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Department of Pediatric Surgery, Children's Hospital,
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HIRSCHSPRUNG’S DISEASE

Long-term outcomes and pathophysiology of bowel
dysfunction

Malla Salli (née Neuvonen)

Academic Dissertation

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To the patients with Hirschsprung's disease
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ABSTRACT

Background. Patients with Hirschsprung's disease (HD) have significant post-operative morbidity due to Hirschsprung-associated enterocolitis (HAEC) and other disturbances in bowel function, but their exact etiology is still unknown. Transanal endorectal pull-through (TEPT) has suggested to interfere with anal sphincter integrity and cause some of these problems, but controlled long-term studies are lacking. Additionally, the relation of bowel dysfunctions and quality of life (QoL) is unclear. Moreover, despite that gut microbial dysbiosis has been associated with the development of HAEC, its impact on other postoperative bowel symptoms is unclear.

Aim. To systematically define bowel, urinary and sexual functional outcomes and QoL after TEPT in HD patients up to adulthood in relation to healthy controls. To characterize the microbiota profiles of HD patients in relation to healthy controls, HAEC and other bowel dysfunctions.

Methods. A single-institution, cross-sectional, retrospective study including all HD patients with a TEPT performed between 1987–2011. Two case-control surveys regarding bowel, urinary and sexual functions and QoL. Voluntary patients participated also in a clinical follow-up with clinical investigations including flowmetry, ultrasound and laboratory samples. Regarding surveys, patients aged ≥4 without an enterostomy/ACE were included in the bowel function analysis, and patients aged ≥4 and ≥16 without an associated syndrome were included in urinary and sexual functional analysis, respectively. The gut microbiota and fecal calprotectin (FC) were analyzed from stool samples of voluntary patients and compared to the microbiota of healthy controls. The ethics committee of the Helsinki University Hospital approved the research protocol.

Results. Overall survival was 98% (n=143/146; median age 15 (3–33); 83% rectosigmoid aganglionosis; 29% had an associated syndrome; 69% underwent transabdominal and 20% totally transanal TEPT). At the last follow-up, 42% and 12% had occasional and frequent fecal soiling, and 8% and 1% had occasional or frequent constipation, respectively, which were predicted by an associated syndrome (p<0.05). Of all, 44% developed recurrent HAEC, which was predicted by extended aganglionosis and an associated syndrome (p<0.05 for both).
Response rate was 64% (n=87; median age 15 (4–32); 86% rectosigmoid aganglionosis). Compared to controls, patients had more impairments in all other aspects of fecal control (p<0.05 for all) than constipation. However, all of the impairments, except abnormal stooling frequency, improved with increasing age to the level of controls in adulthood. The total QoL scores were equal to controls in all age groups, but adult patients had lower emotional scores and increased personal limitations compared to controls (p<0.05). The urinary and sexual functions were at least equal between patients and controls, except that fewer female patients were in stable relationships (p<0.05).

The microbiota analysis of HD patients (n=34; median age 12 (3–25); 77% have had HAEC) showed decreased microbial richness associated with no normal maturation compared to controls. Patients with a history of recurrent HAEC had even more decreased microbial richness and expanded abundance of Proteobacteria among other changes in taxa compared to patients without HAEC (p<0.005). FC level was normal in all patients.

Conclusions. After HD surgery, HAEC and fecal soiling were the most significant problems. An associated syndrome predicted HAEC and bowel dysfunctions, and an extended aganglionosis predicted HAEC. Impairments in fecal control decreased with increasing age. Urinary and sexual functions and total QoL were equal in patients and controls. The gut microbiota of patients with HD and HAEC differed from controls, especially by decreased richness and pathologic expansions of taxa, particularly Enterobacteria and Bacilli.
ORIGINAL PUBLICATIONS

This thesis is based on the following publications:


The publications are referred to in the text by their Roman numerals and are reprinted here with the permission of the publishers.
ABBREVIATIONS

ACE  antegrade continence enema
AChE  acetylcholine esterase
BFS  the Bowel Function Score
EHS  Erectile Hardness Score
ENS  enteric nervous system
FC  fecal calprotectin
GIQLI  the Gastrointestinal Quality of Life Index
HAEC  Hirschsprung’s-associated enterocolitis
HD  Hirschsprung’s disease
HRQoL  health-related quality of life
IBD  inflammatory bowel diseases
IQR  interquartile range
LCT  brush border lactase
LUTS  lower urinary tract symptoms
PedSQL  Pediatric Quality of Life
PT  pull-through
QoL  quality of life
TCA  total colonic aganglionosis
TEPT  transanal endorectal pull-through
1. INTRODUCTION

Hirschsprung’s disease (HD) is one of the most common congenital intestinal motility disorders, affecting approximately 1:5000 live births (1-3). It is characterized by the absence of ganglion cells of the intestine (aganglionosis) in various lengths, resulting in functional obstruction (4). HD is treated with definitive surgery including removal of the aganglionic segment and anastomosis of the innervated proximal bowel to the anus (5). During the past decades, one-stage transanal endorectal pull-through (TEPT) has become one of the most commonly performed operations in HD, which may be performed either totally transanally or in combination with transabdominal colonic mobilization using laparotomy, laparoscopy or umbilical incision (6-8). The long-term outcomes following HD surgery have shown to be imperfect, as more than half of all HD patients experience varying degrees of bowel dysfunction, especially fecal incontinence (9) and Hirschsprung-associated enterocolitis (HAEC) (10-12), causing long-term morbidity and impairments in quality of life (QoL) (13-17). However, due to the marked variation in the previous series and the lack of controlled long-term follow-up studies, the exact outcomes are still debatable. Today, as the first patients at our center with a performed TEPT have reached adulthood and as the first patients with a performed totally transanal TEPT are about to reach adulthood, the long-term outcomes after TEPT procedures can be evaluated.

So far, the pathophysiology of HAEC and other bowel functional impairments has remained unclear, despite several theories. Among other proposed factors, transanal surgery has suggested to interfere with anal sphincter integrity and rectal sensation, resulting in bowel dysfunctions (18-20). Gut microbial dysbiosis have also proposed to play a role especially in the development of HAEC (21-23), but the significance of dysbiosis in the development of other bowel dysfunctions is unknown. Furthermore, food-allergies, particularly to cow’s milk
(24-27), have proposed to lead to HAEC and other bowel functional impairments; however, no publications clarify how HAEC or other bowel symptoms in patients with HD are influenced by the \textit{LCT} genotype C/C, which leads to lactase deficiency (28,29), and fecal calprotectin (FC) levels, which is a sensitive marker of intestinal inflammation (30,31).

So far, the long-term outcomes of urinary and sexual functions in HD patients have been insufficiently explored, despite the possibility for iatrogenic injuries to the genitourinary tract or its innervation during pull-through (PT) surgery for HD (16,32).

The aim of the thesis is to systematically define the long-term outcomes after TEPT in HD patients regarding bowel, urinary and sexual functions as well as QoL in relation to age- and gender-matched healthy controls. Furthermore, the thesis aims to contribute to better understanding of the pathogenesis of HAEC and functional gastrointestinal problems in HD patients by characterizing their gut microbiota profiles, FC levels and the prevalence of the \textit{LCT} genotype C/C.
2. LITERATURE REVIEW

2.1 ENTERIC NERVOUS SYSTEM

The human enteric nervous system (ENS), which is the largest part of the human autonomic nervous system, provides rich bowel innervation in two networks extending from the esophagus to the anal verge (4,33,34). The nerve fibers are also located around blood vessels, near cells of the immune system, and in close apposition to the epithelium, expressing a vast array of neurotransmitters and receptors (33). Thus, the ENS is able to receive signals and modulate the activity of every bowel cell (33). The ENS arises from neural crest cells that migrate into and colonize the developing gastrointestinal tract (35,36). In a healthy human fetus, the neural crest-derived neuroblasts first appear in the developing esophagus by the first weeks of gestation and migrate into the rest of the developing gut from the 5th to 12th week of gestation (33). However, the development of the ENS still continues in the postnatal period by further differentiating and maturing together with intestinal barrier and mucosal enteric glial cells (37). Therefore, the intrinsic neural development of the gut is not determined until the time of the colonization of immune cells and microbiota in the gut (35); thus, also the gut microbial population may influence the formation of the ENS and vice versa (37).

2.2 ETIOLOGY OF HIRSCHSPRUNG’S DISEASE

In HD, the enteric neurons are absent at least from the distal bowel (aganglionosis), leading to a persistently constricted aganglionic region because the extrinsic excitatory innervation is still present (38,39). The exact pathogenesis of HD is still unknown, but currently its primary etiology is considered to be the events that disrupt the formation of the ENS, which requires coordination of the survival, migration, proliferation, and differentiation of the progenitor cells within the gastrointestinal tract (36,39,40). Alternatively, the neural crest cells can fail to
survive, proliferate or differentiate after migration due to abnormalities in the microenvironment (36,39,40). Nevertheless, several details regarding the pathogenesis of HD are yet to be discovered.

2.3. GENETIC BACKGROUND AND INHERITANCE
HD has been associated with at least 15 gene mutations, but they cover only about 30% of all cases of HD (41,42). The known genes associated with HD have roles in the enteric neural crest cell proliferation, maintenance of the ENS progenitors, and neuronal or glial differentiation, thus influencing the ENS migration (40,42). The gene mutations are mostly located in RET proto-oncogene and its ligands, followed by the cell line–derived neurotrophic factor, the endothelin family of genes, SOX-10, and SIP1 Smad interacting protein 1 (40,42). Altogether, HD occurs as an isolated disorder in 70% of cases; in the remaining cases, it is part of either a chromosomal abnormality (12%), including trisomy 21, congenital central hypoventilation syndrome, cartilage-hair hypoplasia, and Waardenburg-Shah syndrome, or other congenital anomalies (18%) (41). The inheritance of HD is complex, with sex-dependent penetrance that may be familial and autosomal dominant or autosomal recessive (43).

2.4 EPIDEMIOLOGY AND MORTALITY
In Western countries, HD occurs in approximately 1 to 5000 live births, and its female-to-male ratio is 1:3–4 in rectosigmoid disease and 1:1–2 in longer-segment diseases (41,44,45). In previous series, the mortality has ranged between 0% and 9% (2,45-47), with higher rates in patients with an associated syndrome, HAEC, and extended aganglionosis (46), especially panintestinal aganglionosis, in which mortality may be as high as 66% (48).
2.5 CLASSIFICATION

Hirschsprung’s disease is commonly classified by the extent of the aganglionic segment of the distal gut: 1. rectosigmoid (classic) aganglionosis (74%–85% of patients); 2. long-segment aganglionosis, in which aganglionosis extends more proximal to the sigmoid colon, but does not affect the entire colon (12%–22%); 3. total colonic aganglionosis (TCA) (4%–13%); and 4. aganglionosis extending more proximal to the ileum (3,33,36,41). The rarer forms of the disease are total intestinal (panintestinal) aganglionosis and ultrashort-segment aganglionosis (3).

2.6 DIAGNOSIS

In most cases, HD is diagnosed in the neonatal period (3). Most symptoms leading to diagnosis are obstructive symptoms caused by the tonic contraction and the lack of motility in the aganglionic bowel segment, including distended abdomen, failure to pass meconium in the first 48 hours, bilious aspirates or vomiting, and feeding intolerance in neonates (3,12,33). Children may also present with HAEC before the diagnosis of HD, which may be a life-threatening condition characterized by fever, abdominal distension, and diarrhea (49,50). After the suspicion of HD, the diagnosis requires clinical investigations including plain abdominal radiographs, contrast enema, anorectal manometry, and full-thickness rectal biopsy or rectal suction biopsy (3,51). Of these, rectal biopsy is considered confirmatory, when it demonstrates the absence of ganglion cells and presence of acetylcholine esterase (AChE)-positive hypertrophic nerve fibers (52).
2.7. SURGICAL MANAGEMENT

2.7.1. Normal anatomy of the anal canal

The anal canal is the terminal and extraperitoneal part of the colon (53,54). It is surrounded by the anal sphincter complex, which is composed of involuntary internal sphincter, longitudinal muscle layer, and voluntary external sphincter (Figure 1) (53,54). Internal sphincter is innervated by the superior hypogastric and pelvic splanchnic nerves and external sphincter by the pudendal nerve (53,54), which is crucial for defecatory, urinary and sexual functions (54). The dentate line marks the transition between the visceral area above with true mucosa and the somatic area below (55) with a highly sensitive mucosa (53). Iatrogenic injuries to the dentate line may have detrimental consequences for fecal continence due to its impact on the sensation of the anal canal (6).

![Figure 1. Normal anatomy of the anal canal.](https://healthjack.com/encyclopedia/pictures-of-anal-canal)

2.7.2. Surgical principles

Today, primary PT procedures, which refer to Swenson’s, Duhamel’s and Soave’s procedure as well as TEPT and their several modifications, are standard for HD (3,56-58). They consist
of the removal of the aganglionic segment and the hypoganglionic transition zone and anastomosis of the normally innervated bowel (5), which is performed above the dentate line in order to preserve normal sphincter function (6). Also other anatomical structures, including the inferior hypogastric plexus, should be maintained intact in order to avoid postoperative defecatory, voiding and sexual dysfunctions (59).

2.7.3. Swenson’s, Duhamel’s and Soave procedure

In Swenson’s procedure (56,60), which is the original operation for HD, the defective distal colon is freed within the pelvis by extrarectal dissection down to 2 cm above the dentate line. The dissection continues proximally in a plane outside the serosa until distended bowel is reached; thereafter, the normally innervated bowel segment is pulled through the anus, and an end-to-end anastomosis is performed to the dentate line (Figure 2) (56,60). The subsequent procedures were developed to protect the pelvic structures during the dissection.

In Duhamel’s procedure (57), the native rectum is left in situ, involving mobilization of the ganglionic bowel down to the dentate line through a plane created behind the rectum and an end-to-side anastomosis to the back of the native rectum. Therefore, a pouch is formed, which consists of aganglionic bowel anteriorly and ganglionic bowel posteriorly (Figure 2) to act as a reservoir for stool (57), which may be helpful for children with a longer aganglionic segment (61). As disadvantages, the remaining aganglionic bowel segment may increase the risk of obstructive features and the development of HAEC, and the pouch can become distended and form a spur at the junction of the ganglionic and aganglionic bowel, leading to obstructive features and stasis (61).
A Soave procedure (58), or endorectal pull-through procedure, involves resection of the rectal mucosa and preservation of the muscular cylinder, which is followed by pulling the healthy proximal bowel down to the dentate line through the “cuff” formed by the aganglionic muscle (Figure 2). In the original description, the pull-through bowel was left hanging out for several weeks to support the anastomosis, but in Boley's modification (62), which is employed by most surgeons today, a primary anastomosis is performed.

Figure 2. Main approaches to PT. Adapted from: Green HL, Rizzolo D, Austin M. Surgical management for Hirschsprung disease: A review for primary care providers. (Journal of the American Academy of PAs 2016; 29(4):24-29.)

2.7.4. Transanal endorectal pull-through (TEPT)

In a Soave procedure, the mucosectomy of the aganglionic bowel segment is performed transabdominally, but, in TEPT (5,6,63), the submucosal endorectal dissection is performed from below (Figure 3). To prevent the loss of anal sensation, the mucosal incision is performed 0.5–1cm above the dentate line to maintain the transitional epithelium undamaged (6). Subsequently, the rectal muscle is incised circumferentially, and the dissection is
continued proximally along the rectal wall (6). The vessels are divided as they enter the bowel wall to avoid injuries to the nerves and vessels as well as the prostate and the vagina (6). After reaching the normally innervated bowel, the bowel is divided above the transition zone, pulled through the muscular cuff, and an anastomosis is performed above the dentate line (6). In addition to the totally transanal approach, the colon may be mobilized in assistance of laparotomy, laparoscopy or transumbilical incision (5,7,8,55,64-66). Moreover, because a long muscular rectal cuff contains a risk of causing obstructive symptoms and an increased tendency for HAEC, a shorter muscular cuff extending 1–2 cm above the dentate line have gained popularity (6,19,65,67-69). However, a too short or nonexistent cuff may contain an increased risk of fecal incontinence.

Figure 3. Technique of the transanal pull-through. First, a circumferential mucosal incision is made, and the mucosa is dissected from the underlying muscle to a distance of several centimeters. The muscle cuff is then incised circumferentially, and the dissection is continued along the rectal wall, dividing the vessels as they enter the rectum. When the normally innervated bowel is reached, the colon is divided, and the anastomosis is performed. (Dasgupta R, Langer JC. Transanal pull-through for Hirschsprung disease. Semin Pediatr Surg 2005; 14: 64–71.)
Within the past decades, minimal-access surgical techniques have gained popularity, especially for patients with rectosigmoid aganglionosis (7,8,19,68,70-72). In relation to transabdominal approaches, totally transanal techniques have the advantages of avoiding intra-abdominal and pelvic dissection and their typical complications as well as having shorter hospitalization periods, less pain, earlier feeding, lower costs and better cosmetic results (7,18,71). However, some concerns exist regarding the possibility of injuring the anal sphincteric muscles due to excessive or prolonged retraction during the procedure (10,19,20). Other disadvantages include the inability to confirm the histological transition zone prior to starting mobilization of the colon and the possibility of colonic torsion.

2.7.5. Surgical techniques for TCA and extended aganglionosis

To preserve absorptive capacity in patients with TCA and more extended aganglionosis, various surgical techniques have been described, including ileoanal pull-through, traditional PT procedures, side-to-side anastomoses between ganglionic and aganglionic intestine, colon-patch modifications (73), and restorative proctocolectomy with J-pouch (74). Patients with panintestinal aganglionosis, in turn, may be salvaged by myectomy-myotomy, distention of the small bowel, and performing a permanent jejunostomy (75). Without intestinal transplantation, however, these patients will most likely be dependent on parenteral nutrition (76,77).

2.8. COMPLICATIONS RELATED TO HD SURGERY

Complications related to HD surgery can be classified as “early” (weeks to months) and “late” complications (months to years) (3). Early complications include not only the general complications of abdominal surgery, including bleeding, infection, injury to adjacent organs, and intestinal obstruction (78), but also anastomotic leaks, cuff abscesses, bowel obstructions,
perineal excoriation, stoma-specific complications, retraction of the pulled-through colon, and wound infections and dehiscence (3,12,79). Late complications include bowel obstructions, anastomotic strictures, HAEC, and bowel functional impairments (3). Cases requiring a complete redo pull-through operation include retained aganglionic tissue or transitional zone pathology, recalcitrant anastomotic stricture for dilatation, anastomotic leak, twisted pull-through and dilated colon (12). In previous series, the occurrence of postoperative complications requiring surgical treatment has varied between 1%–28% in all HD patients (6,12,20,24,60,65,80-85) and between 25%–48% in patients with TCA (86,87).

2.9. BOWEL FUNCTIONAL OUTCOMES

2.9.1. Evaluation of bowel functional outcome

Today, the scoring systems used in previous publications for the assessment of bowel functional outcomes include the Krickenbeck criteria (88), Drossman classification (89), Catto-Smith classification for fecal soiling (24), and the Bowel Function Score (BFS) (90). The BFS is frequently used (11,19,19,83,91,92), as it has the advantages of being observer-independent, previously validated with healthy subjects (93), simple to complete by the child or parent, and having a good correlation with bowel functional outcomes and no requirement of a physical examination (90).

2.9.2. Problems related to the evaluation of the bowel functional outcomes in HD patients

In patients with HD, the long-term bowel functional impairments usually entail persistent obstructive symptoms, fecal incontinence or soiling, HAEC, and combinations of these problems (81). However, their exact frequencies are unclear, as, firstly, different follow-up studies have varied figures due to the various study designs, scoring systems and definitions. Secondly, the divergences in patient characteristics, inclusion criteria, lengths of the follow-up
periods and surgical techniques complicate the comparison of the studies even further (18). Lastly, several studies display too short follow-up periods for reliable assessment of the bowel functional outcomes (63,64,95,96), and most of the series reporting long-term outcomes into adolescence and adulthood deal only with open techniques due to their longer practice (60,91,92,97).

2.9.3. Bowel functional outcome in HD patients aged ≥4

In previous series, any degree of fecal soiling occurred in 10%-76% of HD patients aged ≥4 (16,20,24,55,60,65,85,91,97-99), any degree of fecal accidents/incontinence occurred in 7%-56% of patients (11,24,60,80,91,97,100), and constipation occurred in 7%-42% of patients (10-12,14,20,60,80,85,91,97). In adult patients with HD, 9%-45% had fecal soiling (15,72,91,92,97) and 12%-33% had constipation (15,72,91,97). All studies with a healthy reference group displayed higher rates of bowel functional impairments in HD patients compared to controls (15,91,92,100,101). Furthermore, studies showed that stooling frequency of the patients was mostly normal or frequent (11,12,97), 56% had dietary restrictions, 90% had significant changes in bowel movements in response to foods, and 73% had increased sensitivity to foods (100). Moreover, 16%-58% of HD patients had normal stool consistency (11,16), and 56% experienced abdominal pain more than once a week (99). Normal anorectal sensation was found in 71%-96% of HD patients (97,100), and 71%-93% distinguished stool conditions (16,97).

2.9.4. Prognostic factors

In previous series, poor bowel functional outcome was the most consistently predicted by an associated syndrome (26,85) and long-segment aganglionosis (12,16,24,55,102). Other, less consistently mentioned and somewhat contradictory, predictors included surgical
complications such as anastomotic stricture (12), a redo pull-through procedure (7),
prematurity (12), young age at surgery (55,103), Duhamel procedure when compared to
Swenson procedure (16), and a history of HAEC (85). No surgical approach seemed superior
compared to the others in terms of bowel functional outcomes (80,84,104,105), and also
totally transanal TEPT was associated with both negative and positive bowel functional
outcomes when compared to transabdominal approaches (10,18,24,72,82). Good bowel
functional outcome was predicted consistently only by increasing age
(7,10,14,16,24,46,83,85,98,102,106-109). However, Järvi et al. (91) detected deterioration in
the bowel functional outcome after the age of 35.

2.10. SECONDARY MEASURES TO IMPROVE FECAL CONTROL

2.10.1. Evaluation of bowel functional outcomes

Fecal continence is defined as the ability to have voluntary bowel movements without soiling
and without the need for enemas (110). It may be achieved with normal anal sensation,
voluntary sphincter control, appropriate colonic motility, and co-operation by the patient;
thus, disruption of any of these may lead to fecal incontinence (110). However, although
resection of the bowel and the natural fecal reservoir always causes changes in colonic
motility, good fecal control may be achieved if the dentate line and sphincter muscles are
intact (6). Fecal soiling/incontinence may occur if the mucosal dissection has been performed
too low or the sphincters have been excessively distended (110). It can also be due to
mechanical obstruction, persistent aganglionosis, overflow incontinence due to constipation,
HAEC, colonic excessive motility, and unknown causes (110). Obstructive symptoms, in turn,
may be caused by mechanical obstruction, intestinal dysmotility, residual aganglionosis,
transition zone pull-through, internal sphincter achalasia, or stool holding behavior (111). Not
only should HAEC be ruled out, the specific anatomic and functional characteristics should be
investigated and clear anatomical causes requiring redo pull-through identified before proceeding with other treatments for bowel dysfunctions (6).

2.10.2. Treatment methods for bowel functional impairments

Depending on the type of motility disorder (hypomotility/hypermotility), the conservative management of bowel dysfunctions include laxatives, loperamide, pectin and dietary modifications (6,112). Enemas should be offered only for incontinent patients who fail conservative management (6,112). Botulinum toxin type A has been used to manage children with defecation disorders, including internal anal sphincter achalasia and recurrent HAEC, as it is a minimally invasive method and has a temporary paralytic effect on the internal anal sphincter (6,111,113,114). However, patient selection may be problematic (112). Myotomy/myectomy and internal sphincterotomy have been applied with similar indications, especially if the patient has responded well to botulinum toxin-injections (6,111,115). However, they contain a risk of fecal incontinence (115). Patients with an absent or damaged anal canal and who have been treated with enemas may be managed with antegrade continence enema (ACE) (111,116), as it provides predictability of fecal continence by a continent catheterizable stoma (116). It is also safe, effective and QoL improving (117).

2.11. HIRSCHSPRUNG’S-ASSOCIATED ENTEROCOLITIS

2.11.1. Clinical presentation

Hirschsprung’s-associated enterocolitis (HAEC) is a potentially life-threatening complication of HD with about 5% mortality (118-120). It is a clinical condition of intestinal inflammation characterized by abdominal distention, diarrhea and lethargy, which may be combined with vomiting, bloody stools and fever (118). However, its presentation can vary from mild abdominal distention with loose stools and perianal excoriation to life-threatening toxic
megacolon with explosive diarrhea, rectal bleeding, lethargy, and impending shock (118,121). The onset of HAEC may be rapid or it can be chronic, which usually presents as persistent diarrhea, soiling, intermittent abdominal distention and failure to thrive (49,118,121). The tendency for HAEC usually declines with age, being the highest within the first years after the PT procedure (9,103,121). The definition of HAEC varies in different studies, causing variation in the reported occurrences of HAEC (18). The Delphi method (122) tried to standardize the diagnostic criteria, but it was not widely accepted in clinical work due to its complexity. Recently, the HAEC Collaborative Research Group developed a simpler clinical criterion in the attempt to improve the accuracy of the HAEC diagnosis, showing that diarrhea with explosive stools, decreased peripheral perfusion, lethargy and distended abdomen were the most significant symptoms related to HAEC (118).

2.11.3. Incidence of HAEC

In previous literature, the occurrence of preoperative HAEC has ranged between 6%–29% (9,16,46,122,123) and postoperative HAEC between 3%–54% (7,10-12,15,16,19,24,46,60,65,72,82-85,124). However, the occurrence of recurrent HAEC is rarely mentioned.

2.11.4. Prognostic factors

The most confirmed risk factors for HAEC include trisomy 21 (124,125) followed by longer-segment diseases (9,124). The occurrences of postoperative and recurrent HAEC in patients with TCA have been as high as 76% and 57%, respectively (86,126). Other risk factors for HAEC include family history of HD, low weight (9), young age at diagnosis (12,124), primary PT performed in the neonatal period (12,124), low-level Immunoglobulin A (9), performed TEPT (72,127), and postoperative complications including strictures or obstruction (12,124).
Previous HAEC episodes have also noted to increase the risk to develop further HAEC episodes, indicating that the early onset of HAEC may alter the defense mechanisms of the intestine and predispose the patient to further HAEC episodes (9, 49).

2.11.5. Treatment and prevention

The treatment of children with HAEC includes resuscitation, decompression of the gastrointestinal tract with rectal washouts, and antibiotics according to the severity of the episode (49). However, in cases of fulminant disease or sepsis, diversion with a levelling colostomy may be required (49). Mechanical causes for partial bowel obstruction and retained or secondary aganglionosis should be ruled out and treated before proceeding with other HAEC treatments (49), including prophylactic antibiotics and botulinum toxin-injections (114). As a preventive method, rectal washouts should be performed when surgical management is delayed, as they limit colonic distention and fecal stasis (121, 128).

2.11.6. Proposed etiological factors

Despite ongoing research, the pathogenesis of HAEC remains poorly understood. Nevertheless, HAEC has been associated with decreased bowel blood flow due to bowel distention, abnormalities in the epithelial barrier function, innate immune response, dysbiosis of the gut microbiota, and gene defects, all of which degenerate the epithelial cells’ ability to protect the body from intraluminal microorganisms (Figure 4) (33, 50, 129).
Figure 4. Mechanisms that may underlie HAEC. Bowel distention reduces bowel blood flow, dysmotility alters the gut microbiota, the support of the ENS to epithelial repair and barrier function is disrupted, and impaired bowel immune function may lead to the development of HAEC. (Heuckeroth RO. Hirschsprung disease – integrating basic science and clinical medicine to improve outcomes. Nature 2018. Published online 4 Jan. Available at: doi: 10.1038/nrastro.2017.149)

a. Abnormal intestinal motility and reduced bowel blood flow

Abnormal intestinal motility may lead to bacterial stasis, overgrowth and translocation as well as reduced bowel blood flow (130). However, ENS dysfunction may also reduce bowel blood flow, because normally ENS controls intestinal blood flow (130).

b. Dysfunctions of epithelial barrier, repair processes and mucus secretion

Enteric neurons and glia have shown to affect intestinal epithelial proliferation and barrier function in complex ways (33,131). Therefore, due to the abnormal ENS, HD patients have found to have varied alterations in the epithelial barrier function, repair processes, and mucus secretion from goblet cells (33,50). The reduced expression of caudaltype homeobox gene-1 and -2 may also result in immature mucosa, which has been associated with defective epithelial cell proliferation and differentiation, contributing to prolonged mucosal damage.
and weakening of the intestinal barrier (132). All of these changes may increase enterocyte adherence and lead to abnormal immune responses against the gut microbiota, initiating intestinal inflammation (33,50,129).

c. **Abnormal innate immune response**

A central part of the human immune system, consisting of a large compartment of immune cells comprising Peyer’s patches, follicles and lymphoid cells, resides in the bowel and functions together with the ENS (50). Thus, aganglionosis and an abnormal ENS may cause alterations in the immune responses via several mechanisms, resulting in increased susceptibility to intestinal inflammation (131,133-135). Defective white blood cells and transfer mechanisms may also, at least partly, explain the patient’s increased susceptibility to HAEC, especially in patients with trisomy 21 and other associated syndromes (136-138).

d. **Genetic abnormalities**

Genetic predisposition to HAEC has been suggested to exist via impaired leukocyte function and T-cell regulation (127) and via decreased proliferation and differentiation of the mucosal cells by reduced expression of caudal type homeobox gene-1 and -2 (132). Moreover, the gene defects in **RMRP, RET, Ednrd/-** and **Edn3/-**, which have been associated with HD, may also affect bowel immunology negatively (133,134).

e. **Abnormal gut microbiota composition**

Under normal conditions, humans live in a symbiotic relationship with their commensal gut microbiota (139). Gut microbiota is known to affect several important aspects of its host’s health and development, including protection against pathogens, nutrient processing, maturation of the mucosal immunity, early programming of the epithelial function and
angiogenesis, among many others (139). It interacts bidirectionally with both the central nervous system and the ENS by neural, endocrine, immune, and humoral means to influence its composition and behavior (140,141). Gut microbiota is acquired from the environment starting at birth, and its diversity builds up over the first few years of life (139,140). It is largely shaped by environmental factors such as age, diet, use of antibiotics, disease state, abnormalities in the ENS, epithelial mucins, and bowel motility (139), which are often abnormal in patients with HD. Therefore, HD patients may be prone to develop gut microbial dysbiosis, which is a disease-promoting state associated with several disorders including inflammatory bowel diseases, type 2 diabetes, necrotizing enterocolitis, irritable bowel syndrome and mental health diseases (140,142), potentially causing wide health consequences in HD patients.

The few publications that exist regarding the gut microbiota in HD patients have revealed associations between dysbiosis and intestinal aganglionosis and HAEC (21-23,141,143). Li et al. (21) showed that the most abundant phylum among HD patients without HAEC was *Bacteroidetes* followed by *Firmicutes* and *Proteobacteria*; patients with HAEC, in turn, had *Proteobacteria* as their most abundant phyla, which was associated with decreased microbial richness. Frykman et al. (22) found similar changes in addition to an increased abundance of *Candida sp*. However, the association of dysbiosis and postoperative bowel symptoms other than HAEC is unknown, despite the known ability of the gut microbiota to influence intestinal motility (144-146). All in all, many aspects regarding the relation of the gut microbiota and HD are yet to be discovered.
2.11.7. Fecal calprotectin (FC)

Fecal calprotectin is released extracellularly to feces in times of cell stress or damage; thus, it is a sensitive marker of intestinal inflammation (30,147). However, no published series exist regarding the FC levels in HD patients.

2.11.8. LCT genotype

The genotype C/C-13910 leads to lactase deficiency and, thus, frequently to lactose intolerance (28,29), which may cause bowel symptoms resembling bowel dysfunctions related to HD. Although functional symptoms of lactose intolerance and food-allergies, especially to cow's milk, have been found to be related to an increased frequency of HAEC and other bowel symptoms in HD (24-27), the influence of the LCT genotype C/C on HAEC and other postoperative bowel symptoms in HD has not been determined.

2.13. URINARY AND SEXUAL FUNCTIONAL OUTCOMES

Most of the innervation of the bladder and genitals are located close to the surgical area of HD patients. Thus, surgical treatment of HD could cause iatrogenic damage to these structures and lead to significant urinary and/or sexual dysfunctions (16,32,46). However, only a few studies have focused on urinary and sexual functions in patients with HD. So far, studies have shown a low overall frequency of lower urinary tract symptoms (LUTS) in patients with HD, between 0%–12% (15,16,60,80,92,97,100), which is comparable to the incidence in healthy controls (80,92,148). Also urodynamic results have found to be normal during the first years after TEPT (149). However, compared to TEPT, the PT techniques with open pelvic dissection have shown to lead to higher rates of LUTS (16,32,80). The frequency of infertility and erectile dysfunctions, in turn, have ranged between 0%–11% in males, occurring mostly after
Duhamel and Swenson procedures (16,99,148,150). In females, sexual dysfunctions (dyspareunia and infertility) and sexual distress have occurred in 10%–53% and 20%–45% of patients, respectively (16,150,151). In a more detailed study, decreased perceived self-competence was found to be associated with increased rate of sexual distress in females, problems with forming romantic relations in adolescent males, problems with making close friendships and decreased sensation of sexual desire in adult males, and decreased intercourse satisfaction in both sexes (151).

2.14. QUALITY OF LIFE


Quality of life can be measured by two main approaches: General QoL instruments assess the general health and wellbeing of the patients independently of the disease, while symptom-specific, health-related QoL (HRQoL) measurement focuses on the specific issues relevant to the disease. Numerous validated tools are available for the measurement of HRQoL, of which the PedsQL Generic Core Scales are commonly used for children (152,153) and the Gastrointestinal Quality of Life Index (GIQLI) for adults (154). To measure general QoL, SF-36 is commonly used in adults (155).

2.14.2. Quality of life of HD patients

Patients with HD are known to endure a number of diagnostic procedures, operations and hospital admissions during childhood, and they encounter often diverse long-term bowel functional problems. All of these may have consequences in social functioning, emotional well-being and QoL (11,156,157). All in all, patients with HD have shown to have comparable educational, professional and marital status to the healthy population (92,97,99,158), but the
level of QoL is still unclear due to the contradictory research outcomes. On one hand, most studies have presented equivalent or higher generic QoL scores in HD patients compared to controls (14,15,92,100,159,160), which has often been associated with comparable or even higher physical scores in HD patients compared to controls (14,92,100,159). These paradoxical results may be explained by stronger psychological coping strategies that HD patients develop due to their illness compared to their healthy peers (161,162). This phenomenon has been recognized also in other patient groups with chronic illnesses (163). However, on the other hand, some studies have presented decreased generic QoL scores in HD patients compared to controls (11,156), and some studies have revealed slightly decreased scores in psychosocial and/or mental health domains, including perceived self-competence, in HD patients than in controls (15,92,100).

2.14.3. Prognostic factors

Poor QoL has shown to be predicted by female sex (15,159,164), male sex (14), increasing age (156,159,165), a stoma (165), another congenital disease associated with HD (165), and longer-segment aganglionosis (15,159), which, however, have been contradictory in some cases. The association between bowel symptoms and generic QoL scores in HD patients has also remained obscure (156). Impaired fecal control, especially the presence of fecal soiling and incontinence, has previously been associated with decreases in both disease-specific QoL scores and generic QoL scores (14,100) or only in disease-specific QoL scores (91,156,161,166) or generic QoL scores (11,92,159). However, increasing number of recent studies have not found a direct association between bowel symptoms and disease-specific or generic QoL scores, as patients with existing bowel symptoms have had equal or even higher QoL scores than their healthy peers (15,92,100,159,160,162). This parallels with the theories that suggest the association between bowel symptoms and generic QoL to be mediated by
personal and social resources, including self-satisfaction, emotions and self-acceptance of one’s own body image, rather than having a direct relation between them (11,14,91,98,100,101,156,157,159-161,164). Therefore, parental warmth may also have a strong impact on the child’s total QoL (167).
AIMS OF THE THESIS:

The specific aims of the present study are the following:

1. To determine the long-term bowel functional outcomes up to adulthood in patients with HD after TEPT, and to specify the prognostic factors of HAEC and other bowel functional impairments
2. To determine the long-term urinary and sexual functional outcomes after TEPT in patients with HD
3. To define the QoL of patients with HD, and to clarify the impact of bowel symptoms and other prognostic factors on it
4. To characterize the intestinal microbiota composition of patients with HD and to define its impact on the development of HAEC and other bowel functional impairments
5. To define the levels of FC and the LCT genotype in patients with HD and to unravel their significance on the development of HAEC
4. METHODS

4.1. PATIENTS (I-IV)

We identified all patients who had undergone TEPT for histologically verified HD at our center between 1987-2011. Clinical details were reviewed from case records. Demographic data included gestational age, birth weight, family history, associated syndromes, anomalies and other illnesses, details of growth, and nutritional status. Surgical data included age at operation, operative details, bowel histology, anatomy of the remaining bowel, postoperative complications, and other surgical procedures including ACE and enterostomy. Measures regarding HAEC and bowel function included the frequency of pre- and postoperative HAEC; medications including use of antibiotics; medications for bowel function; impairments in bowel function including fecal soiling, incontinence, constipation and stooling frequency. Retrospectively, bowel function was analyzed in HD patients aged ≥3 without an ACE or enterostomy. Regarding the questionnaire results, bowel function was analyzed in HD patients aged ≥4 without major cognitive impairment due to an associated syndrome and without an ACE or enterostomy. Urinary and sexual functions were analyzed in patients aged ≥4 and ≥16, respectively, without major cognitive impairment due to an associated syndrome.

4.2. DEFINITIONS (I-IV)

The definition of extended aganglionosis was aganglionosis extending proximal to the rectosigmoid colon, and the definition of transitional zone pull-through was aganglionosis or pronounced AChE-staining and immature and small ganglion cells in the pull-through bowel. Fecal soiling was defined as small amounts of fecal staining on underclothing, and fecal accident was defined as involuntary loss of feces. Occasional fecal soiling/accidents indicated a symptom occurring less than once a week, and frequent soiling/accident indicated a
symptom occurring at least once a week. Social continence was defined as fecal soiling or fecal accidents occurring less than once a week with no requirement for protective aids (93). Normal defecation frequency was defined as stooling every other day to twice per day. HAEC was defined based on clinical symptoms including abdominal distension, diarrhea, vomiting and, in some cases, bloody stools and/or fever (118). Recurrent HAEC was defined as symptoms leading to two or more successive treatment episodes.

4.3. QUESTIONNAIRES (II,III)

Between years 2012-2013, an independent investigator contacted patients or their caregiver by post with questionnaires regarding bowel, urinary and sexual functional outcomes and QoL. Additionally, patients were invited to participate in a clinical follow-up, including investigations presented in Section 4.4.

4.3.1. Enquiry of bowel functional outcomes and HAEC (II)

The Bowel Function Score (BFS), which was developed to analyze bowel functional outcomes in pediatric anorectal disorders, is an established 7-item scoring system with a maximum score of 20 (Appendix 1) (90,91,93). It is an observer-independent survey, as the child or their parents may complete it without a requirement for clinical examination. The BFS scores have revealed a correlation with clinical outcomes, and good functional outcomes have been defined as a BFS score ≥17 (90,91,93). Stool consistency was classified as formed, loose and liquid. Moreover, all treatments received within the past year and after the definitive surgery for HAEC were enquired (response scale: yes/no. If yes: once/2-3 times/≥4 times).
4.3.2. Enquiry of LUTS and sexual functions (III)

Items 3 to 9, regarding the enquiry for LUTS (Appendix 2), were adapted from the previously validated Danish Prostatic Symptom Score (168). Items on sexual function (Appendix 3) included the previously validated Erectile Hardness Score for males (EHS) (169).

4.3.3. Enquiry of QoL (II)

Quality of life was analyzed by age groups based on previously validated questionnaires: Pediatric Quality of Life (PedSQL 4.0) (152,153) for patients aged 4–17, and the Gastrointestinal Quality of Life Index (GIQLI) (170) and SF-36 health surveys (155) for patients aged ≥18. For children aged 4–7, PedSQL includes a parent-proxy report only, and for children aged 8–17, it includes both a child self-report and a parent-proxy report. Moreover, GIQLI is a 36-item questionnaire for HRQoL measurement, with a score below 105 indicating constant gastrointestinal symptoms (highest score 144) (170), and SF-36 is a survey for global health-related QoL, with 36 questions (highest score 100 for each scale) (155).

4.4. CLINICAL INVESTIGATIONS (III,IV)

Clinical investigations were performed between years 2013-2014 to all voluntary patients.

4.4.1. Clinical investigations regarding bowel function (IV)

To further investigate bowel symptoms, the brush border lactase (LCT) gene, fecal calprotectin (FC) level, and gut microbiota based on fecal DNA extraction were determined. Because dilutional effects render the fecal sample invalid, patients with an ACE and an enterostomy were excluded from the gut microbiota analysis.
a. **Fecal DNA extraction and sequencing (IV)**

Fecal samples were collected and frozen at −20°C for 4h after collection and stored at −70°C until analysis. Using a repeated bead-beating method, the bacterial DNAs were extracted from the fecal samples (171); using 16S rDNA amplicon sequencing, the microbiota compositions were analyzed. Illumina HiSeq with the forward primer CTACGGGNGGCWGCAG and the reverse primer GACTACHVGGGTATCTAATCC were used to sequence the V3-V4 variable region, but only the V3 region analysis was used in this study. The R package mare was used to process the DNA amplicon sequences (172), but only forward reads were analyzed and trimmed to 150 bases. As potential errors, the reads that represented less than 0.001% of all unique reads were discarded. For taxonomic annotation, the reads were mapped to the Silva reference database for taxonomic annotation, and the abundances were summarized at different taxonomic levels. Clustering for operational taxonomic units (OUTs) with USEARCH (173) were performed only in richness analysis.

b. **Analysis of carbohydrate-active enzyme levels and carbohydrate degradation potential (IV)**

In order to analyze gut microbiota functions, carbohydrate-active enzymes and the carbohydrate degradation potential were assessed using the CAZy database (174). Carbohydrate active enzyme abundances were assessed using predictions based on observed taxonomic profiles.
c. **Fecal calprotectin (FC) (IV)**

A quantitative, enzyme-linked immunoassay (PhiCal Test, Calpro AS, Oslo, Norway; NovaTec Immunodiagnostica, Dietzenbach, GmbH, Germany) was used to measure the FC level. A level of 100 microg/g was taken as the upper limit of normal (29).

d. **LCT genotype (IV)**

Genomic DNA was extracted from blood samples, and the lactase gene *LCT* genotype (LCT-13910) was genotyped using standard methods (175). The *LCT* genotype C/C indicates lactase deficiency and potential lactose intolerance (175).

4.4.2. **Clinical investigations for urine function analysis (III)**

Urinary functions were examined by urine flowmetry (spinning disc transducer URODYN® 1000 (© Mediwatch Plc 2008)), renal tract ultrasound, and laboratory tests including infection parameters, electrolytes, creatinine, blood count, and urinalysis. The flowmetry curves were classified as belly-shaped (normal) or tower-, plateau-, staccato- and interrupted-shaped (abnormal) as defined by International Children’s Continence Society (164).

4.5. **CONTROLS (II-IV)**

4.5.1. **Controls for the survey analysis (II-III)**

The age- and gender-matched normal population controls, who were randomly selected from a previously collected pool of 594 respondents aged 4–26 years and obtained from the Population Register Centre of Finland (93,148), had previously responded to the questionnaires presented in Section 4.3. As the oldest available controls from this pool were 26 years of age, 3 patients aged >27 years had to be matched to 26-year-old controls.
4.5.2. Controls for the microbiota analysis (IV)

The control population for the microbiota analysis consisted of a previously collected cohort of 236 normal Finnish children aged 2–7 without known illnesses (176), of which 141 children had donated fecal samples. Two samples per healthy control were analyzed using a phylogenetic microarray, the HITChip, which resulted in 282 control samples. A small subset of control samples was also sequenced using the same methods as with the patient samples for the standardization of the microarray data (177).

4.6. STATISTICS (I-IV)

4.6.1. General statistics (I-IV)

Statistics were calculated using SPSS Version 21.0 (I-IV). Data are presented as medians and ranges (I,IV), percentages and frequencies (I,II,III,IV), or means ± standard deviations (II,III), if not stated otherwise. A chi-square test or, where appropriate, Fisher’s exact test was used to compare categorical variables, and Mann-Whitney U test was used to compare continuous variables (I-IV). Univariate logistic-regression analysis was used to assess predictors for defective bowel function and recurrent HAEC (I-IV). A $p$ value less than 0.05 was considered significant (I-IV).

4.6.2. Statistics regarding the microbiota analysis (IV)

Statistical analyses were conducted in R, using the package mare, including tools from packages vegan (178), metacoder (179), spatial data (180), MASS (181), and nlme (182). The taxa that occurred in less than 30% of the samples were excluded from the taxon-specific analyses. As the control data derived from a microarray experiment, the tests comparing
patients and controls had no omitted taxa and did not contain zeros. The microbiota composition and relative abundances of bacterial taxa were analyzed by age, stool consistency, histories of antibiotic treatment and HAEC, \textit{LCT} genotype, and FC, as appropriate, and negative binomial models were used to explore associations between them. All analyses were adjusted for patient age, level of aganglionosis, and history of HAEC, apart from the patient-control comparisons. However, if the models were unfit for the data because of heteroscedasticity, general least squares models were used. A \textit{p} value less than 0.005 was considered significant.

4.7. ETHICAL CONSIDERATIONS (I-IV)

The study protocols were approved by the ethics committee for Pediatric, Adolescent Medicine and Psychiatry in the Hospital District of Helsinki and Uusimaa. The participants and their parents were fully informed about the study protocol, data collection, and use of collected data. All patients participated in the studies on a voluntary basis. An informed consent was received from all patients and/or their parents. All patients were informed about the findings of the investigations, and appropriate care was arranged when needed.
5. RESULTS

5.1. RETROSPECTIVE DATA (I)

5.1.1. Patients, surgical complications and requirements for enterostomy and ACE (I)

Overall survival was 98% (n = 143/146) (I). The main patient characteristics, lengths of aganglionosis, and surgical details are presented in Table 1.

Table 1. Patient characteristics and operative details (I)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>146</td>
</tr>
<tr>
<td>Males/females</td>
<td>112/34 (3.3:1)</td>
</tr>
<tr>
<td>Gestation age (weeks)</td>
<td>39 (30–42)</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>3400 (1565–4840)</td>
</tr>
<tr>
<td>Familial disease (%)</td>
<td>15 (10)</td>
</tr>
<tr>
<td>Syndrome* (%)</td>
<td>42 (29)</td>
</tr>
<tr>
<td>Associated isolated congenital anomalies (%)</td>
<td>16 (11)</td>
</tr>
<tr>
<td>Level of aganglionosis</td>
<td></td>
</tr>
<tr>
<td>Rectosigmoid (%)</td>
<td>121 (83)</td>
</tr>
<tr>
<td>Long segment (%)</td>
<td>10 (7)</td>
</tr>
<tr>
<td>Total colonic (%)</td>
<td>6 (4)</td>
</tr>
<tr>
<td>Extending to small bowel (%)</td>
<td>9 (6)</td>
</tr>
<tr>
<td>Age, last outpatient visit (years)</td>
<td>15 (3–33)</td>
</tr>
<tr>
<td>Overall survival (%)</td>
<td>143 (98)</td>
</tr>
<tr>
<td>Age at primary pull-through surgery (weeks)</td>
<td>13 (0.4–722)</td>
</tr>
<tr>
<td>Post pull-through hospital stay (days)</td>
<td>9 (1–1053)</td>
</tr>
<tr>
<td>Transanal pull-through with abdominal operation (%)</td>
<td>100 (69)</td>
</tr>
<tr>
<td>Laparotomy</td>
<td>87 (60)</td>
</tr>
<tr>
<td>Concomitant stomal closure</td>
<td>16 (11)</td>
</tr>
<tr>
<td>Laparoscopy</td>
<td>3 (2)</td>
</tr>
<tr>
<td>Umbilical incision</td>
<td>10 (7)</td>
</tr>
<tr>
<td>Totally transanal pull-through (%)</td>
<td>29 (20)</td>
</tr>
<tr>
<td>Proctocolectomy with ileoanal anastomosis (%)</td>
<td>13 (9)</td>
</tr>
<tr>
<td>Permanent endostomy (%)</td>
<td>4 (3)</td>
</tr>
</tbody>
</table>

Data are presented as frequencies and percentage or medians and range.


In total, 23% (n = 33) of all patients had at least one postoperative complication that required surgical intervention, including six patients requiring redo pull-through operations. Surgical complications occurred more often in patients with extended aganglionosis, a performed transabdominal TEPT (in relation to transanal TEPT), and a history of recurrent HAEC compared to others (p < 0.05 for all). Eight patients required enterostomies, of whom most (n
= 7/8) had aganglionosis extending beyond the rectosigmoid, and 4 had an associated syndrome. Eleven patients required an ACE conduit (n = 11).

5.1.2. Bowel functional outcomes and its predictors (I)

Of all 117 eligible patients, 45% (n = 54) had no soiling, 42% (n = 49) had occasional soiling, 12% (n = 14) had frequent soiling. Constipation occurred in 9% of patients (n = 11). Of all patients without an associated syndrome (n = 89), 51% (n = 45) had no soiling, 45% (n = 40) had occasional soiling, 5% (n = 4) had frequent soiling, and 5% (n = 5) had constipation that occurred occasionally only. In a univariate regression analysis, an associated syndrome was the only significant predictor for postoperative soiling or constipation (p < 0.001; OR 4.5; 95% CI 1.5–12.3), as the level of aganglionosis, age at surgery, totally transanal TEPT, transition zone pull-through, and follow-up age were not (p > 0.05 for all).

5.1.4. HAEC and its predictors (I)

Altogether, 17% of patients had preoperative HAEC and 44% had recurrent HAEC. The respective figures in patients without an associated syndrome were 19% and 38%, respectively. In a univariate regression analysis, significant predictors for recurrent HAEC were extended aganglionosis (p < 0.001; OR 6.9; 95% CI 2.4-19.7) and an associated syndrome (p < 0.019; OR 2.4; 95% CI 1.2-5.0), while age at operation, totally transanal TEPT, and transition zone pull-through were not (p > 0.05 for all).
5.2. DATA OBTAINED FROM THE QUESTIONNAIRES (II,III)

5.2.1. Participants (II,III)

The total response rate was 64% (n = 79/123) (II,III). The characteristics and operative details of respondents and non-respondents are shown in Table 2 (II,III). Baseline characteristics were comparable between the groups (p > 0.05). Altogether, bowel function was analyzed of 57 respondents (median age 15 (range, 4–32) years; 74% male)(II); urinary function and QoL were assessed of 61 respondents (median age 14 (IQR 9–20) years; 72% male), of whom 32 (median age 13 (IQR 8–21) years; 52%) also participated in the clinical follow-up (II,III); sexual function and fertility were analyzed of 24 respondents (median age 22 (IQR 18–24) years; 67% male; 92% rectosigmoid aganglionosis; none had an ACE)(III).

Table 2. Characteristics of the study population (II,III)

<table>
<thead>
<tr>
<th></th>
<th>Respondents (n = 79)</th>
<th>Non-respondents (n = 44)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>15 (4-32)</td>
<td>16 (4-30)</td>
</tr>
<tr>
<td>Sex (M:F)</td>
<td>59:20 (3:1)</td>
<td>35:9 (4:1)</td>
</tr>
<tr>
<td>Level of aganglionosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rectosigmoid</td>
<td>66 (84)</td>
<td>36 (82)</td>
</tr>
<tr>
<td>Long segment</td>
<td>7 (9)</td>
<td>3 (7)</td>
</tr>
<tr>
<td>Total colonic</td>
<td>3 (4)</td>
<td>2 (5)</td>
</tr>
<tr>
<td>Extended</td>
<td>3 (4)</td>
<td>3 (7)</td>
</tr>
<tr>
<td>Operation type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TEPT*</td>
<td>15 (19)</td>
<td>11 (25)</td>
</tr>
<tr>
<td>TEPT with laparotomy/laparoscopy</td>
<td>55/3 (73)</td>
<td>27 (61)</td>
</tr>
<tr>
<td>Ileoanal PT</td>
<td>3 (5)</td>
<td>5 (11)</td>
</tr>
<tr>
<td>Definitive endostomy</td>
<td>3 (4)</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Enterostomy</td>
<td>4 (5)</td>
<td>2 (5)</td>
</tr>
<tr>
<td>ACE</td>
<td>9 (11)</td>
<td>6 (14)</td>
</tr>
<tr>
<td>An associated syndrome</td>
<td>20 (24)</td>
<td>14 (32)</td>
</tr>
<tr>
<td>Recurrent enterocolitis</td>
<td>36 (46)</td>
<td>16 (36)</td>
</tr>
</tbody>
</table>

Data are frequencies (percentage) or medians (range).
* includes procedures with colon biopsies through umbilical incision, and procedures with a stoma closure in conjunction.

5.2.2. Bowel functional outcomes (II)

The impairments of fecal control in HD patients and controls are displayed in Table 4. Patients had higher impairment rates in all aspects of fecal control (p < 0.001 for all) except constipation. Additionally, 54% of patients had abnormal stooling frequency, which was increased in 92% of patients. Altogether, 75% of patients (n = 43) were socially continent (vs
98% of controls; *p < 0.001). Bowel functional outcome was good (BFS ≥17) in 63% of patients (n = 36), moderate (BFS 12–16) in 26% of patients (n = 15), and poor (BFS<12) in 11% of patients (n = 6). The respective figures in controls were 96%, 4% and 0% (*p < 0.001 for all compared to patients).

Table 4. Reported bowel symptoms among HD patients and controls aged ≥4 years. Patients with an intellectual disability, an ACE, or an enterostomy were excluded. (II)

<table>
<thead>
<tr>
<th></th>
<th>HD (n = 57)</th>
<th>Controls (n = 171)</th>
<th>P - value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feels the urge to defecate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Always</td>
<td>40 (70)</td>
<td>158 (92)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Most of the time</td>
<td>13 (23)</td>
<td>12 (7)</td>
<td>0.006*</td>
</tr>
<tr>
<td>Uncertain</td>
<td>4 (7)</td>
<td>1 (1)</td>
<td>0.019*</td>
</tr>
<tr>
<td>Absent</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Mean score ± SD</td>
<td>2.63 ± 0.62</td>
<td>2.92 ± 0.30</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Ability to hold back defecation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Always</td>
<td>42 (74)</td>
<td>163 (95)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Problems less than once a week</td>
<td>10 (18)</td>
<td>8 (5)</td>
<td>0.013*</td>
</tr>
<tr>
<td>Weekly problems</td>
<td>5 (9)</td>
<td>0 (0)</td>
<td>0.001*</td>
</tr>
<tr>
<td>No voluntary control</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Mean score ± SD</td>
<td>2.65 ± 0.64</td>
<td>2.95 ± 0.21</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Normal defecation frequency **</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean score ± SD</td>
<td>1.46 ± 0.50</td>
<td>1.90 ± 0.30</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Fecal soiling</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>18 (32)</td>
<td>118 (69)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Staining less than once a week</td>
<td>25 (44)</td>
<td>50 (29)</td>
<td>0.101</td>
</tr>
<tr>
<td>Frequent soiling, change of underwear required</td>
<td>14 (25)</td>
<td>3 (2)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Daily soiling, protective aids required</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Mean score ± SD</td>
<td>2.07 ± 0.75</td>
<td>2.67 ± 0.51</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Fecal accidents</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>37 (65)</td>
<td>157 (92)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Less than once a week</td>
<td>14 (25)</td>
<td>14 (8)</td>
<td>0.006*</td>
</tr>
<tr>
<td>Weekly accidents, protective aids often required</td>
<td>5 (9)</td>
<td>0 (0)</td>
<td>0.001*</td>
</tr>
<tr>
<td>Daily, protective aids required day and night</td>
<td>1 (2)</td>
<td>0 (0)</td>
<td>0.563</td>
</tr>
<tr>
<td>Mean score ± SD</td>
<td>2.53 ± 0.73</td>
<td>2.92 ± 0.28</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Constipation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No constipation</td>
<td>55 (97)</td>
<td>162 (95)</td>
<td>0.455</td>
</tr>
<tr>
<td>Manageable with diet</td>
<td>0 (0)</td>
<td>7 (4)</td>
<td>0.268</td>
</tr>
<tr>
<td>Manageable with laxatives</td>
<td>0 (0)</td>
<td>2 (1)</td>
<td>1.000</td>
</tr>
<tr>
<td>Manageable with enemas</td>
<td>2 (4)</td>
<td>0 (0)</td>
<td>0.101</td>
</tr>
<tr>
<td>Mean score ± SD</td>
<td>2.89 ± 0.56</td>
<td>2.94 ± 0.29</td>
<td>0.633</td>
</tr>
<tr>
<td>Social problems</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No social problems</td>
<td>40 (70)</td>
<td>163 (95)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Sometimes (foul odors)</td>
<td>10 (18)</td>
<td>8 (5)</td>
<td>0.005*</td>
</tr>
<tr>
<td>Problems causing restrictions in social life</td>
<td>7 (12)</td>
<td>0 (0)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Major social/psychosocial problems</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Mean score ± SD</td>
<td>2.58 ± 0.71</td>
<td>2.95 ± 0.21</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Total bowel function score</td>
<td>16.81± 2.95</td>
<td>19.25 ± 1.14</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

Data are frequencies (percentage) or means ± SD.

* *p < 0.05.
** Normal defecation frequency is defined as stooling more often than every other day or less frequently than twice daily.
Effects of increasing age on bowel functional outcomes (II)

As shown in Figure 5, fecal control improved significantly with increasing age ($p < 0.001$). In addition, the mean total BFS improved with increasing age ($p < 0.001$). In adulthood, all parameters of fecal control were equal between patients and controls ($p > 0.05$), except abnormal stooling frequency. In a univariate regression analysis, only increasing patient age predicted good functional outcome (BFS≥17) ($p < 0.05$), while patient gender, level of aganglionosis, and surgical approach (totally transanal or combined) did not.

**Figure 5.** Prevalence of impairment of fecal control among patients and controls by age group. (II)

NS = non-significant, $p > 0.05$

The number mentioned beneath the age groups equals the number of patients in each age group.

* $P < 0.05$ between patients and controls
Effects of HAEC on bowel functional outcomes (II)

The overall rates of any degree of fecal soiling or fecal accidents obtained from the hospital records did not differ between patients with and without a history of recurrent HAEC ($p > 0.05$ for both), but patients with a history of recurrent HAEC had less good outcome by BFS ($\geq 17$) ($45\% (n = 9/20)$) compared to the patients without HAEC ($72\% (n = 26/36); p = 0.044$). As reported by patients, $42\% (n = 24/57)$ had $\geq 1$ episode of HAEC, and $21\% (n = 12/57)$ had experienced $\geq 4$ treated episodes after their definitive surgery. Within the past year, $13\%$ of patients ($n = 5$) had experienced an episode of HAEC, including 3 patients aged 4–12 years (17%), 2 patients aged 13–17 years (10%), and no adults (0%). The patients aged $\leq 12$ years who have had $\geq 4$ postoperative episodes of HAEC ($n = 7$) reported more frequently fecal accidents (100%) and difficulties withholding defecation (86%) compared to others ($n = 17$; the respective figures were 47% and 29%; $p < 0.05$ for both). However, among patients aged $> 12$ years, the reported HAEC episodes were no longer associated with bowel functional impairments ($p > 0.05$ for all).

5.2.3. Lower urinary tract symptoms (LUTS) (III)

As shown in Figure 6, the overall LUTS profiles in HD patients were at least comparable to controls. Overall, $64\%$ of patients ($n = 39/61$) and $79\%$ of controls ($n = 144/183$) reported at least one type of LUTS ($p = 0.070$), but frequent symptoms occurred only in $15\%$ of patients ($n = 9/61$) and $16\%$ of controls ($n = 29/183$) ($p = 0.84$). Urine flowmetry was performed on 30 patients (2 patients declined), of whom $53\% (n = 16/30)$ had a belly-shaped flowmetry curve and the rest had tower-shaped ($n = 2/30; 6\%$), staccato-shaped ($n = 3/30, 9\%$) or interrupted ($n = 5/30; 16\%$) curves. The type of flowmetry curve was not related to the frequency of LUTS ($p > 0.05$). However, a 21-year-old patient, who had undergone a complication-free laparotomy-assisted TEPT, had significant difficulties emptying the bladder and had a
staccato-shaped curve. He was later diagnosed with a stricture of the bladder neck. The renal tract ultrasound, residual urine volume (median of 6 (IQR 3-11) ml), and laboratory values were normal in all 32 studied patients (median age 13 years).

Figure 6. Prevalence of any impairment in urinary function (%). Patients with an intellectual disability or a definitive endostomy were excluded. (III)

5.2.4. Sexual functional outcomes (III)

As shown in Figure 7, the parameters of sexual functions were otherwise comparable in patients and controls ($p > 0.10$), except that female patients were currently less in a stable relationship compared to female controls ($25\%: n = 2/8$ vs $83\%: n = 20/24$, $p = 0.005$). Furthermore, none of the patients had fertility problems; all three male patients who had attempted to become parents had a healthy biological child.
5.2.5. Quality of life (II)

The QoL scores are presented in Table 5. The parents and children responded differently in items regarding depression/sadness (in which parents gave lower scores than children) and keeping up with the pace of peers at school (in which parents gave higher scores than their children) ($p < 0.05$ for both). Adult patients had lower scores in the emotional subscale of GIQLI compared to adult controls ($p < 0.001$). Additionally, patients reported a greater need to restrict diet due to bowel symptoms (3.1±1.0 vs 3.5±0.8 (response scale 0–5; 5=no effect from illness or no symptoms)), less happiness within the past two weeks (1.1±0.9 vs 3.0±0.8), more strain from changes in the appearance caused by bowel symptoms (3.1±1.3 vs 3.7±0.9), and greater limitation of sexual activities due to illness (3.2±1.3 vs 3.8±0.5) than controls ($p < 0.05$ for all). Furthermore, the mean score for limitations to relationships with friends and family was lower in patients than controls (3.8±0.4 vs 3.9±0.2; $p = 0.039$).
Table 5. Results of QoL questionnaires. Patients with an intellectual disability due to an associated syndrome, an ACE, or an enterostomy were excluded (II).

<table>
<thead>
<tr>
<th></th>
<th>Patient parent-proxy report (n = 12)</th>
<th>Control parent-proxy report (n = 36)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PedsQL</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age &lt; 8 years</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical score</td>
<td>79.17 (16.39)</td>
<td>87.00 (8.75)</td>
<td>0.116</td>
</tr>
<tr>
<td>Emotional score</td>
<td>77.08 (17.38)</td>
<td>71.67 (15.07)</td>
<td>0.382</td>
</tr>
<tr>
<td>Social score</td>
<td>82.08 (22.41)</td>
<td>82.36 (14.62)</td>
<td>0.664</td>
</tr>
<tr>
<td>School score</td>
<td>96.26 (7.93)</td>
<td>90.34 (9.36)</td>
<td>0.013*</td>
</tr>
<tr>
<td>Age 8–17 years</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient self-report</td>
<td>(n = 27)</td>
<td>Control self-report (n = 81)</td>
<td></td>
</tr>
<tr>
<td>Physical score</td>
<td>88.77 (12.97)</td>
<td>87.77 (14.20)</td>
<td>0.844</td>
</tr>
<tr>
<td>Emotional score</td>
<td>82.78 (15.46)</td>
<td>75.62 (14.65)</td>
<td>0.014*</td>
</tr>
<tr>
<td>Social score</td>
<td>94.26 (16.56)</td>
<td>85.19 (17.69)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>School score</td>
<td>80.19 (12.29)</td>
<td>77.28 (15.85)</td>
<td>0.451</td>
</tr>
<tr>
<td><strong>PedsQL</strong></td>
<td></td>
<td>Patient self-report (n = 27)</td>
<td></td>
</tr>
<tr>
<td>Physical score</td>
<td>88.77 (12.97)</td>
<td>89.47 (14.27)</td>
<td>0.250</td>
</tr>
<tr>
<td>Emotional score</td>
<td>82.78 (15.46)</td>
<td>80.93 (15.45)</td>
<td>0.336</td>
</tr>
<tr>
<td>Social score</td>
<td>94.26 (16.56)</td>
<td>94.07 (16.59)</td>
<td>0.739</td>
</tr>
<tr>
<td>School score</td>
<td>80.19 (12.29)</td>
<td>83.89 (13.54)</td>
<td>0.104</td>
</tr>
<tr>
<td><strong>Age ≥ 18 years</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient self-report</td>
<td>(n = 18)</td>
<td>Control self-report (n = 54)</td>
<td></td>
</tr>
<tr>
<td>GiQLI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Symptom score</td>
<td>120.06 (13.11)</td>
<td>125.45 (12.38)</td>
<td>0.100</td>
</tr>
<tr>
<td>Physical score</td>
<td>64.47 (6.49)</td>
<td>65.94 (6.37)</td>
<td>0.391</td>
</tr>
<tr>
<td>Emotional score</td>
<td>22.65 (4.46)</td>
<td>23.29 (3.59)</td>
<td>0.689</td>
</tr>
<tr>
<td>Social score</td>
<td>148.88 (2.09)</td>
<td>17.18 (2.90)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Medical score</td>
<td>14.12 (2.23)</td>
<td>15.06 (1.26)</td>
<td>0.177</td>
</tr>
<tr>
<td>SF-36</td>
<td>3.94 (0.24)</td>
<td>3.98 (0.14)</td>
<td>0.411</td>
</tr>
<tr>
<td></td>
<td>102.59 (2.48)</td>
<td>101.98 (8.63)</td>
<td>0.115</td>
</tr>
</tbody>
</table>

Data are presented as means (standard deviation).
* P < 0.05
5.3. THE GUT MICROBIOTA ANALYSIS (IV)

5.3.1. Patients and bowel functional outcomes (IV)

Altogether, of the 89 survey respondents, 48 patients (54%) participated in the clinical follow-up, of whom 38 (43%) gave stool samples and 37 (41%) blood samples for laboratory analysis. Of the stool samples, 34 remained for the microbiota analysis (median age 12 (range 3–25) years; rectosigmoid aganglionosis 88%; an associated syndrome 18%), as patients with ACE (n = 3) or small bowel enterostomy (n = 1) were excluded. Of these, 28 patients also participated in the survey regarding bowel functional outcomes, of whom half (n = 19/28) and 33% (n = 9/28) reported any and frequent fecal soiling, respectively, 36% (n = 10/28) had increased stooling frequency, and 56% (n = 19/34) experienced loose or liquid stools. None had constipation. Seventy-seven percent of patients (n = 26/34) and 53% (n = 18/34) have had ≥1 episode and recurrent HAEC after the definitive surgery, respectively. All patients with a history of recurrent HAEC (n = 26) had received oral antibiotics, and 83% (n = 15/18) had a history of long-term use of prophylactic oral antibiotics. At the time of the survey, two patients (n = 2/34; 6%) were taking prophylactic oral metronidazole, but none reported having symptoms of HAEC.

5.3.3. The gut microbiota composition (IV)

As shown in Figure 8, relative abundances of *Escherichia, Pseudomonas, Dialister, Actinomyces, Bacilli, and Prevotella* were increased, and most normally abundant taxa, including *Bacteroidales, Ruminococcaceae, Roseburia, and Lachnospiraceae* were decreased in HD patients (*p* < 0.005 (FDR-corrected *p*-value < 0.01 for all)). Furthermore, patients with HD had lower abundances of predicted carbohydrate-active enzymes compared to controls (*p* < 0.005). The microbiota composition of HD patients changed slightly with age: the abundance of *Christensenella* decreased, and the abundance of *Clostridia, Asaccharobacter, Collinsella* and
*Lactococcus* increased ($p < 0.005$). However, the DNA richness stayed constant up to the age of 25 years ($p > 0.05$).

**Figure 9.** Comparison of the microbiota compositions of the patients ($n = 34$) and the controls ($n = 141$). The colors represent the strength of the association: blue color indicates higher abundance in controls and red higher abundance in patients. The size of the nodes represents the relative abundance of the taxonomic group. (IV)
**Effects of recurrent HAEC on the microbiota composition**

A history of recurrent HAEC was associated with lower microbial richness (mean 112 OTUs vs mean 150 OTUs in those without recurrent HAEC; \( p = 0.004 \)), increased abundances of Proteobacteria, *Lactobacillus, Lactococcus* and *Escherichia*, and decreased abundances of Clostridia, *Prevotella, Oscillospira* and *Holdemania* (\( p < 0.05 \) for all, compared to those without a history of recurrent HAEC; **Figure 10**). Additionally, the predicted enzyme-level for carbohydrate degradation was decreased in these patients (\( p < 0.005 \)).

**Figure 10.** Comparison of microbiota compositions between patients with \( n = 18 \); Group 2 in the figure) and without a history of recurrent Hirschsprung-associated enterocolitis (HAEC; \( n = 16 \); Group 1 in the figure), presented as a heat tree. Significantly \( (p < 0.005) \) increased relative abundances of the bacteria in the HAEC group are shown as red, and significantly decreased relative abundances as blue. (IV)
Effects of bowel functional impairments on the microbiota composition

Patients with an abnormal stool consistency had a higher abundance of Proteobacteria and lower abundances of Actinomyces compared to those with normal stool consistency ($p < 0.005$). Additionally, compared to controls, the patients with frequent soiling had decreased abundance of Clostridia ($p < 0.005$). However, increased stooling frequency was not reflected on the microbiota composition ($p > 0.005$).

5.3.5. Fecal calprotectin (FC) level (IV)

The FC level (median 9 (range 0–65) microg/g) was within the normal range in all patients with HD.

5.3.6. LCT genotype (IV)

Altogether, 35/38 blood samples were available for $LCT$ genotyping (1 refusal, 2 missing samples). Six participants (17%) had the $LCT$ genotype C/C, which was not associated with the frequency of HAEC, fecal soiling, increased stooling frequency or abnormal stool consistency ($p < 0.05$ for all).
6. DISCUSSION

6.1. METHODOLOGY

To our best knowledge, this was one the few population-based controlled long-term follow-up studies of HD patients continuing into adulthood (32 years). Voluntary patients were included in the surveys and clinical investigations. Bowel function analysis was performed among patients without an enterostomy or an ACE, and the lower age limits were 3 (I) and 4 years (II,IV), beyond the age when toilet training is normally complete (183). Moreover, in order to avoid the effects of additional major factors, the bowel functional outcomes of patients with and without major cognitive impairment due to an associated syndrome were mainly discussed separately (I,II,IV).

The first article (I) is a single-center, population-based, cross-sectional follow-up study that defined the frequency of long-term bowel functional impairments and their predictors in patients with HD, based on the data obtained from hospital records. As the surgical treatment of HD patients in Finland is mostly centralized in Helsinki, the data used in this study may be considered as nationwide. Moreover, none of the patients were lost to follow-up, and, thus, the patient-sample is all-inclusive. However, the retrospective study design and the data collected from hospital records causes some limitations.

The second article (II) is a case-control survey, which assessed bowel functional impairments, QoL, and their mutual relation in HD patients, based on previously validated patient and parent-proxy questionnaires for different age groups. The results were compared to the previously collected respective results of age- and gender-matched healthy controls (93). This defined the baseline of normality in all ages and, thus, gave the ability to distinguish the abnormal results from normal variations. The patient sample in this study gave a satisfactory
representation of the whole study group, as the response rate was 64%. As the main characteristics between participants and non-participants did not show a statistically significant difference, a significant selection bias was unlikely.

The third article (III) is a case-control survey, which defined the long-term urinary and sexual functional outcomes in HD patients aged ≥4 and ≥16, respectively, without major cognitive impairment due to an associated syndrome, using previously validated questionnaires. As in study II, the response rate was 64% with an unlikely selection bias. The results were compared to respective, previously collected data of age- and gender-matched healthy controls (148). Clinical investigations, including renal tract ultrasound, urine flowmetry and blood samples, were performed on voluntary patients, and the results were analyzed. In terms of clinical investigations, the low participation rate (49% of the survey respondents) causes the main limitation.

The fourth article (IV) went deeper into the etiology of HAEC and other bowel functional impairments in HD patients by defining the gut microbiota composition of the stool samples given by voluntary HD patients via bacterial DNA analysis. The results were compared to previously collected gut microbiota data of healthy controls aged 2–7 (204) and analyzed in relation to HAEC and bowel functional impairments. The analysis of the stool samples was performed in a laboratory dedicated to microbiota analyses. Moreover, the FC level and LCT genotype were determined and analyzed in relation to HAEC and bowel functional impairments. This study provided valuable information of the gut microbiota in HD patients and its significance to the development of HAEC and other bowel dysfunctions. However, the low number of samples (43% of survey respondents gave their stool samples for the microbiota analysis) is an obvious weakness in this study.
6.2. PATIENTS, SURGICAL TREATMENT AND EARLY POSTOPERATIVE COMPLICATIONS (I)
The median age of all patients was 15 years, which compares favorably with several other follow-up studies regarding patients with HD (10,11,14,19,20,24,72,83,84,100,102,184). The ratio of males to females (3.3:1) and distribution of the lengths of aganglionosis (83% rectosigmoid) are proportional to the generally presented ratios (3,33,45,57). However, the proportions of patients with an associated syndrome to HD (29%) and with a familial disease are slightly higher in our cohort compared to the figures in previous studies (7,10,83). This could result to generally worse outcomes compared to respective studies with fewer of these patients. However, to avoid the bias, the functional results of patients with and without an associated syndrome have mainly been discussed separately in our studies. Most patients (69%) underwent TEPT with an abdominal approach, and 20% underwent totally transanal TEPT. The number of patients who underwent transanal TEPT is not sufficiently high to draw definite conclusions regarding its outcomes and the concerns related to it (20). Nevertheless, our studies contribute valuable preliminary knowledge regarding the long-term outcomes after totally transanal TEPT, which is currently a subject of great interest.

Altogether, 23% of all patients had at least one postoperative complication requiring surgical intervention, which is consistent with previous literature (12,63,72,83-85). Surgical complications occurred mainly in patients with a performed transabdominal procedure; only 2 patients who had undergone a totally transanal procedure had postoperative complications requiring surgical treatments, which emphasize the advantage of the totally transanal approach in reducing postoperative complications, which has been noted early in several previous studies (7,18,71). Moreover, the noted relation of extended aganglionosis and a history of recurrent HAEC with surgical complications most likely is also explained by the
performed transabdominal surgery, as transabdominal TEPT was performed on most patients with extended aganglionosis, which, in turn, was associated with recurrent HAEC.

6.3. BOWEL FUNCTIONAL OUTCOMES (I,II)

Our results confirmed that bowel functional impairments are frequent late complications after PT surgery in patients with HD; based on the reviewed hospital records, 55% of all patients, including those with an associated syndrome, had any degree of fecal soiling, and 12% had frequent fecal soiling (I), which parallels with some previous studies (24,72,91). However, after excluding patients with an associated syndrome, the respective rates were 50% and 5%, showing a marked decrease in the frequency of fecal soiling (I) emphasizing the significance of an associated syndrome on the functional results. However, as reported by patients, the rate of frequent soiling was markedly higher, up to 25% (II), which may suggest that the patients’ symptoms have been underestimated in the hospital records, potentially due to the modesty and politeness of the patients towards the operating surgeon, the underestimation of the symptoms by the surgeon, or the insufficient notes made during the outpatient visit. It may also be an indication of an increased willingness of the patients with postoperative problems to participate in surveys compared to the patients without significant problems. Furthermore, despite the increased rates of bowel functional impairments among HD patients compared to healthy controls found in study II, which indicates the need to improve the postsurgical outcome, the finding that none of the patients had absent fecal sensation is noteworthy when assessing the safety of TEPT. Furthermore, constipation was only a minor problem after TEPT, comparing favorably with previous studies (10-12,14,20,60,85,91,97).
Prognostic factors (I,II)

An associated syndrome was the only significant predictor for poor fecal control noted in both study I and II, which corroborates previous studies (24,85). However, in several previous publications, longer-segment aganglionosis has also shown to predict worse functional outcome (12,16,24,55,102), which was not the case in our series (I,II). This may have been caused by the high proportion of excluded patients with extended aganglionosis (44% (I)) from the bowel functional analysis due to the exclusion criteria (I,II). Moreover, fecal control was shown to improve with increasing age, which is a phenomenon recognized in several previous studies (7,10,14,16,24,46,83,85,98,102,106-109) and which may reflect an actual improvement in sphincter function or, at least partly, the individual's adaptation to the imperfections in their fecal control. The finding, that most parameters of fecal control in adulthood were comparable between HD patients and controls, is encouraging knowledge for patients, their families, and healthcare providers and, thus, it may be important to bring up while discussing the prospects of the disease. However, study II includes patients only up to 32 years old and, thus, it cannot exclude the possibility of bowel functional deterioration later in life, as suggested by a study by Järvi et al (91) regarding HD patients aged 35–48 years with a performed Duhamel's procedure.

To our best knowledge, only one previous study has explored the impact of recurrent HAEC on bowel functional results (85), showing a negative association between them. Our results verify this finding, as fewer patients with a history of recurrent HAEC had good bowel functional outcome by BFS (II). However, the effect seemed only mild and temporary, as the history of recurrent HAEC did not have an impact on the overall rates of fecal soiling or fecal accidents, and its associations with the frequency of fecal accidents and difficulties withholding defecation were present only in patients aged ≤12 (II).
As presented in studies I and II, the surgical approach (transanal/transabdominal TEPT) did not have a significant impact on the bowel functional outcome, and, despite the high frequency of other bowel functional impairments, most patients (93%) had a close to normal rectal sensation. Therefore, our findings do not show clear evidence to support the suggestions regarding the unsafety of totally transanal TEPT (20), but, instead, they emphasize the disadvantage of the transabdominal approach of causing an increased occurrence of surgical complications (I). However, further controlled long-term studies with a higher number of patients with a performed transanal TEPT are required to take a definitive stand on its long-term consequences. Recently, attention has also been drawn to surgery performed in the neonatal period, which has suggested to cause increased occurrence of early postoperative morbidity, including anastomotic strictures and leakages, postoperative HAEC, and worse bowel functional results due to the lower immunity, poorer tolerance for infection and surgery, poorer tissue tolerance, and shorter bowel length of the neonates compared to older children (185,186). However, also opposite results exist (187). Our findings did neither support these suggestions (I), although this was not one of our studies’ main research questions.

6.4. HAEC AND ITS PREDICTORS (I,II)

As presented in study I, 44% of all patients had experienced recurrent HAEC during their follow-up period, and the respective figure in study II was 35%, consistent with other reports with similarly low diagnostic thresholds (7,10,12,24). Moreover, study II showed a tendency towards an age-related decline regarding the frequency of recurrent HAEC, parallel to previous studies (9,103,121). However, in contrast to previous literature (9,103,121), HAEC episodes were prevalent also beyond the first years after primary PT, even though no adult patient had experienced an episode of HAEC during the past year (II).
The strongest predictors for recurrent HAEC were both an extended aganglionosis beyond the rectosigmoid and an associated syndrome (I,II), which led to a 7 and 4 times higher occurrence of recurrent HAEC compared to other patients (I), respectively, supporting previous literature (9,124). However, totally transanal TEPT (in relation to transabdominal approaches) and operation age did not have an impact on the frequency of HAEC (I), unlike previous suggestions (19,186,188).

6.5. GUT MICROBIOTA COMPOSITION (IV)

The gut microbiota of HD patients

Study IV displayed an immature gut microbiota composition in all patients with HD, indicated by decreased microbial richness and high abundances of Actinobacteria and Escherichia, which are normally highly abundant in infants, but not in adults (141). The immature microbiota may result from intestinal aganglionosis itself (141) or from the medications or dietary restrictions that are present in many HD patients during the first years of life, which is the most critical phase of gut microbiota maturation (139). Furthermore, as microbial richness is essential for the stability and resilience of the gut microbiota, the decreased microbial richness found in HD patients may render their microbiota vulnerable to the colonization of pathogenic bacteria and the development of pathological outgrowth (189). Moreover, study IV showed that the microbiota of HD patients changed only slightly with increasing age and had constant microbial richness, unlike in healthy individuals (139). This may, at least partially, explain the increased susceptibility of HD patients to develop varied bowel functional imperfections and HAEC. However, the diminishing clinical bowel symptoms with increasing age most likely is explained by other factors than the microbiota.
Patients with a history of recurrent HAEC and other bowel functional impairments (IV)

Study IV showed clear differences in the microbiota compositions between HD patients with and without a history of recurrent HAEC. However, while discussing these results, it must be acknowledged that the all patients with a history of recurrent HAEC used oral antibiotics, as it most likely has a significant impact on the changes found in the microbiota. Firstly, patients with a history of recurrent HAEC had an even more decreased microbial richness than patients without HAEC. Additionally, they had increased abundances of Proteobacteria, Lactobacillus, Lactococcus and Escherichia and decreased abundances of Clostridia, Prevotella, Oscillospira and Holdemania, parallel to previous findings regarding patients with HAEC (21-23). Similar changes have also been found in healthy children with previous use of oral antibiotics (190).

The alterations induced by oral antibiotics have been shown to be largely reversible, at least after single courses of antibiotics (190). However, as the full recovery of the microbiota after a course of antibiotics has shown to take up to 2 years (190), the microbiota may not have time to recover between repeated courses, and, thus, the dysbiosis may become established. This may lead to permanent disturbances in the metabolic and immunological health of the child and manifest as asthma or IBD, amongst other conditions (140,142). These insights are essential, as HD patients are often prescribed multiple courses of antibiotics and long-term prophylactic antibiotics, as in study IV, without knowledge of the potentially harmful consequences.

The expansion of Proteobacteria has suggested to be central in the development of dysbiosis and, thus, in the development of HAEC (191,192). Proteobacteria are Gram-negative opportunistic pathogens with significant pathogenic importance, as they produce potent
inflammatory compounds, lipopolysaccharides (191,192). In a healthy individual, Proteobacteria form a small proportion (1–2%) of the microbiota, but expanded abundances have suggested to be a diagnostic microbial marker for epithelial dysfunction, dysbiosis, and the risk of disease development, including intestinal inflammation and HAEC (191,192).

*Patients with other bowel functional impairments (IV)*

Intestinal motility and the gut microbiota are known to have a bidirectional relation; transit time has a defining influence on the gut microbiota, and the gut microbiota may also modify intestinal motility via serotonin signaling, stimulation of macrophage-ENS crosstalk, and metabolism of bile salt (193,194). Therefore, the nonexistent association between increased stooling frequency and the gut microbiota composition found in study IV was unexpected. However, a link between abnormal stool consistency and expansion of Proteobacteria was stated (IV), which is consistent with previous literature showing similar changes in patients with loose or diarrheal stools and in patients with inflammatory and diarrheal gastrointestinal diseases (195,196) as we found in our patients with loose stools. Therefore, the expansion of Proteobacteria may have a wider pathogenic impact on the bowel functional parameters in HD patients than merely the development of HAEC. All in all, based on our findings, the gut microbiota seems to have an impact on the development of the long-term postoperative complications in patients with HD.

*The carbohydrate-active enzymes (IV)*

As showed in study IV, patients with HD had lower levels of predicted carbohydrate-active enzymes compared to healthy controls. This may result from the low abundances of Clostridia and Bacteroidia, as they are the dominant carbohydrate-degrading taxa. Furthermore, the patients with a history of long-term use of prophylactic antibiotics had even more decreased
levels of carbohydrate-active enzymes than others, which, in turn, may be caused by the
expansion of Proteobacteria, as they participate only little in the digestion and fermentation
of carbohydrates. The reduction of the carbohydrate-active enzymes undoubtedly affects the
host’s ability to derive energy and nutrients from food, as the host intrinsically has a very
limited repertoire of carbohydrate-active enzymes; however, it may also induce bowel
functional changes, as short fatty acids, which are the end products of the fermentation of
complex dietary carbohydrates by the carbohydrate-active enzymes, have also a marked
functional value to the epithelium (197). Therefore, the changes in the levels of carbohydrate-
active enzymes caused by the altered microbiota may participate in creating the clinical bowel
symptoms.

*Fecal calprotectin and LCT genotype (IV)*

As showed in study IV, all patients in our series had FC levels within the normal range.
However, none of our patients had active HAEC at the time of this investigation, which may
have influenced the results. Furthermore, 16% of the patients had the *LCT* genotype C/C,
which did not correlate with the frequency of postoperative HAEC or bowel function
impairments, potentially due to the diet that HD patients at our center follow that exclude
foods with lactose content, especially in the presence of bowel symptoms. Thus, no definitive
conclusion can be drawn from these results.

6.6. LOWER URINARY TRACT SYMPTOMS (III)

Study III showed an equal frequency of LUTS in HD patients compared to healthy controls,
indicating that TEPT is safe regarding the preservation of urinary functions. However, one
patient, who primarily underwent a complication-free laparotomy-assisted TEPT and who
had neither postoperative HAEC or long-term bowel dysfunctions, was diagnosed with
urethral stricture, emphasizing the possibility of iatrogenic injuries during all surgical approaches to HD.

6.7. SEXUAL FUNCTIONAL OUTCOMES (III)

In our series, patients with HD did not have defects regarding physical sexual functioning (III). However, previous studies have shown increased rates of sexual dysfunction and distress in female HD patients (16,150,151), suggesting that the psychosocial aspect of sexual health is more complicated. Patients with HD are exposed to several factors during childhood, including bowel management programs, that may have a negative impact on their perceived self-confidence, sexual development and sexual activities later in life (150). Perceived self-confidence, especially, has shown to have wide-ranging effects on the psychosexuality in HD patients, in addition to its effects on the general QoL (100,151,159-161,167). The psychosocial comorbidity that is related to impaired fecal control may further restrict the establishment of close relationships (198-200). However, study III showed that the only deviating parameter in the items concerning sexual health between HD patients and controls was the lower number of female patients being currently in stable relationships compared to controls. All other items of sexual health (sexual interest, sexual satisfaction and sexual relationships) were similar in patients and controls. These findings present no firm evidence of a broader negative impact of the disease on the patients’ sexual health or psychosexuality. However, our series included only 8 female patients aged ≥16 years, and, thus, definitive conclusions cannot be drawn. Nevertheless, an appropriate follow-up is essential to be arranged, especially in adolescence and early adulthood, to address the potential issues regarding sexuality and its development that HD patients are predisposed to (150).
6.8. QUALITY OF LIFE (II)

As displayed in study II, HD patients in all age groups had equal general QoL scores to their healthy peers. Moreover, HD patients had significantly higher scores than their healthy peers in the subscales of PedsQL concerning school among patients aged <8 years and in the emotional and social domains among patients aged 8–17 years. Additionally, although not on a statistically significant level, the scores of all other domains in PedsQL were also higher in patients than in controls, except the physical domain in <8-year-olds, which was lower in patients (II). These findings confirm the theory of the development of firm coping strategies in children with HD and other chronic illnesses (159,163,165,201). Moreover, patients with HD were shown to have equal QoL scores to their healthy peers despite their frequent bowel symptoms (II), which confirm the understanding that the physical symptoms of HD patients are not directly related to the general QoL (15,92,100,159,160,162,163). The higher scores in the emotional and social domains in HD patients (II), in turn, support the theory that the association of bowel symptoms and general QoL may be mediated by personal and social resources (100,159,160,167), which may compensate the shortages in the physical domain and, thus, maintain the patients’ good QoL. Therefore, healthy self-esteem and self-competence may be a precondition for a good QoL, because they decrease the disease invasiveness, which refers to the negative impact of the disease on the general QoL (163). The child’s self-esteem and self-competence, in turn, is for the most part built on the positive support of the parents, which may, therefore, be the key element of the child’s general QoL (162).

However, parents with HD children may experience concerns and anxiety due to their child’s situation. This may be reflected in the differences in the scores given by the child and by the parents (II). Parental anxiety, in turn, may have a negative effect on the relationship between
the parent and the child, which, in the worst case, may lead to the child's decreased self-competence and QoL (162). Therefore, active psychological support should be offered to the parents during the child's treatment process (202,203). Insufficient knowledge of the child's illness and its treatments given by the medical team has also been associated with increased parental anxiety, which emphasizes the importance of thorough communication between the medical team and the parents (204). Lastly, the knowledge of the decreasing bowel function impairments with increasing age (II) may encourage families with HD children to maintain positivity and hopefulness throughout the demanding treatments of HD.

Adult patients were shown to have lower scores on the emotional subscale than controls, mainly due to restrictions caused by bowel symptoms, despite that the bowel symptoms were significantly diminished in adulthood (II). This may be explained by the higher demands of adulthood compared to childhood in terms of working life, forming lifelong relationships, starting a family, among others. Thus, the existing impairments of fecal control in adults, despite their decreased frequency, may cause more anxiety and restrictions in life than in children, and, thus, have also greater emotional consequences. Moreover, the illness itself as well as the treatments related to it may also contribute to the development of psychological issues in adulthood. However, importantly, our study showed that the decreased emotional scores in adult patients did not affect negatively the total QoL scores, which were equal to adult controls.

6.9. FUTURE CONSIDERATIONS

Teenagers and young adults pose a unique challenge to the medical care, as they transition physically and mentally into adulthood and become independent from parental supervision and guidance in self-care. This population has shown to be vulnerable to lapses in self-care
while being transferred from child to adult healthcare, as transitional programs are often imperfectly adopted to practice (205,206). The requirement of adequate transitional care with a thorough understanding of the long-term physiologic complications is emphasized in patients with HD (205,207), as they frequently experience bowel functional impairments and other difficulties into adulthood that require continued evaluation and management. Moreover, transitional care should consist of multidisciplinary care teams, coordinated support systems and educated adult surgeons for pediatric surgical diseases and their complications to have the ability to address potential psychological and sexual issues that occur in adolescence and early adulthood (205,207,208). In addition, the conversations regarding the transition to and identification of adult providers should be initiated several years in advance to ensure a smooth transition between the child and adult healthcare (205,207,208), as it has shown to have long-term health benefits for these patients (206).

The research concerning HD is undergoing a great transition. Several new details regarding the pathogenesis of HD, HAEC and other bowel functional impairments are being revealed, which may bring along new methods to treat HD primarily and postoperatively, including stem cell treatments (209). In addition, as the impact of the gut microbiota on HAEC and the postoperative bowel dysfunctions in HD patients is being discovered, new means to affect and modify the microbiota may lead to the resolution of the long-term postoperative complications of HD.
CONCLUSION

The aim of this thesis was to define the long-term outcomes after TEPT for HD. The findings confirmed that HD patients experience high frequencies of postoperative bowel functional impairments, especially HAEC and fecal soiling (I,II). Patients with an associated syndrome were found to be prone to develop recurrent HAEC and other bowel dysfunctions, and patients with extended aganglionosis were shown to be susceptible to develop recurrent HAEC (I). Importantly, all aspects of bowel symptoms, except abnormal stooling frequency, diminished to the level of healthy controls by adulthood (II). The findings in study III confirmed the safety of TEPT in terms of preserving urinary and sexual functions, as they were at least comparable to controls. However, fewer female patients were currently in stable relationships, which may indicate increased psychosexual problems caused by HD and its treatments (III). The results regarding QoL are encouraging; the total generic QoL scores in HD patients were at least at the level of their healthy peers, despite existing bowel symptoms. However, the lower emotional scores and the increased personal and sexual limitations shown in adult HD patients are noteworthy, and they may require consideration for the arrangement of future follow-ups and transition in care (II,III). Moreover, families and patients with HD would most likely benefit from psychological support and multidisciplinary care teams throughout the treatments and follow-ups. Especially in adolescence, HD patients require understanding and knowledge of treatments not only for surgical postoperative problems but also for other, including sexual and psychological, issues.

The characterization the gut microbiota of patients with HD revealed clear differences between patients and controls; the most significant alterations in HD patients were with decreased microbial richness and the lack of normal maturation of the microbiota with increasing age (IV). Moreover, the patients with a history of HAEC and use of oral antibiotic
had even more decreased microbial richness, which was associated with the expansion of
Proteobacteria, among other changes (IV). The expansion of Proteobacteria was significant
also in patients with loose stools, indicating that alterations in the gut microbiota may also
participate in the development of other bowel functional impairments than HAEC (IV). These
findings may also suggest that the frequent use of oral antibiotics in HD patients require re-
evaluation in the attempt to avoid the development of established dysbiosis and its lifelong,
harmful, consequences on the patients’ bowel function and health. However, further
prospective studies with larger patient cohorts are required to draw definitive conclusions.
Lastly, the FC level was within the normal range in all patients, and the LCT genotype was
associated with neither HAEC nor other bowel dysfunctions (IV).
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Malla Salli
Helsinki, December 2018
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# APPENDIX 1

## Evaluation on Fecal Continence

<table>
<thead>
<tr>
<th>Description</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feels the urge to defecate</td>
<td></td>
</tr>
<tr>
<td>Always</td>
<td>3</td>
</tr>
<tr>
<td>Most of the time</td>
<td>2</td>
</tr>
<tr>
<td>Uncertain</td>
<td>1</td>
</tr>
<tr>
<td>Absent</td>
<td>0</td>
</tr>
<tr>
<td>Ability to hold back defecation</td>
<td></td>
</tr>
<tr>
<td>Always</td>
<td>3</td>
</tr>
<tr>
<td>Problems less than once a week</td>
<td>2</td>
</tr>
<tr>
<td>Weekly problems</td>
<td>1</td>
</tr>
<tr>
<td>No voluntary control</td>
<td>0</td>
</tr>
<tr>
<td>Frequency of defecation</td>
<td></td>
</tr>
<tr>
<td>Every other day—twice a day</td>
<td>2</td>
</tr>
<tr>
<td>More often</td>
<td>1</td>
</tr>
<tr>
<td>Less often</td>
<td>1</td>
</tr>
<tr>
<td>Soiling</td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>3</td>
</tr>
<tr>
<td>Staining less than once a week, no change of underwear required</td>
<td>2</td>
</tr>
<tr>
<td>Frequent staining/soiling, change of underwear required</td>
<td>1</td>
</tr>
<tr>
<td>Daily soiling, requires protective aids</td>
<td>0</td>
</tr>
<tr>
<td>Accidents</td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>3</td>
</tr>
<tr>
<td>Less than once a week</td>
<td>2</td>
</tr>
<tr>
<td>Weekly accidents, often requires protective aids</td>
<td>1</td>
</tr>
<tr>
<td>Daily, protective aids required day and night</td>
<td>0</td>
</tr>
<tr>
<td>Constipation</td>
<td></td>
</tr>
<tr>
<td>No constipation</td>
<td>3</td>
</tr>
<tr>
<td>Manageable with diet</td>
<td>2</td>
</tr>
<tr>
<td>Manageable with laxatives</td>
<td>1</td>
</tr>
<tr>
<td>Manageable with enemas</td>
<td>0</td>
</tr>
<tr>
<td>Social problems</td>
<td></td>
</tr>
<tr>
<td>No social problems</td>
<td>3</td>
</tr>
<tr>
<td>Sometimes (foul odors)</td>
<td>2</td>
</tr>
<tr>
<td>Problems causing restrictions in social life</td>
<td>1</td>
</tr>
<tr>
<td>Major social/psychosocial problems</td>
<td>0</td>
</tr>
</tbody>
</table>
APPENDIX 2

Lower Urinary Tract Symptoms Questionnaire

1. Have you (Has your child)* had urinary organ diseases?
   - Yes
   - No

2. Have you (Has your child) had a urinary tract infection?
   - Yes
   - No

3. How many times do you (does your child) pass urine each day?
   - 1-3 times
   - 4-8 times
   - More than 8 times

4. Do you (Does your child) need to strain to start/continue urination?
   - No
   - Seldom
   - Often
   - Always

5. Do you (Does your child) get a sudden urge to pass urine?
   - No
   - Seldom
   - Often
   - Always

6. Is the urge so strong that urine escapes before reaching the toilet?
   - No
   - Seldom
   - Often
   - Always

7. Does urine ever leak upon straining (e.g. laughing, sneezing or coughing)?
   - No
   - Seldom
   - Often
   - Always

8. Does urine ever leak without physical activity or need to urinate?
   - No
   - Seldom
   - Often
   - Always

9. Do you (Does your child) have to strain to start/continue voiding?
   - Never
   - Seldom (less than once a week)
   - Often (more than once a week)
   - Always

10. Do you (Does your child) wet bed at night?
    - Never
11. Do you (Does your child) have to wake up at night to urinate?
   - Never
   - Once per night
   - Twice per night
   - Three times per night or more

12. Do you (Does your child) have social problems due to urinary incontinence?
   - No
   - Yes, due to daytime urinary incontinence only
   - Yes, due to night-time urinary incontinence
   - Yes, due to day- and night time urinary incontinence

13. How satisfied are you to your bladder function on a scale 1-5?

* Questions phrased “your child” for parents of respondents less than 8 years of age.
APPENDIX 3

Sexual Health Questionnaire

14. How often do you feel sexual desire or interest?
   - In most cases
   - Sometimes
   - Seldom

15. Are you in a regular relationship?
   - Yes
   - No

16. Have you been so far in a sexual relationship?
   - No
   - Yes

17. If you have had sexual activity, how often do you get satisfaction of it?
   - No sexual activity
   - Seldom
   - Often
   - Most of the times

Females

18. In what age did you have your first menstruation?

19. The length of menstruation (days)?

20. Menstruation
   - Regular
   - Irregular
   - Currently using contraceptive pills: yes / no

Males

1. In what age did you have your first ejaculation?

2. Does your penis harden during the sexual excitement?
   - Does not swell up nor stiffen
   - Swells up but does not stiffen
   - Stiffens but does not harden
   - Stiffens and hardens

Both genders

3. Have you tried to have a child with your partner?
   - No
   - Yes, my spouse (men)/I (women) have not conceived yet
   - Yes, my spouse (men)/I (women) have been conceived
   - My spouse is (men)/I am (women) pregnant at the moment

4. Do you have children?
   - No
   - Yes, how many children?
   - Yes, my spouse is (men)/I am (females) pregnant at the moment
5. Have you needed examinations or treatments for your fertility?
   - I have not tried to get conceived yet
   - There has been no need for it – pregnancies have succeeded
   - No, but we are considering the option
   - Yes