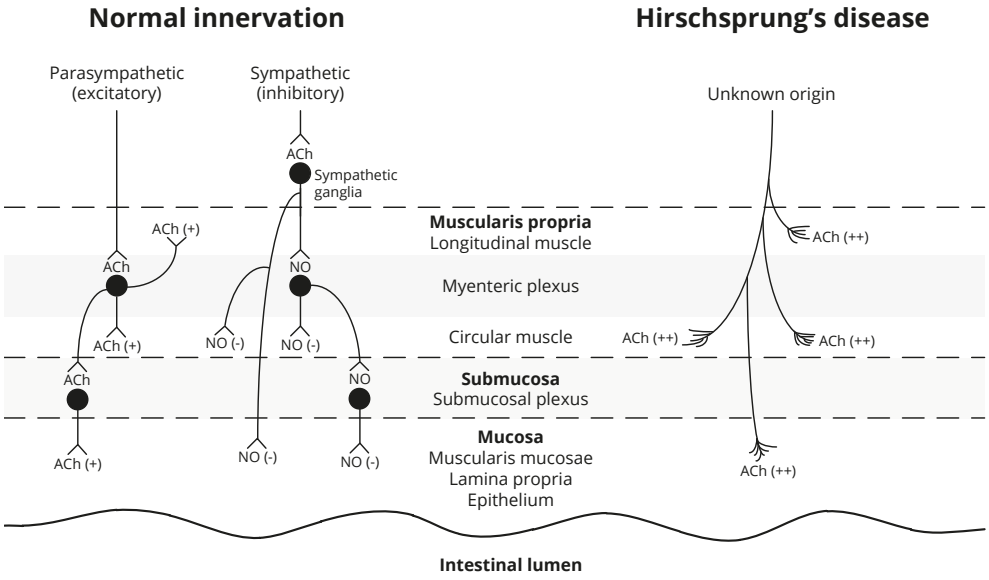

Figure 2

A schematic illustration of the layers of the intestinal wall with normal innervation (left) and innervation in case of Hirschsprung's disease (right). In normal innervation excitatory neurons and inhibitory neurons are balanced and coordinated by ganglion cells (●) of the enteric nervous system. In Hirschsprung's disease ganglion cells are absent in both plexuses, while a proliferation of extrinsic nerve fibers of unknown origin is present. The increased release of acetylcholine (ACh) by these fibers, in combination with the absence of inhibition by nitric oxide (NO), is thought to be responsible for the constant increased tonus of the intestines seen in Hirschsprung's disease.

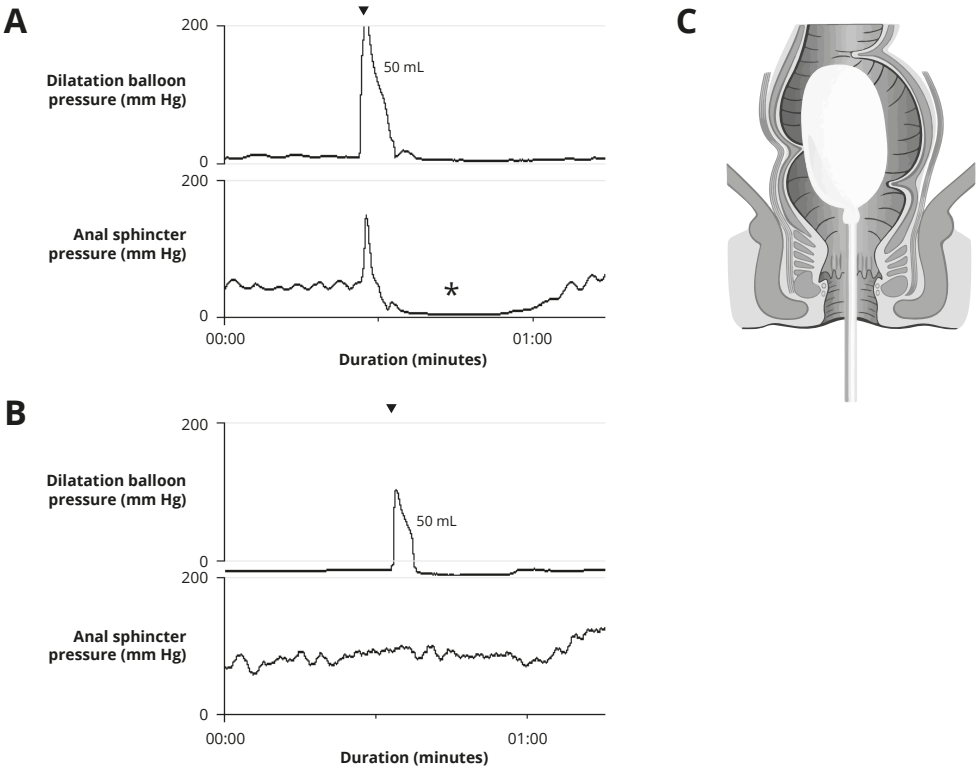

Figure 3

Anorectal manometry measurements.

A: Measurement in a healthy control showing a rectoanal inhibitory reflex (asterisk) following rectal dilatation (arrowhead).

B: Measurement in a patient with Hirschsprung's disease showing no response in anal sphincter pressure following rectal dilatation.

C: Illustration of the anorectal manometry catheter with a dilatation balloon at the tip of the catheter. The catheter is equipped with a pressure sensor at its tip to measure the dilation balloon pressure, as well as multiple pressure sensors at the level of the anal canal to measure changes in sphincter pressure.

Consequently, the absence of this reflex in HD contributes to the constipation complaints experienced by HD patients (Figure 3B). The absence of the rectoanal inhibitory reflex in HD was first reported by Schnaufer and collegues[20] and Lawson and Nixon[21] in 1967, and was later revealed to be caused by a lack of NO-producing inhibitory neurons.[22]

CLASSIFICATION

The classification of HD depends on the length of intestinal aganglionosis, which in turn depends on the developmental stage at which the migration of neural crest cells fails. If migration fails in an early stage of embryonic development, the aganglionic segment may be as long as the complete colon and part of the small intestines. If it fails at a later stage, the aganglionic segment may be limited to the anal canal and/or to the anal sphincter alone. The following types of HD are distinguished: the short-segment variant limited to the rectum and sigmoid (roughly 80% of the patients), the long-segment variant up to the splenic flexure or transverse colon (15%) and lastly, the total colonic variant (5%) (Figure 1).

In addition, there is a rare variant of HD, known as internal anal sphincter achalasia, previously ultrashort HD, in which aganglionosis is limited to the anal sphincter. This variant is characterized by normally innervated intestines but an absent rectoanal inhibitory reflex. Preferably this variant is treated conservatively with laxatives.[23] Lastly, the most extreme and rare variant of HD is total intestinal aganglionosis. Patients suffering from this type of HD have a very poor prognosis and the mortality rate is high.[24]

CLINICAL PRESENTATION

HD is a relatively rare cause of constipation and occurs in an estimated 1 to 2 cases per 10.000 live births.[25–27] Boys are affected more often than girls, especially in case of the shorter variant of HD in which the male-to-female ratio is 3:1.[25,28]

In the majority of the patients, HD presents shortly after birth with a failure to pass meconium during the first 24 to 48 hours. Nowadays, on account of this early presentation and overall increased awareness of the disease, HD is diagnosed in the neonatal period in 91% of the patients.[29] While a failure to pass meconium during the first 48 hours is not uncommon in otherwise healthy newborns, other symptoms such as a distended abdomen, feeding intolerance, and bilious vomiting often contribute to raising the suspicion of HD. Despite increasingly earlier diagnoses, still approximately 5% of patients with HD are diagnosed after the first year of life.[25] Especially patients with a shorter segment of aganglionosis, who suffer less severe symptoms of constipation,

are at higher risk of being diagnosed at a later age.[30] Patients in whom the disease has gone unnoticed present with symptoms such as chronic constipation with intermittent episodes of diarrhea, acute enterocolitis, or a sigmoid volvulus.[31–33]

Several other diagnoses may be associated with a delayed meconium passage and therefore mimic the symptoms of constipation seen in HD. Important diagnoses that should be considered in the differential diagnosis are: meconium ileus caused by cystic fibrosis, intestinal atresia, malrotation, anorectal malformation, and small left colon syndrome (associated with maternal diabetes). Additionally, several systemic disorders should be kept in mind as they could also be responsible for constipation, for example: electrolyte disorders, hypothyroidism, or constipation caused by maternal medication or drug use.

While most cases of HD seem to occur sporadically and isolated, it is estimated that 10% to 20% of the cases present with associated congenital anomalies, predominantly in the gastrointestinal tract, cardiovascular system, and urinary tract.[25,26,34] Chromosomal anomalies are also often seen in patients with HD. Especially the connection with Down syndrome seems significant, as Down syndrome accounts for 94% of all chromosomal anomalies in HD and has an incidence of 6% to 9% in the HD patient population.[25,26,34]

DIAGNOSTIC INVESTIGATIONS

While clinical presentation only suggests HD, the final diagnosis must be confirmed by the outcomes of rectal suction biopsy, anorectal manometry, and/or contrast enema. These three tests have been shown to have similar sensitivity and specificity, with the rectal suction biopsy considered to be the gold standard.[35,36]

Rectal suction biopsy

The rectal suction biopsy procedure entails extracting rectal tissue consisting of mucosal and submucosal material with a rectal suction biopsy tube. Generally, the procedure can be carried out without sedation or anesthesia. Rectal tissue is extracted at multiple levels above the anal verge and sent to a pathology laboratory for histologic examination. The tissue is examined for the presence of intrinsic ganglion cells and the proliferation of extrinsic nerve fibers. Absence of ganglion cells combined with proliferation of nerve fibers is compatible with HD. Rectal suction biopsies should be taken at least 2 cm from the edge of the pectinate line, as the first 1 to 2 cm physiologically have a reduced number of ganglion cells.[37] Furthermore, it is important to critically define the quality of the biopsy, as the extracted tissue should consist of sufficient submucosa for the appraisal of intrinsic ganglion cells.

Over the years various staining techniques have been introduced to analyze the tissue for intrinsic absence of ganglion cells and extrinsic proliferation of nerve fibers. The variety of staining options implies that no uniform approach to the analysis of rectal suction biopsies exists and, as a consequence, the approaches at different institutions vary. One approach is to only assess tissue on the presence of ganglion cells by staining with hematoxylin and eosin (H&E). Historically this proved effective but requires complete dedication and much time of the responsible pathologist, because many sections have to be inspected before a reliable diagnosis can be made. Another approach is to use more advanced staining techniques to make diagnosing quicker and easier. One of these staining techniques is acetylcholinesterase (AChE) histochemistry that was introduced by Meier-Ruge in 1972.[38] This technique can be used to judge the proliferation of extrinsic nerve fibers in HD that are typically rich in ACh and AChE (the enzyme which catalyzes the breakdown of ACh). AChE histochemistry increases the specificity of the rectal suction biopsy by reducing the number of false positive outcomes.[36] Despite making the diagnosis of HD quicker and easier, AChE histochemistry is generally considered to be a more sophisticated staining technique making it less suitable perhaps for institutions without high-end laboratory equipment. Besides, it has been shown that this attaining technique is difficult to interpret in neonates, which possibly leads to a higher false negative rate at these ages.[39,40] Therefore, newer staining techniques have been introduced, such as calretinin immunohistochemistry, which are believed to further increase the diagnostic accuracy of the rectal suction biopsy.[41] While there is increasing advocacy for calretinin immunohistochemistry,[42–44] a recent analysis pointed out that this technique might be associated with higher risks of false positive diagnoses, leading to unnecessary surgical intervention.[45] These new staining techniques might not completely replace AChE, but certainly constitute an important addition to the routine repertoire of stains used in the diagnosis of HD.

While the rectal suction biopsy is considered safe and reliable a small risk of complications, such as persistent rectal bleeding, remains.[46,47]

Anorectal manometry

Anorectal manometry can be used to examine anorectal physiology, including the presence of the rectoanal inhibitory reflex. As previously explained, absence of this reflex is a distinguishing feature of HD (Figure 3A).[19–21] The anorectal manometry procedure consists of inserting a catheter, equipped with pressure sensors and a small dilatation balloon at its tip, into the anal canal of the patient. The balloon is placed in the rectum and inflated slightly to simulate stool and to stimulate the rectal wall. As the rectal balloon is inflated the pressure sensors at the level of the anal canal should measure a decrease in

internal anal sphincter pressure, also known as the rectoanal inhibitory reflex (Figure 3B).

Several studies have demonstrated the value of anorectal manometry as a screening tool for HD, especially on account of its being non-invasive and having little to no risks.[48–51] It has been disputed, however, that it is generally more difficult to interpret in newborns, which in turn increases the risk of false negative and false positive test results.[36,48] As a consequence, few pediatric surgeons still use anorectal manometry for the purpose of diagnosing HD, while the majority opts for rectal suction biopsies as the diagnostic procedure of first choice.[52,53] Recent technological advances, however, such as the introduction of new catheters and high-resolution anorectal manometry, have increased diagnostic accuracy.[54] Anorectal manometry could therefore still be a valuable screening tool for HD, especially because it is non-invasive and its use could serve to reduce the number of invasive rectal suction biopsies.

Contrast enema

The last technique used in the diagnosis of HD is the contrast enema. This technique entails injecting a barium enema followed by an abdominal X-ray. A contrast enema carried out in a HD patient typically shows a contracted distal colon, a transition zone, and a distended colon in the caudal direction due to obstruction. Unfortunately, this characteristic image is not seen in all HD patients. For example, a contrast enema taken in a patient with a total colonic or an ultrashort variant of HD does not show the transition zone and the difference in intestinal caliber, which would lead to a false negative test result. The contrast enema has therefore lost its popularity as a diagnostic technique for HD, as its accuracy was shown to be inferior to anorectal manometry and rectal suction biopsy.[35,36] Accuracy also greatly depends on the expertise of the radiologist. For example, forceful injection of the contrast will distend the bowel and diminish accuracy of interpretation. It is therefore not uncommon to record a 24-hour delayed radiograph, which negates this effect. Indeed, Wong and colleagues found that a delayed radiograph can be useful to rule out HD.[55] Nevertheless, they suggested that it remains necessary to carry out a rectal suction biopsy to either exclude or confirm the diagnosis of HD. It therefore remains questionable whether the outcome of a 24-hour delayed radiograph following contrast enema actually has any clinical implications.

The contrast enema, despite its flaws as a diagnostic procedure, still remains the only investigation that can be used to evaluate the extent of aganglionosis and helps preoperative planning. A recent publication by Muller and colleagues, however, showed that the correlation of the radiographic transition zone with the level of aganglionosis remains low.[56] Their conclusion was that a biopsy remains mandatory to define the transition zone.

SURGICAL TREATMENT

A surgical reconstruction is usually performed to treat HD after the diagnosis is established. Reconstruction consists of removing the majority of the aganglionic intestines in order to restore bowel functionality. Nowadays, there are two major kinds of surgical strategies: the abdominal approach and the transanal approach.

The abdominal approach consists of surgical techniques such as the Swenson, Rehbein, Duhamel, and Soave procedures (Figure 4).[3,5–7] Most of these procedures have undergone alterations and modifications over the years, including the addition of laparoscopy.[57,58] In 1948, Swenson introduced a technique to resect aganglionic intestines.3 His procedure consisted of mobilizing and resecting the complete aganglionic intestines followed by an end-to-end anastomosis of normal colon to the anal canal. Many surgeons, however, faced postoperative problems such as pelvic nerve damage as a consequence of this surgical procedure. Hence, other techniques were introduced, such as the one described by Rehbein.[5] Rehbein's procedure avoided the pelvic nerves by only resecting the upper aganglionic colon. The remaining aganglionic rectum and anal canal were dilated afterwards. This too was not entirely satisfactory and in turn led to newer techniques such as the ones described by Duhamel6 and Soave.[7] Duhamel opted for a retrorectal

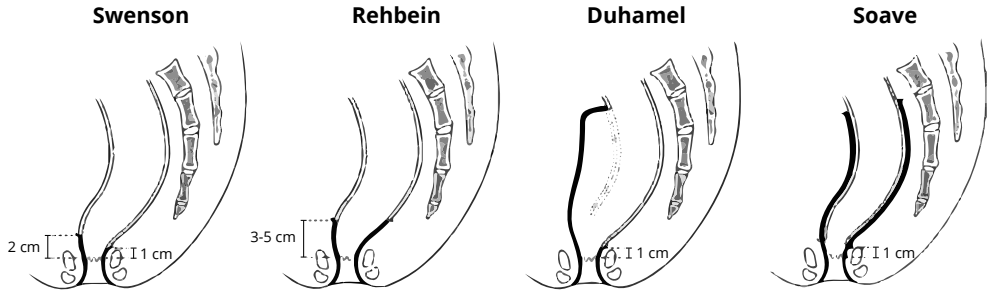

Figure 4

A schematic illustration of four of the most common surgical procedures that use an abdominal approach. Swenson's procedure consists of mobilizing and resecting the complete aganglionic intestines relatively close to the dentate line, followed by an end-to-end anastomosis of healthy colon to the anal canal. Rehbein's procedure consists of resecting the upper aganglionic colon, leaving 3 to 5 cm of distal aganglionic colon in situ, which is usually dilated afterwards. Duhamel's procedure consists of a retrorectal approach followed by a side-to-side anastomosis of healthy colon to the posterior of the aganglionated rectum. Finally, Soave's procedure consists of dissecting a rectal mucosal tube off the submucosal plane after which the ganglionic colon is pulled through the rectal sleeve.

Adapted from Figure 44-1 in the chapter on Hirschsprung Disease. In: Ziegler MM et al, editors. Operative Pediatric Surgery. New York, NY: McGraw-Hill Education; 2014.

approach followed by a side-to-side anastomosis of ganglionic colon to aganglionated rectum thereby completely avoiding the pelvic nerves anterior of the rectum. Soave's solution to avoid damaging the innervation of the pelvic floor was to devise an endorectal pull-through procedure whereby a rectal mucosal tube was dissected off the submucosal plane.

The transanal approach that has gained in popularity over the last few years is the transanal endorectal pull-through (TERPT) described by De la Torre-Mondragón and Ortega in 1998.[59] The TERPT procedure consists of a transanal pull-through of ganglionic intestines followed by a very low, direct anastomosis just above the dentate line (Figure 5).[59] The latter can be done by using a short aganglionic muscular cuff created by a transanal submucosal dissection (Soave-like)[59,60] or by a full-thickness dissection of the bowel wall (Swenson-like).[61] By avoiding extensive manipulation in the peritoneal cavity, this approach is thought to reduce the risk of postoperative adhesions. At the same time damage to the pelvic floor innervation is prevented by avoiding extensive pelvic dissection outside the rectum. Short-term outcomes of this technique seem favorable.

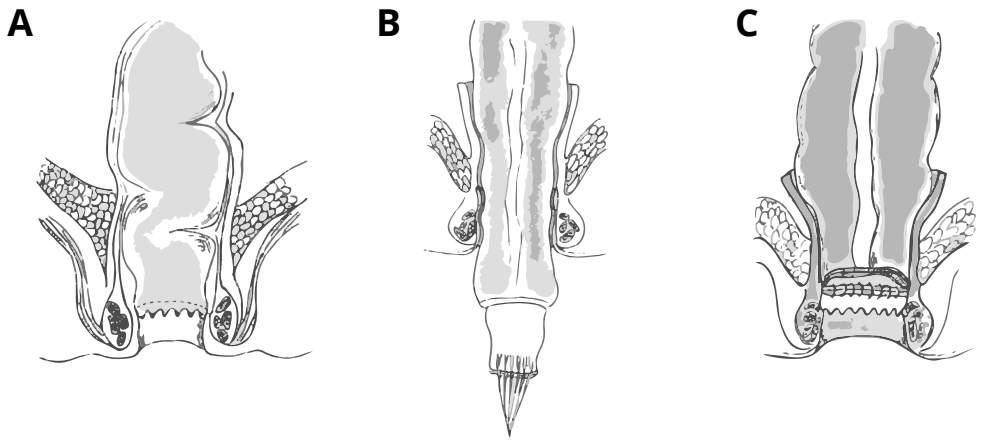

Figure 5
A schematic representation of the transanal endorectal pull-through (TERPT) procedure.
A: The location of the intended transanal circumferential incision, approximately 5 mm above the dentate line, marked by the dotted line.
B: An endorectal dissection is made following the submucosal plane of the rectum until the level of the peritoneal cavity is reached. Next, the division of the muscular rectal wall is continued circumferentially, freeing the intra-abdominal colon from the muscle sleeve. The colon is then pulled through the anus.
C: The pulled-through aganglionic colon is resected and an anastomosis of the healthy colon and the anus is made.
Adapted from Figures 2, 4, and 5 in the article Hirschsprung disease. Haricharan RN, Georgeson KE. Semin Pediatr Surg. 2008 Nov;17(4):266-75.

Some studies reported outcomes comparable to other techniques and other studies even reported better results.[60,62–64] Nevertheless, concerns remain, such as that during the TERPT procedure the anal sphincter may be damaged by overstretching.[65] Despite this concern, a manometric study performed by van Leeuwen and colleagues in 2002, found no differences regarding sphincter functioning between abdominal and transanal approaches.[66] The authors subsequently concluded that the transanal approach did not pose an increased risk of sphincter damage. A more recent study by Stensrud and colleagues, however, showed that sphincter damage and incontinence are in fact seen more often following the anal approach in comparison to the abdominal approach.[67] It is important to note, however, that for this technique extensive long-term clinical results are not yet available. It remains to be seen what the impact of the different surgical approaches is on long-term anorectal functioning and fecal continence.

LONG-TERM OUTCOMES

Despite the best surgical efforts, studies often emphasize that HD is an incurable disease. This is illustrated by various reports reporting that after surgical reconstruction, a large group of patients continue to suffer from defecation disorders, such as constipation and fecal incontinence.[68–72] To date, it is not clear why some patients experience more complaints than others. What is clear, is that these disorders may have far reaching consequences, because both constipation and fecal incontinence are known to negatively influence the quality of life.[73,74]

Constipation

Constipation is the chief complaint of HD patients. Often the complaints are so severe that surgical reconstruction of the affected intestines is required to restore bowel continuity. Without this intervention, intestinal obstruction could eventually lead to abdominal distension, Hirschsprung's disease-associated enterocolitis, growth failure, and in severe cases, mortality. Even after surgical reconstruction, however, the majority of HD patients retain a lifelong tendency towards constipation.

The tendency towards constipation may have several causes. First, and most importantly, patients with HD will never develop a functional rectoanal inhibitory reflex.[19–21] This reflex and subsequent relaxation of the internal anal sphincter are vital for the smooth passage of stool. Second, incomplete resection may result in residual aganglionic intestines that could continue to hinder the passage of stool. Last, constipation is a common complaint in the general population with an estimated prevalence of 16%.[75] After excluding secondary causes for constipation, the majority of these complaints can

be explained either as a functional defecation disorder (dyssynergic defecation), slow-transit constipation, or irritable bowel syndrome.[76] On account of the high prevalence of these disorders in the general population, it is likely that these disorders may also play a role in the constipation complaints of HD patients. Further research is necessary to determine to what extent other causes of constipation play a role in the complaints of patients with HD.

Fecal incontinence

Fecal incontinence is a frequent complaint of patients with HD, particularly after surgical reconstruction. The prevalence of fecal incontinence, mostly limited to soiling, in the general population is estimated at approximately 8%,[77] whereas it may be as high as 40% in patients with HD.[78,79] It has been postulated that the fecal incontinence complaints of HD patients may be a consequence of damage to the anal sphincter or innervation of the pelvic floor during surgery, or from a reduced rectal reservoir as a result of surgical reconstruction.[80] There are several known risk factors for poor fecal continence in HD patients, such as total colonic aganglionosis and the combination of HD with Down syndrome.[68,81] Another potential risk factor for fecal incontinence may be constipation in association with fecal incontinence, a phenomenon often seen in pediatric and geriatric populations.[82] Further research on this subject is needed because as the cause for fecal incontinence in the majority of HD patients remains unclear.

Quality of life

Quality of life plays an increasingly important aspect in the assessment of long-term outcomes, especially in chronic illnesses such as HD. Quality of life is a broad concept, subjective by definition. It is often subdivided into various domains, often including the physical, psychosocial, and social domains, as well as environment, level of independence, and spirituality. Defecation disorders, such as constipation and fecal incontinence, are known to influence the quality of life.[73,74] The prevalence of these disorders in HD patients is relatively high[68–72] and one may assume that it negatively influences their quality of life. This line of thought has prompted various studies on the long-term functional outcomes and quality of life in HD patients.[70,72,83,84] Unfortunately, it is still unclear how these complaints and their influence on quality of life develop with aging.[83] Additional research is therefore needed to determine how the influence of defecation disorders on quality of life varies in different age groups.

AIMS OF THIS THESIS

Both the diagnosis and treatment of HD have improved vastly over the last few decades. Nevertheless, diagnosing HD remains troublesome, especially in very young infants. Recent studies have shown that the rectal suction biopsy is not entirely satisfactory and that caution is required when interpreting the outcome. Although surgical techniques are being perfected and outcomes are improving, proper follow-up studies are necessary to assess the differences between the various techniques in terms of long-term functional outcomes and quality of life.

From this follows the twofold aim of this thesis. First, to improve the diagnostic process of determining HD with the aim to increase accuracy and to reduce the number of invasive biopsy procedures. Second, to perform long-term follow-up studies of HD patients to assess their functional outcomes and quality of life.

The first part of this thesis focusses on the diagnostic process of determining HD, starting with a study on the accuracy of rectal suction biopsies in **Chapter 2**. For this study we investigated, in retrospect, all rectal suction biopsies performed at University Medical Center Groningen between 1975 and 2011, and analyzed at what age rectal suction biopsies gave an accurate diagnosis. On the basis of this study we hypothesized that anorectal manometry could be both a viable and safe screening tool for HD, and that it could be used to reduce the number of invasive rectal suction biopsy procedures in the diagnosis of HD. Prospectively, we gathered the results of 105 patients suspected of HD who had undergone anorectal manometry. The results of this study are presented and discussed in **Chapter 3**. In our study on anorectal manometry we found that even in patients with normally developed ganglion cells, that is patients in whom HD was excluded on the basis of rectal suction biopsy, the rectoanal inhibitory reflex could be absent at birth. We hypothesized that the absence of this reflex might play a role in the constipation complaints experienced by these patients. In addition, we hypothesized that this reflex might mature and develop after birth. The development of this reflex in newborns and its role with regards to constipation complaints are discussed in **Chapter 4**. In **Chapter 5**, the last chapter in this part of the thesis, we describe two extraordinary cases of HD. In these two patients the disease had gone unnoticed until adolescence, when they both presented with a solitary rectal ulcer.

The second aim of this thesis is to assess the long-term functional outcomes of HD patients. Traditionally, the main outcome parameters in the treatment of HD are constipation and fecal incontinence, that is, the inability to evacuate and retain stool. Unfortunately, the questionnaires currently available for assessing these complaints are often limited in the number of items and focus on quality of life rather than on factors

that influence anorectal functioning.[85–90] The second part of this thesis is therefore dedicated to the detailed questionnaire we developed to assess anorectal functioning. The contents, applicability, and validity of the questionnaire are explained in **Chapter 6**. To obtain reference data for our study on HD patients, we performed an extensive survey of the Dutch population, the analysis of which is presented in **Chapter 7**.

The third and last part of this thesis focuses on the long-term outcomes of HD patients. Based on our clinical observations we hypothesized that a significant number of HD patients who reach adulthood continue to experience functional complaints such as constipation and fecal incontinence. To test this hypothesis we performed a study together with all six pediatric surgery institutes in the Netherlands. The resulting nation-wide, cross-sectional study consisted of investigating the medical records of all known HD patients and inviting eligible patients to complete our newly developed questionnaire on anorectal functioning and a questionnaire on quality of life. The results of this study are discussed in **Chapters 8 and 9**. In **Chapter 8** we analyze the results of the anorectal functioning and quality of life questionnaires, with a subanalysis to determine factors associated with poor outcomes, and an analysis on the influence of poor outcomes on quality of life. In **Chapter 9** we use a subgroup of patients collected from the nation-wide study to perform a matched comparison of patients treated with the Duhamel procedure and the TERPT procedure. In the final chapter of this section, **Chapter 10**, we report on a study in which we show that dyssynergic defecation can play an important role in the postoperative constipation complaints of HD patients. We hypothesized that not all postoperative defecation complaints were attributable to HD and that dyssynergic defecation – for which viable treatment options are available – may increase the severity of the constipation in these patients.

Finally, we discuss the main findings of this thesis in a general discussion in **Chapter 11**, thereby reflecting on the hypotheses laid down at the beginning of the thesis. We also discuss the implications of this work and directions for future research. A summary of the main findings and conclusions is given in **Chapters 12 and 13**, in English and Dutch respectively.

REFERENCES

1 Hirschsprung H. Stuhlträgheit Neugeborener in Folge von Dilatation und Hypertrophie des Colons. Jahrb für Kinderheilkd und Phys Erziehung. 1887;27:1–7.

2 Swenson O, Neuhauser EB, Pickett LK. New concepts of the etiology, diagnosis and treatment of congenital megacolon (Hirschsprung's disease). Pediatrics. 1949;4:201–9.

3 Swenson O, Bill AH. Resection of rectum and rectosigmoid with preservation of the sphincter for benign spastic lesions producing megacolon; an experimental study. Surgery. 1948;24:212–20.

4 Whitehouse FR, Kernohan JW. Myenteric plexus in congenital megacolon; study of 11 cases. Arch Intern Med (Chicago, Ill 1908). 1948;82:75–111.

5 Rehbein F. Operative Behandlung der Hirschsprungschen Krankheit. Langenbecks Arch fur Klin Chir. 1953;276:540–3.

6 Duhamel B. A new operation for the treatment of Hirschsprung's disease. Arch Dis Child. 1960;35:38–9.

7 Soave F. Une nouvelle technique chirurgicale pour le traitment de la maladie de Hirschsprung. J Chir (Paris). 1963;86:451–64.

8 Goldstein AM, Hofstra RMW, Burns AJ. Building a brain in the gut: development of the enteric nervous system. Clin Genet. 2013;83:307–16.

9 Wallace AS, Burns AJ. Development of the enteric nervous system, smooth muscle and interstitial cells of Cajal in the human gastrointestinal tract. Cell Tissue Res. 2005;319:367–82.

10 Webster W. Embryogenesis of the enteric ganglia in normal mice and in mice that develop congenital aganglionic megacolon. J Embryol Exp Morphol. 1973;30:573–85.

11 Hoehner JC, Wester T, Pahlman S, et al. Alterations in neurotrophin and neurotrophin-receptor localization in Hirschsprung's disease. J Pediatr Surg. 1996;31:1524–9.

12 Langer JC, Betti PA, Blennerhassett MG. Smooth muscle from aganglionic bowel in Hirschsprung's disease impairs neuronal development in vitro. Cell Tissue Res. 1994;276:181–6.

13 Langer JC. Hirschsprung disease. Curr Opin Pediatr. 2013;25:368–74.

14 Furness JB. Types of neurons in the enteric nervous system. J Auton Nerv Syst. 2000;81:87–96.

15 Watanabe Y, Ito F, Ando H, et al. Extrinsic nerve strands in the aganglionic segment of Hirschsprung's disease. J Pediatr Surg. 1998;33:1233–7.

16 Frigo GM, Tacca M Del, Lecchini S, et al. Some observations on the intrinsic nervous mechanism in Hirschsprung's disease. Gut. 1973;14:35–40.

17 O'Kelly TJ, Davies JR, Tam PK, et al. Abnormalities of nitric-oxide-producing neurons in Hirschsprung's disease: morphology and implications. J Pediatr Surg. 1994;29:294–9.

18 Vizi ES, Zseli J, Kontor E, et al. Characteristics of cholinergic neuroeffector transmission of ganglionic and aganglionic colon in Hirschsprung's disease. Gut. 1990;31:1046–50.

19 Scharli AF. Pathophysiology of Classical Hirschsprung's disease. In: Holschneider AM, Puri P, editors. Hirschsprung's Disease and Allied Disorders. Frankfurt, Germany: Springer; 2000. p. 109–25.

20 Schnaufer L, Talbert JL, Haller JA, et al. Differential sphincteric studies in the diagnosis of ano-rectal disorders of childhood. J Pediatr Surg. 1967;2:538–43.

21 Lawson JO, Nixon HH. Anal canal pressures in the diagnosis of Hirschsprung's disease. J Pediatr Surg. 1967;2:544–52.
22 Rattan SS. The internal anal sphincter: regulation of smooth muscle tone and relaxation. Neurogastroenterol Motil. 2005;17 Suppl 1:50–9.
23 Puri P, Gosemann JH. Variants of Hirschsprung disease. Semin Pediatr Surg. 2012;21:310–8.
24 Ruttenstock E, Puri P. A meta-analysis of clinical outcome in patients with total intestinal aganglionosis. Pediatr Surg Int. 2009;25:833–9.
25 Suita S, Taguchi T, Ieiri S, et al. Hirschsprung's disease in Japan: analysis of 3852 patients based on a nationwide survey in 30 years. J Pediatr Surg. 2005;40:197–201.
26 Best KE, Addor MC, Arriola L, et al. Hirschsprung's disease prevalence in Europe: a register based study. Birth defects Res A, Clin Mol Teratol. 2014;100:695–702.
27 Bradnock TJ, Knight M, Kenny S, et al. Hirschsprung's disease in the UK and Ireland: incidence and anomalies. Arch Dis Child. 2017;102:722-7.
28 Ryan ET, Ecker JL, Christakis NA, et al. Hirschsprung's disease: associated abnormalities and demography. J Pediatr Surg. 1992;27:76–81.
29 Singh SJ, Croaker GD, Manglick P, et al. Hirschsprung's disease: the Australian Paediatric Surveillance Unit's experience. Pediatr Surg Int. 2003;19:247–50.
30 Stensrud KJ, Emblem R, Bjornland K. Late diagnosis of Hirschsprung disease--patient characteristics and results. J Pediatr Surg. 2012;47:1874–9.
31 Doodnath R, Puri P. A systematic review and meta-analysis of Hirschsprung's disease presenting after childhood. Pediatr Surg Int. 2010;26:1107–10.
32 Sharma S, Gupta DK. Hirschsprung's disease presenting beyond infancy: surgical options and postoperative outcome. Pediatr Surg Int. 2012;28:5–8.
33 Zeng M, Amodio J, Schwarz S, et al. Hirschsprung disease presenting as sigmoid volvulus: a case report and review of the literature. J Pediatr Surg. 2013;48:243–6.
34 Moore SW. The contribution of associated congenital anomalies in understanding Hirschsprung's disease. Pediatr Surg Int. 2006;22:305–15.
35 Lorijn F De, Reitsma JB, Voskuijl WP, et al. Diagnosis of Hirschsprung's disease: a prospective, comparative accuracy study of common tests. J Pediatr. 2005;146:787–92.
36 Lorijn F De, Kremer LCM, Reitsma JB, et al. Diagnostic Tests in Hirschsprung Disease : A Systematic Review. J Pediatr Gastroenterol Nutr. 2006;42:496–505.
37 Aldridge RT, Campbell PE. Ganglion cell distribution in the normal rectum and anal canal. A basis for the diagnosis of Hirschsprung's disease by anorectal biopsy. J Pediatr Surg. 1968;3:475–90.
38 Meier-Ruge W, Lutterbeck PM, Herzog B, et al. Acetylcholinesterase activity in suction biopsies of the rectum in the diagnosis of Hirschsprung's disease. J Pediatr Surg. 1972;7:11–7.
39 Nakao M, Suita S, Taguchi T, et al. Fourteen-year experience of acetylcholinesterase staining for rectal mucosal biopsy in neonatal Hirschsprung's disease. J Pediatr Surg. 2001;36:1357–63.
40 Bagdzevicius R, Gelman S, Gukauskiene L, et al. Application of acetylcholinesterase histochemistry for the diagnosis of Hirschsprung's disease in neonates and infants: a twenty-year experience. Medicina (Kaunas). 2011;47:374–9.
41 Kapur RP, Reed RC, Finn LS, et al. Calretinin immunohistochemistry versus acetylcholinesterase histochemistry in the evaluation of suction rectal biopsies for Hirschsprung Disease. Pediatr Dev Pathol. 2009;12:6–15.

42 Guinard-Samuel V, Bonnard A, Lagausie P De, et al. Calretinin immunohistochemistry: a simple and efficient tool to diagnose Hirschsprung disease. Mod Pathol. 2009;22:1379–84.
43 Morris MI, Soglio DB-D, Ouimet A, et al. A study of calretinin in Hirschsprung pathology, particularly in total colonic aganglionosis. J Pediatr Surg. 2013;48:1037–43.
44 de Arruda Lourencao PL, Takegawa BK, Ortolan E V, et al. Does calretinin immunohistochemistry reduce inconclusive diagnosis in rectal biopsies for Hirschsprung disease? J Pediatr Gastroenterol Nutr. 2014;58:603–7.
45 Takawira C, D'Agostini S, Shenouda S, et al. Laboratory procedures update on Hirschsprung disease. J Pediatr Gastroenterol Nutr. 2015;60:598–605.
46 Pini-Prato A, Martucciello G, Jasonni V. Rectal suction biopsy in the diagnosis of intestinal dysganglionoses: 5-year experience with Solo-RBT in 389 patients. J Pediatr Surg. 2006;41:1043–8.
47 Friedmacher F, Puri P. Rectal suction biopsy for the diagnosis of Hirschsprung's disease: a systematic review of diagnostic accuracy and complications. Pediatr Surg Int. 2015;31:821–30.
48 Iwai N, Yanagihara J, Tokiwa K, et al. Reliability of anorectal manometry in the diagnosis of Hirschsprung's disease. Z Kinderchir. 1988;43:405–7.
49 Emir H, Akman M, Sarimurat N, et al. Anorectal manometry during the neonatal period: its specificity in the diagnosis of Hirschsprung's disease. Eur J Pediatr Surg. 1999;9:101–3.
50 Huang Y, Zheng S, Xiao X. Preliminary evaluation of anorectal manometry in diagnosing Hirschsprung's disease in neonates. Pediatr Surg Int. 2009;25:41–5.
51 Noviello C, Cobellis G, Romano M, et al. Diagnosis of Hirschsprung's Disease: an age-related approach in children below or above one year. Colorectal Dis. 2010;12:1044–8.
52 Bradnock TJ, Walker GM. Evolution in the management of Hirschsprung's disease in the UK and Ireland: A national survey of practice revisited. Ann R Coll Surg Engl. 2011;93:34–8.
53 Zani A, Eaton S, Morini F, et al. European Paediatric Surgeons' Association Survey on the Management of Hirschsprung Disease. Eur J Pediatr Surg. 2016;27:96–101.
54 Tang YF, Chen JG, An HJ, et al. High-resolution anorectal manometry in newborns: normative values and diagnostic utility in Hirschsprung disease. Neurogastroenterol Motil. 2014;26:1565–72.
55 Wong CW, Lau CT, Chung PH, et al. The value of the 24-h delayed abdominal radiograph of barium enema in the diagnosis of Hirschsprung's disease. Pediatr Surg Int. 2015;31:11–5.
56 Muller CO, Mignot C, Belarbi N, et al. Does the radiographic transition zone correlate with the level of aganglionosis on the specimen in Hirschsprung's disease? Pediatr Surg Int. 2012;28:597–601.
57 Hoffmann K, Schier F, Waldschmidt J. Laparoscopic Swenson's procedure in children. Eur J Pediatr Surg. 1996;6:15–7.
58 de Lagausie P, Berrebi D, Geib G, et al. Laparoscopic Duhamel procedure. Surg Endosc. 1999;13:972–4.
59 la Torre-Mondragón L De, Ortega-Salgado JA. Transanal endorectal pull-through for Hirschsprung's disease. J Pediatr Surg. 1998;33:1283–6.
60 la Torre L De, Ortega A. Transanal versus open endorectal pull-through for Hirschsprung's disease. J Pediatr Surg. 2000;35:1630–2.
61 Langer JC. Laparoscopic and transanal pull-through for Hirschsprung disease. Semin Pediatr Surg. 2012;21:283–90.
62 Tannuri ACA, Tannuri U, Romão RLP. Transanal endorectal pull-through in children with

Hirschsprung's disease—technical refinements and comparison of results with the Duhamel procedure. J Pediatr Surg. 2009;44:767–72.
63 Stensrud KJ, Emblem R, Bjørnland K. Functional outcome after operation for Hirschsprung disease—transanal vs transabdominal approach. J Pediatr Surg. 2010;45:1640–4.
64 Giuliani S, Betalli P, Narciso A, et al. Outcome Comparison Among Laparoscopic Duhamel, Laparotomic Duhamel, and Transanal Endorectal Pull-Through: A Single-Center, 18-Year Experience. J Laparoendosc Adv Surg Tech. 2011;21:859–63.
65 El-Sawaf MI, Drongowski RA, Chamberlain JN, et al. Are the long-term results of the transanal pull-through equal to those of the transabdominal pull-through? A comparison of the 2 approaches for Hirschsprung disease. J Pediatr Surg. 2007;42:41–7.
66 van Leeuwen K, Geiger JD, Barnett JL, et al. Stooling and manometric findings after primary pull-throughs in Hirschsprung's disease: Perineal versus abdominal approaches. J Pediatr Surg. 2002;37:1321–5.
67 Stensrud KJ, Emblem R, Bjornland K. Anal endosonography and bowel function in patients undergoing different types of endorectal pull-through procedures for Hirschsprung disease. J Pediatr Surg. 2015;50:1341–6.
68 Menezes M, Corbally M, Puri P. Long-term results of bowel function after treatment for Hirschsprung's disease: a 29-year review. Pediatr Surg Int. 2006;22:987–90.
69 Ieiri S, Nakatsuji T, Akiyoshi J, et al. Long-term outcomes and the quality of life of Hirschsprung disease in adolescents who have reached 18 years or older—a 47-year single-institute experience. J Pediatr Surg. 2010;45:2398–402.
70 Jarvi K, Laitakari EM, Koivusalo A, et al. Bowel function and gastrointestinal quality of life among adults operated for Hirschsprung disease during childhood: a population-based study. Ann Surg. 2010;252:977–81.
71 Aworanti OM, McDowell DT, Martin IM, et al. Does Functional Outcome Improve with Time Postsurgery for Hirschsprung Disease? Eur J Pediatr Surg. 2015;26:192–9.
72 Niramis R, Watanatittan S, Anuntkosol M, et al. Quality of life of patients with Hirschsprung's disease at 5 - 20 years post pull-through operations. Eur J Pediatr Surg. 2008;18:38–43.
73 Bartlett L, Nowak M, Ho Y-H. Impact of fecal incontinence on quality of life. World J Gastroenterol. 2009;15:3276–82.
74 Belsey J, Greenfield S, Candy D, et al. Systematic review: Impact of constipation on quality of life in adults and children. Aliment Pharmacol Ther. 2010;31:938–49.
75 Mugie SM, Benninga MA, Lorenzo C Di. Epidemiology of constipation in children and adults: A systematic review. Best Pract Res Clin Gastroenterol. 2011;25:3–18.
76 Sharma A, Rao S. Constipation: Pathophysiology and Current Therapeutic Approaches. In: Handbook of experimental pharmacology. 2016. p. 59–74.
77 Whitehead WE, Borrud L, Goode PS, et al. Fecal Incontinence in US Adults: Epidemiology and Risk Factors. Gastroenterology. 2009;137:512–517.e2.
78 Catto-Smith AG, Trajanovska M, Taylor RG. Long-term continence after surgery for Hirschsprung's disease. J Gastroenterol Hepatol. 2007;22:2273–82.
79 Neuvonen MI, Kyrklund K, Rintala RJ, et al. Bowel Function and Quality of Life After Transanal Endorectal Pull-through for Hirschsprung Disease: Controlled Outcomes up to Adulthood. Ann Surg. 2017;265:622–9.
80 Heikkinen M, Rintala R, Luukkonen P. Long-term anal sphincter performance after surgery for Hirschsprung's disease. J Pediatr Surg. 1997;32:1443–6.
81 Moore SW. Total colonic aganglionosis in Hirschsprung disease. Semin Pediatr Surg.

2012;21:302–9.
82 Nurko S, Scott SM. Coexistence of constipation and incontinence in children and adults. Best Pract Res Clin Gastroenterol. 2011;25:29–41.
83 Hartman EE, Oort FJ, Aronson DC, et al. Quality of life and disease-specific functioning of patients with anorectal malformations or Hirschsprung's disease: a review. Arch Dis Child. 2011;96:398–406.
84 Collins L, Collis B, Trajanovska M, et al. Quality of life outcomes in children with Hirschsprung disease. J Pediatr Surg. 2017;52:2006-10.
85 Eypasch E, Williams JI, Wood-Dauphinee S, et al. Gastrointestinal Quality of Life Index: development, validation and application of a new instrument. Br J Surg. 1995;82:216–22.
86 Osterberg A, Graf W, Karlbom U, et al. Evaluation of a questionnaire in the assessment of patients with faecal incontinence and constipation. Scand J Gastroenterol. 1996;31:575–80.
87 Frank L, Kleinman L, Farup C, et al. Psychometric validation of a constipation symptom assessment questionnaire. Scand J Gastroenterol. 1999;34:870–7.
88 Hanneman MJ, Sprangers MA, De Mik EL, et al. Quality of life in patients with anorectal malformation or Hirschsprung's disease: development of a disease-specific questionnaire. Dis Colon Rectum. 2001;44:1650–60.
89 Lehur P-A, Zerbib F, Neunlist M, et al. Comparison of quality of life and anorectal function after artificial sphincter implantation. Dis Colon Rectum. 2002;45:508–13.
90 Adelstein B-A, Irwig L, Macaskill P, et al. A self administered reliable questionnaire to assess lower bowel symptoms. BMC Gastroenterol. 2008;8:8.

PART I

Early diagnosis of Hirschsprung's disease