From the Department of Women's and Children's Health
Karolinska Institutet, Stockholm, Sweden

ASPECTS OF HIRSCHSPRUNG DISEASE

Anna Löf Granström

Karolinska Institutet

Stockholm 2016

Cover: Histopathology specimen of normal colon, stained with hematoxylin and eosin (x100). Photo: Abiel Orrego

Published by Karolinska Institutet.

Printed by E-Print AB 2016

ISBN 978-91-7676-243-1

Institutionen för Kvinnors och Barns Hälsa

Aspects of Hirschsprung Disease

AKADEMISK AVHANDLING
som för avläggande av medicine doktorsexamen vid Karolinska Institutet offentligen försvaras i Skandiasalen, Astrid Lindgrens Barnsjukhus

Fredagen den 20 maj 2016 klockan 9.00

av

Anna Löf Granström
Leg. Läkare

Huvudhandledare:
Professor Tomas Wester
Karolinska Institutet
Institutionen för Kvinnors och Barns Hälsa

Bihandledare:
Professor Agneta Nordenskjöld
Karolinska Institutet
Institutionen för Kvinnors och Barns Hälsa

Dr Britt Husberg Sellberg
Karolinska Institutet
Institutionen för kliniska vetenskaper
Danderyds sjukhus

Fakultetsopponent:
Professor Malin Sund
Umeå Universitet
Institutionen för kirurgisk och perioperativ vetenskap

Betygsnämnd:
Professor Sven Cnattingius
Karolinska Institutet
Institutionen för medicin

Docent Ulrik Lindforss
Karolinska Institutet
Institutionen för klinisk vetenskap, intervention och teknik

Professor David Gisselsson Nord
Lunds Universitet
Avdelning för klinisk genetik

Stockholm 2016

“Most people say that it is the intellect which makes a great scientist.
They are wrong: it is character.”
/Albert Einstein

To A, E and C

ABSTRACT

Hirschsprung disease (HSCR) is a congenital defect of the enteric nervous system characterized by a lack of enteric neurons in the distal hindgut. Motility disturbances in the distal colon usually lead to neonatal intestinal obstruction. The birth prevalence of HSCR has been assessed to 1 in 5,000 live births. HSCR is known to be a multifactorial disease caused by both genetic and environmental factors. HSCR can be part of a syndrome, most commonly Down syndrome (trisomy 21). A majority of the patients undergo surgical treatment during the first year of life. There is a risk of impaired long-term functional outcome, with fecal incontinence and obstructive symptoms.

The aim of this thesis was to assess the functional outcome and quality of life (QoL) after surgery for HSCR. The aim was also to investigate environmental risk factors for HSCR as well as assessing it as a risk factor for a patient's future socioeconomic life. An additional aim was to evaluate the molecular background in a family with HSCR combined with multiple sclerosis (MS).

In Study I, mutation screening was performed using exome sequencing in three females in a family; a girl with HSCR, and also her mother and grandmother, both with HSCR associated with MS. A novel heterozygote mutation in the endothelin receptor B gene was found changing arginine at position 133 into a premature stop codon (p.Arg>*).

Study II was conducted as a longitudinal assessment of bowel function with a three-year interval. Twenty-nine patients operated 1998-2009 with laparoscopic assisted pull-through surgery for HSCR in the Stockholm area were eligible for inclusion. Median age at surgery was 104 days (29 days-8 years) and median age at first follow-up was 4 (2-16) years. Soiling for loose stools was reported by 67% of the patients at the first interview and by 59% three years later, whereas soiling for solid stools was reported by 59% and 56% of the patients respectively. The number of patients suffering from constipation decreased significantly from 41% to 14% (p=0.023).

Study III was a cross-sectional study of bowel function and QoL in patients who underwent surgery for HSCR between 1969 and 1994. Validated questionnaires for bowel function and QoL were sent to 60 patients. Forty-eight responded, of who 39 were finally included. For each patient one age- and gender-matched control was selected. The median age at follow-up was 28 (20-43) years and most of the patients had undergone a Soave procedure. Patients with HSCR reported significantly more constipation symptoms and fecal incontinence than the control group. There were no differences in the generic QoL but the symptom-specific QoL showed significantly lower scores for patients with HSCR.

Studies IV and V were population-based register studies. In Study IV, maternal risk factors, perinatal characteristics and the birth prevalence of HSCR were investigated in a case-control study between 1982 and 2012. Study IV included 600 cases of HSCR and 3,000 controls and

their mothers. The results showed that maternal obesity was associated with an increased risk of the child having HSCR (OR 1.74; CI 1.25-2.44) as well as maternal parity of three or more children (OR 1.25; CI 1.00-1.56). Patients with HSCR were born at an earlier gestational age (OR 1.60; CI 1.18-2.17). The birth prevalence in Sweden between 1987 and 2012 was 1.91/10,000. In Study V the impact of HSCR on future educational level and income was assessed in individuals born between 1964 and 2013. In the cohort, 739 individuals were exposed (having HSCR) and 3,847 individuals were unexposed. The median age of the cohort was 25 (16-49) years. The highest educational level did not differ between the groups (p=0.327). Median individual disposable income was 142,200 (0-817,200) Swedish krona (SEK) in the exposed group and 159,900 (0-3418,900) SEK in the non-exposed group (p=0.615).

POPULÄRVETENSKAPLIG SAMMANFATTNING

Varje år föds det i Sverige cirka 20-25 barn med Hirschsprungs sjukdom (HSCR). Sjukdomen orsakas av att nervceller i tarmväggen saknas, oftast i den sista delen av tjocktarmen. Detta får till följd att tarmens motorik inte fungerar tillfredsställande, vilket medför att barnen får symtom i form av kräkningar, uppblåst mage och oförmåga att tömma tarmen. De flesta fallen diagnostiseras under nyföddhetsperioden och behandlingen är operation, där den sjuka delen av tarmen tas bort och den friska delen kopplas till ändtarmens nedersta del.

Denna avhandling syftar till att utöka kunskaperna om HSCR inom områden som genetik, postoperativ tarmfunktion, livskvalitet samt epidemiologi. Studierna har genomförts på patienter opererade i Stockholm från 1969 till 2009, men omfattar även data från svenska nationella register.

I delarbete I gjordes en mutationsscreening på en familj med HSCR och multipel skleros (MS) för att försöka identifiera den molekylära bakgrunden till dessa sjukdomar. Familjen bestod av mormor och mamma med HSCR och MS samt dotter (probanden) med enbart HSCR. Blodproven analyserades med exomsekvensering och en icke tidigare beskriven mutation hittades i endotelin receptor B genen. Mutationer i andra delar av denna gen har tidigare rapporterats förekomma hos personer med HSCR, men inte hos patienter med MS.

I delarbete II gjordes en uppföljningsstudie av patienter opererade för HSCR med titthålsoperation kombinerat med transanal operation utförd på Astrid Lindgrens Barnsjukhus mellan åren 1998-2009. Syftet var att undersöka hur vanligt det är med förstoppningsbesvär och avföringsläckage efter operation. Strukturerade intervjuer utfördes under 2009 (29 patienter med medianålder 4 år) och under 2012 (27 patienter med medianålder 7 år). Studien visade att frekvensen av avföringsläckage var hög (56-67%) och att frekvensen inte minskade med tiden. Förstoppningstendensen var 41 % vid första mätningen men hade minskat signifikant till 14 % fyra år senare.

I delarbete III gjordes en långtidsuppföljning av patienter opererade för HSCR under åren 1969-1994. Syftet med studien var att utvärdera tarmfunktionen och livskvaliteten hos dessa patienter efter att en längre tid förflutit efter operationen. Av de 48 patienter som svarade på enkäten (60 tillfrågade) med validerade frågor om tarmfunktion och livskvalitet, kunde HSCR bekräftas hos 39 patienter. Dessa patienters resultat jämfördes med en kontrollgrupps. Medianåldern vid uppföljningen var 28 år och de flesta patienter hade opererats med Soaves teknik (74 %). Patienter med HSCR hade signifikant mer besvär med förstoppning och avföringsinkontinens än kontrollgruppen. Det fanns ingen skillnad i den upplevda generella livskvaliteten, men patienter med HSCR rapporterade signifikant sämre symtomspecifik livskvalitet än kontrollgruppen.

I delstudie IV gjordes en nationell registerstudie med syfte att undersöka antalet svenska fall av sjukdomen, maternella riskfaktorer och nyföddhetskaraktäristika hos barnet med HSCR. Med hjälp av koppling mellan Medicinska födelseregistret och Patientregistret mellan år 1982 till 2012 kunde 816 möjliga fall identifieras. Efter att ha säkerställt HSCR diagnosen bland dessa fall inkluderades 600 fall och 3 000 köns-och födelseårs matchade kontroller. Födelseprevalensen av HSCR i Sverige mellan 1987-2012 var 1,91 per 10 000 levande födda. Fetma hos mamman under tidig graviditet ökade risken för barnet att få HSCR samt om barnet föddes som nummer tre eller senare i syskonskaran. Barn med HSCR föddes också vid lägre gestationsålder än friska kontroller. Bland de undersökta fallen hade 34,5 % associerade missbildningar, inklusive Downs syndrom.

I delstudie V användes också nationella svenska register för att undersöka ett möjligt samband mellan HSCR och uppnådd utbildningsnivå respektive inkomst. I en nationell kohort hämtad ur folkbokföringsregistret mellan år 1964 och 2013 hittades 1 267 personer med HSCR, varav 739 inkluderades. Alla personer under 16 års ålder eller med kromosomavvikelser exkluderades innan analysen. I Utbildningsregistret och Inkomst-och taxeringsregistret analyserades 389 exponerade individer och 3 847 oexponerade (10 individer matchade för födelseår och kön mot varje exponerad individ). Medianåldern i kohorten var 25 år. Ingen statistisk skillnad avseende utbildningsnivå eller inkomst kunde påvisas. Exponerade individer hade en medianinkomst på 142 200 kr jämfört med 159 900 kr hos oexponerade.

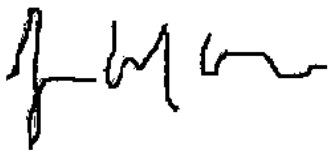
Sammanfattningsvis har avhandlingen visat att det efter operation för HSCR förekommer en hög frekvens av avföringsläckage och förstoppningsbesvär. Den symtomspecifika livskvaliteten är lägre hos patienter som är opererade för HSCR jämfört med en kontrollgrupp, medan den generella upplevda livskvaliteten är likartad mellan de två grupperna. Att leva med HSCR verkar inte påverka möjligheten till utbildning och försörjning. Antalet personer som föds med HSCR i Sverige är cirka en på 5 000 levande födda och har varit oförändrat under åren. Fetma hos mamman under tidig graviditet eller om man föds som tredje barn eller senare ökar risken för sjukdomen. Barn med HSCR föds också vid lägre gestationsålder än friska barn.

PREFACE

Eight years ago, I started to contact patients with Hirschsprung disease (HSCR) for a medical degree project. After a couple of telephone calls, I talked to the mother of a twelve-year-old boy with HSCR. She told me that everything was fine with the boy and his bowel function. But when I specifically asked about soiling problems, she told me that he had a second locker at school where he kept extra clothes in case of soiling. Since then, that boy has remained in my mind along with the following thoughts: how is the bowel function after surgery for HSCR? What are the consequences for a patient's future life and quality of life? How could the boy's mother say that everything was fine? Is what I have learned really true, that patients born with HSCR undergo surgery and then live normal lives thereafter?

Looking back now, I never thought that I would sit here, eight years later, writing the preface to my thesis on HSCR. That young boy is twenty years old now and my deepest wish is that this thesis will help improve healthcare not only for him but also for others in his situation, and that they can live as symptom-free as possible.

Astrid Lindgrens Barnsjukhus, February 15, 2016



Anna Löf Granström

LIST OF SCIENTIFIC PAPERS

This thesis is based on the following studies, which will be referred to in the text by their Roman numerals (I-V).

I. **Granström AL**, Markljung E, Fink K, Nordenskjöld E, Nilsson D, Wester T, Nordenskjöld A.
A novel stop mutation in the EDNRB gene in a family with Hirschsprung's disease associated with multiple sclerosis.
Journal of Pediatric Surgery 2014;49:622-5.

II. **Granström AL**, Husberg B, Nordenskjöld A, Svensson PJ, Wester T.
Laparoscopic-assisted pull-through for Hirschsprung's disease, a prospective repeated evaluation of functional outcome.
Journal of Pediatric Surgery. 2013;48:2536-9.

III. **Granström AL**, Danielson J, Husberg B, Nordenskjöld A, Wester T.
Adult outcomes after surgery for Hirschsprung's disease: Evaluation of bowel function and quality of life.
Journal of Pediatric Surgery. 2015;50:1865-9.

IV. **Granström AL**, Svenningsson A, Hagel E, Oddsberg J, Nordenskjöld A, Wester T.
Maternal risk factors and perinatal characteristics of Hirschsprung disease.
Manuscript submitted.

V. **Granström AL**, Svenningsson A, Nordenskjöld A, Wester T.
Hirschsprung disease; impact on educational level and income.
Manuscript submitted.

LIST OF ABBREVIATIONS

AD	Autosomal dominant
AR	Autosomal recessive
BC	Before Christ
BMI	Body Mass Index
CI	Confidence interval
CNS	Central nervous system
DNA	Deoxyribonucleic acid
DS	Down syndrome
ECE-1	Endothelin converting enzyme 1
EDNRB	Endothelin receptor type B
EDN3	Endothelin 3
EDTA	Ethylene diamine tetraacetic acid
ENS	Enteric nervous system
GDNF	Glial cell-derived neurotrophic factor
GIQLI	Gastrointestinal Quality of Life
GLI	Glioma-associated oncogene homolog
HAEC	Hirschsprung-associated enterocolitis
IBD	Inflammatory Bowel Disease
ICC	Interstitial cells of Cajal
ICD	International Classification of Diseases
KBP	Kinesin-binding protein
LAP	Laparoscopic assisted pull-through
MBR	The Swedish Medical Birth Register
MEN 2	Multiple Endocrine Neoplasia 2
MS	Multiple Sclerosis
MTC	Medullary Thyroid Carcinoma
NANC	Non-noradrenergic, non-cholinergic transmitters
NPR	The Swedish National Patient Register
NRG1	Neuregulin 1

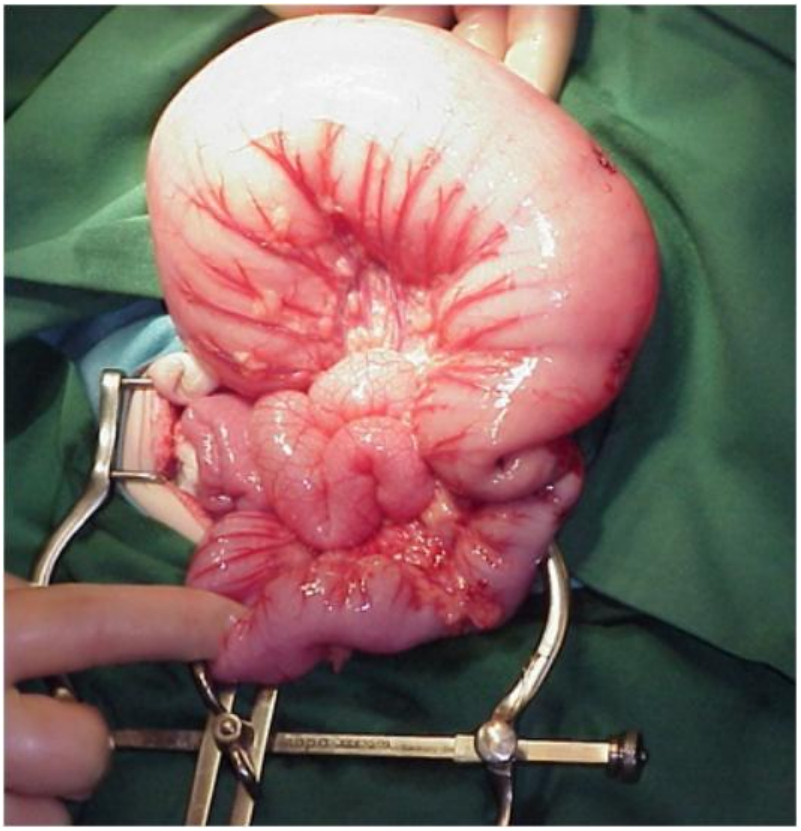
NRTN	Neurturin
OR	Odds Ratio
PCR	Polymerase chain reaction
PHOX2B	Paired-like homeobox 2b
QoL	Quality of Life
RA	Retinoic acid
RET	Rearranged during transfection protooncogene
SEK	Swedish krona
SF-36	The Short Form (36) Health Survey
SGA	Small for gestational age
SOX10	Sex determining region Y box 10
SRY	Sex determining region Y
TCA	Total colonic aganglionosis
TERPT	Total transanal endorectal pull-through
WHO	World Health Organisation
WS4	Waardenburg-Shah Syndrome
ZEB2	Zinc finger homeobox 2

TABLE OF CONTENTS

1 INTRODUCTION

Hirschsprung disease (HSCR) is a congenital disorder characterized by the absence of enteric neurons along variable length of the colon. The aganglionosis leads to a tonic contraction of the affected segment giving rise to an intestinal obstruction. It is known as a multifactorial disease, caused by both genetic and environmental factors. The birth prevalence is 1 in 5,000 live births. Treatment consists of surgical removal of the aganglionic part of the colon.

Figure 1. The photo below shows the contracted aganglionic colon and dilated ganglionic proximal colon. Hirschsprung disease has also been named congenital megacolon.
(Photo: Anna Löf Granström)



Through this thesis you will have the opportunity to follow George. George was born full-term as the first child to two healthy parents in 2008.

1.1 HISTORY OF HIRSCHSPRUNG DISEASE

In Berlin in 1888 the Danish pediatrician Harald Hirschsprung (1830-1926) presented findings of megacolon in two infants with fatal bowel obstructions.[1] Since then, the acronym

Hirschsprung disease has been used for aganglionic congenital megacolon. Megacolon had been described before Dr Hirschsprung's publication and some speculate that even Hindu surgeons about 2,000 BC had some knowledge of the disease.[2]

The doctoral thesis "Megacolon in the newborn", which was defended 70 years ago at Karolinska Institutet by Theodor Ehrenpreis, was one milestone in the history of HSCR. Ehrenpreis had performed repeated contrast enemas in children with HSCR and concluded that the pathology was not located to the dilated part of the colon but to the narrow distal part.[3] Whitehouse and Kernohan first confirmed the histopathological diagnosis characterized by the lack of enteric neurons.[4]

In 1949, Orvar Swenson made another milestone contribution, when he introduced a new surgical technique for HSCR; the Swenson procedure. From this time forward there was no other treatment for HSCR than surgery. He also performed manometery and concluded that there were no peristaltic waves in the aganglionic segment and that the patients had an increased baseline tonus compared with controls.[5]

Figure 2. Photo of Harald Hirschsprung. Published by kind permission of The Royal Library, Copenhagen, The Collection of Prints and Photographs.

1.2 NORMAL DEVELOPMENT OF THE ENTERIC NERVOUS SYSTEM

The fetal gastrointestinal tract is derived from the endoderm and is divided into three segments based on vascular supply. The foregut comprises the esophagus, stomach and proximal part of the duodenum and is supplied by the celiac artery. The midgut consists of the small and large intestine down to the splenic flexure and is supplied by the superior mesenteric artery. The hindgut is the remaining part of the large intestine down to the superior part of the anal canal and is supplied by the inferior mesenteric artery. The enteric

nervous system (ENS) primarily develops from neural crest cells that emigrate from the neural tube somite 1-7, also called the vagal level of the neural crest.[6]

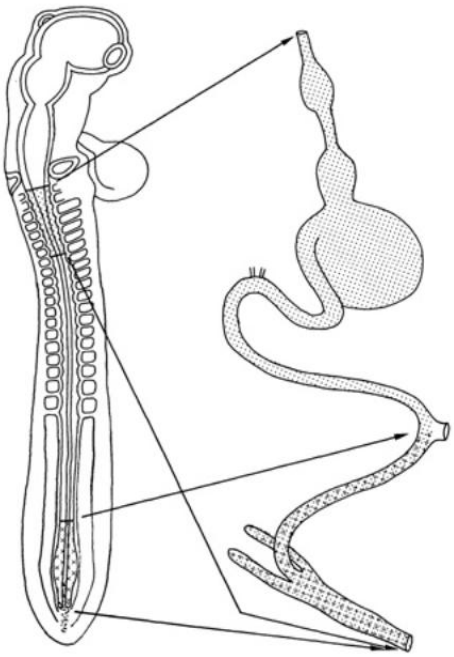
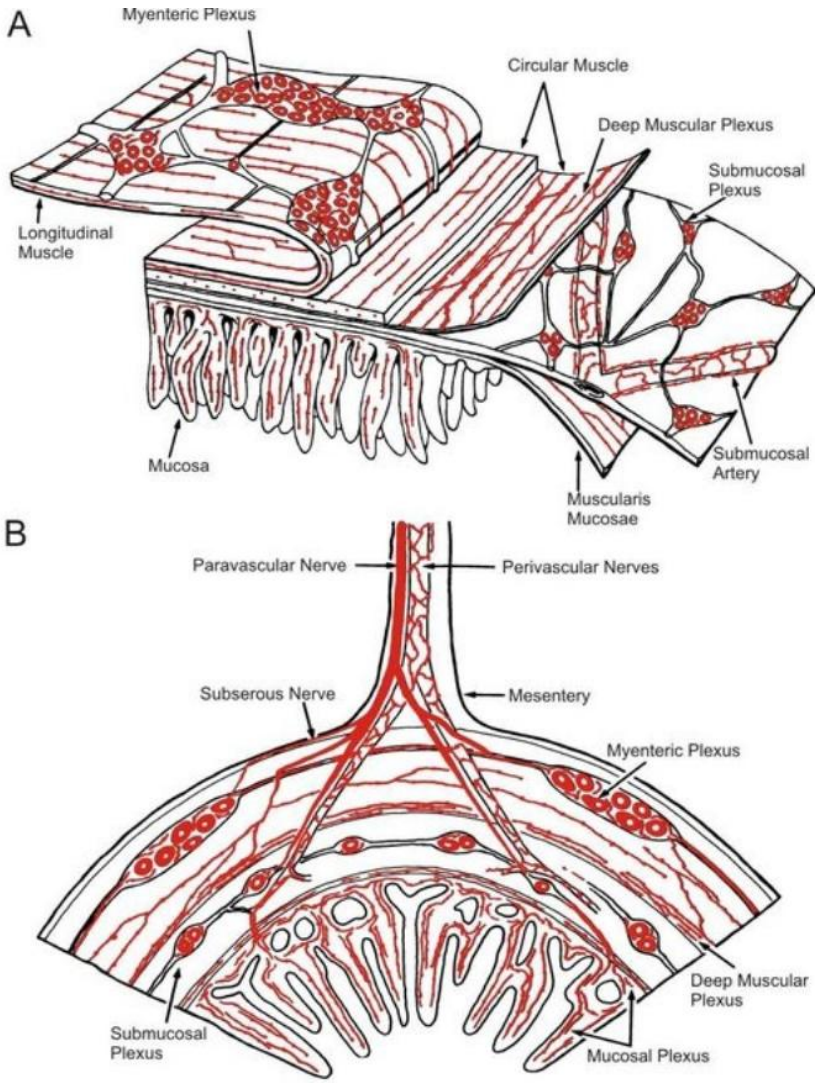


Figure 3. The organization of the developing enteric nervous system.[7] Reproduced by kind permission of Le Douarin and Telliet, Development, 1973;30:44. The Company of Biologists Ltd.

A minority of the neurons in the hindgut derive from the sacral neural crest (behind somite 28).[7-9] The vagal neural crest-derived cells enter the foregut and then migrate caudally along to the hindgut during the 5th and 12th weeks of gestation.[10] During the migration and colonization of the gut most of the neural crest cells are undifferentiated and respond to proliferative signals.[11] During the proliferation and maturation, the neural crest cells differentiate from a stem-cell like state to different types of neurons and glial cells.[12] The neurons (also called ganglion cells) are then organized as intramural ganglia (a cluster of neurons), in the myenteric (Auerbach's plexus) and submucosal (Meissner's plexus) ganglia, and as extramural neurons, which connect neuritis in the gut wall to the cell bodies in autonomic or sensory ganglia.[13] In order to develop normally, migrating neural crest cells interact with the local gut environment for different signalling cues along their way.[14] Different signalling pathways are involved in this process as well as different transcription factors. The composition of the extracellular matrix may also influence gut colonization and maturation of the ENS.[15,16]

Figure 4. Anatomy of the enteric nervous system showing different layers of the intestinal wall. The picture is taken from The Enteric Nervous System, Chapter 1, page 2, John Barton Furness, 2005, and published by kind permission of Johan Wiley and Sons.



1.3 FUNCTIONS OF THE ENTERIC NERVOUS SYSTEM AND ANAL SPHINCTERS

The myenteric plexus innervates the longitudinal and circular muscle layers to obtain bowel movements, while the submucosal plexus innervates the circular layers as well as the mucosa and submucosal vessels and therefore regulates more of the secretion and blood flow.[17] In both the myenteric plexus and the submucosal plexus a meshwork of nerve bundles form a primary plexus connecting the ganglia. Different neurotransmitters play an essential role in the ENS functions such as acetylcholine, adrenaline, norepinephrine and non-noradrenergic, non-cholinergic transmitters (NANC) as nitric oxide and substance P play an essential role for the ENS functions. The ENS can function independently of the central nervous system (CNS) and is therefore also called the brain of the gut, but is also connected to the CNS by motor input and sensory output.[6,18]

Gut motility is a complex process caused by an interaction between intestinal smooth muscle cells, interstitial cells of Cajal (ICC) and the ENS. ICC directs the bowel movements to the enteric motorneurons. The ENS is essential for widespread coordination of the signal to the intestinal smooth muscle cells to generate segmentation and peristaltic waves. Both occur in the absence of extrinsic innervation but require an intact myenteric plexus. Except for gastrointestinal motility, the main functions of the ENS are controlling the mucosal secretory cells, neuroendocrine cells, microcirculation as well as immunomodulatory and inflammatory cells.[19]

The anal canal is composed of the external and the internal anal sphincter and surrounding collagen tissue. Their main functions, to coordinate the act of defecation and to maintain continence in between, are achieved at an average age of 28 months.[20] The external sphincter is a voluntarily regulated striated muscular tube. Its main function is to contract to prevent defecation until appropriate. The internal sphincter, which is involuntarily regulated, is responsible for holding continence by maintaining most of the resting anal canal pressure.[21] It has the ability to transiently relax when the rectum is distended, known as the rectoanal inhibitory reflex, which is missing in patients with HSCR because of the lack of ganglion cells.[22] The congenital absence of enteric ganglia in combination with the possible effect on smooth muscle cells of the hypertrophic nerve trunks results in obstruction.[23] This prevents the colonic mass movements to propagate through the aganglionic segment, which consequently remains in a tonic state. Furthermore, the presence of feces in the rectum fails to elicit relaxation of the internal anal sphincter, which contributes to the clinically observed obstructive symptoms.

1.4 ETIOLOGY

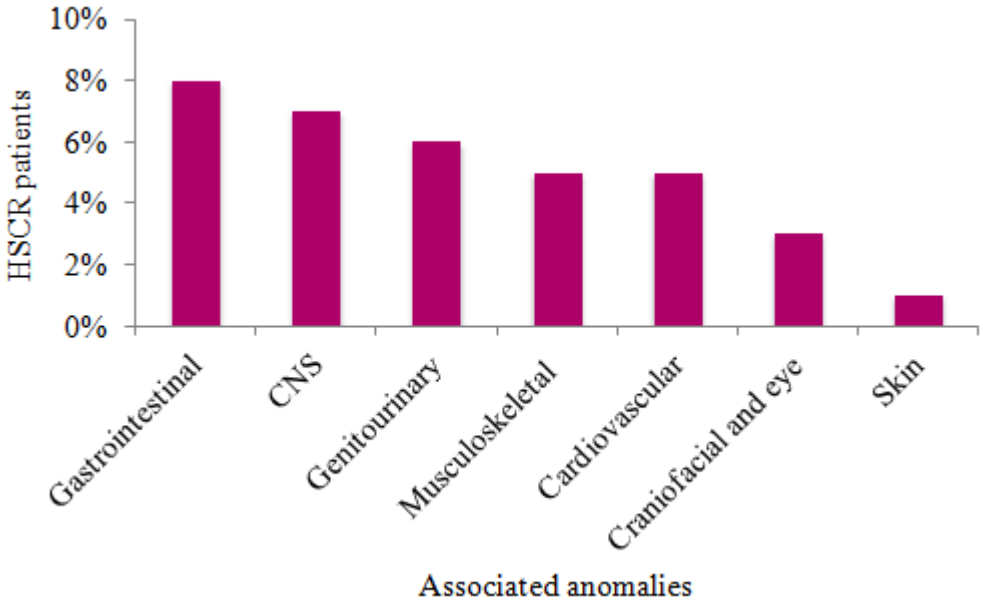
The absence of enteric ganglion cells in the myenteric and submucosal plexi along variable portions of the gastrointestinal tract is pathognomonic for HSCR. The underlying mechanism of HSCR is still unknown, but there is support for three main theories. One is that the migration of neurons from the neural crest is defective and therefore causes aganglionosis. Another hypothesis is the hostile microenvironment (the presence or absence of local factors in the extra cellular matrix) and its possible negative effect on the migration and maturation of the neurons. The third theory concerns a possible defective differentiation and formation of the enteric ganglia, caused by survival and selective death (apoptosis).[24] The exact mechanism is unknown, however, but HSCR is concluded to be a multifactorial disease caused by both genetic and environmental factors.

1.4.1 Genetic factors

HSCR can be inherited as an autosomal dominant, autosomal recessive and even as a polygenic disorder. In the majority of cases HSCR is inherited with low, sex-dependent penetrance, suggesting a complex pattern of inheritance. For short-segment disease, there is a 4:1 male to female predominance compared to with long segment where the ratio is 2:1. The sibling recurrence risk is 4%, giving a relative risk of sibs as high as 200 compared with the

general population.[25] Furthermore, HSCR is associated with other congenital malformations in 5%-32% of cases. The most commonly reported anomalies are found in the gastrointestinal tract (8%) followed by CNS anomalies (7%).[26] The incidence of anomalies associated with HSCR is shown in Figure 7. HSCR may also be syndromic, where the most common syndrome is Down syndrome (DS). Seven to 15% of patients with HSCR also have DS.[27,28] Other syndromes that are associated with HSCR are, for instance, Waardenburg-Shah syndrome (WS4), Mowat-Wilson syndrome, Goldberg-Shprintzen syndrome, Pitt-Hopkins syndrome and Haddad syndrome (congenital central hypoventilation syndrome).

Figure 5. Graph showing the mean incidence of associated anomalies with Hirschsprung disease (HSCR).[26] CNS: central nervous system.



Mutations in gene encoding components in the ENS signalling pathways are associated with HSCR and the genes identified until now are shown in Table 1. The first and major susceptibility gene is the proto-oncogene rearranged during Transfection *(RET*), that is also involved in multiple endocrine neoplasia type 2 (MEN 2), causing medullary thyroid carcinoma, pheochromocytoma and primary hyperparathyroidism.[29,30]A *RET* mutation is identified in 50% of the familiar cases and 15% of the sporadic cases of HSCR.[31] *RET* is a transmembrane tyrosine kinase receptor. Activation of RET promotes proliferation, migration and differentiation of the ENS.[32] *RET* also exerts a pro-apoptotic effect that is inhibited by Glial cell line-derived neurotrophic factor (GDNF).[33]

GDNF is part of a family of neurotrophic factors that bind to a cell surface protein called GDNF family receptor alphas. This ligand complex activates the RET tyrosine kinase. This pathway is essential for the survival, proliferation, migration and differentiation of the neural crest cells.[34,35] HSCR is rarely associated with mutations in *GDNF*.[36]

Endothelin 3 (EDN3) is a ligand for a G protein-coupled endothelin receptor type B (EDNRB) that plays a role in the development of ENS. Mice lacking *edn3* or *ednrb* develop colonic aganglionosis.[37] This signalling is also required for melanocyte development.[34,38] In HSCR patients, *EDNRB* or *EDN3* mutations are found in about 5% of cases.[39]

Sex determining region Y related box 10 (SOX 10) transcription factor plays an essential role in ENS formation since it is necessary for the expression of *RET* and paired-like homeobox 2B (*PHOX2B).*[40,41] Mutations in the *SOX10* have been identified in patients with WS4 including HSCR and deafness with pigmentation abnormalities.[42]

PHOX2B is a homeodomain transcription factor, expressed prior to the entry of vagal neural crest cells into the gut.[43,44] Mutations in the *PHOX2B* gene are rare in patients with HSCR but may cause HSCR, congenital central hypoventilation syndrome, and neuroblastoma in various combinations.[45]

Zinc finger homeobox 2 *(ZEB2)* codes for a zinc finger homeobox that is involved during early neural crest development.[46,47] Mutations in this gene cause Mowat-Wilson syndrome, which is characterized by moderate or severe intellectual disability, a characteristic facial appearance, microcephaly, epilepsy, agenesis or hypoplasia of the corpus callosum, congenital heart defects, HSCR and urogenital/renal anomalies.[48]

To explain the gender disparity among HSCR patients, interference of the sex-determining region Y (*SRY)* gene has been suggested. The *SRY* gene is the sex-determining gene on the Y-chromosome and is a transcription factor gene. Li et al have shown that *SRY* competes with *SOX10* for binding to the *RET* gene, but acts as a negative modifier on *RET* expression.[49]

Hedgehog signals regulate gut organogenesis and ENS development. There is speculation, therefore, about their possible impact on HSCR. It has been shown that Indian hedgehog mutant mice show features typical of HSCR.[50] Also, the Sonic hedgehog protein has been shown to induce intestinal aganglionosis if overexpressed.[51] Another connection between HSCR and the Hedgehog family is the transcription factors glioma-associated oncogene honomlog (GLI), which mediates Hedgehog signalling. Recently published data suggest that a new mutation in the *GLI* genes causes HSCR in mice.[52]

Table 1. Genes in which coding sequence mutations are associated with Hirschsprung disease in humans.[53,54] AD: Autosomal Dominant, AR: Autosomal Recessive, MEN 2: Multiple Endocrine Neoplasia 2, WS4: Waardenburg-Shah Syndrome.

Gene	Map location	Mode of inheritance	Phenotype in mutants
RET	10q11	AD	Non-syndromic, MEN 2
EDNRB	13q22	AD/AR	Non-syndromic/WS4
EDN3	20q13	AD/AR	Non-syndromic/WS4
NRG1	8p12	AD	Non-syndromic
GDNF	5p13	AD	Non-syndromic
NRTN	19p13	AD	Non-syndromic
ECE-1	1p36	AD	Non-syndromic
ZEB2	2q22	AD	Mowat-Wilson syndrome
SOX10	22q13	AD	WS4
PHOX2B	4p12	AD	Haddad syndrome
KBP	10q22	AR	Goldberg-Shprintzen megacolon syndrome

1.4.2 Genetic methods

The study of genes has proven to be a powerful approach to understanding biological systems and disorders. The genetic methods available for use in investigations are developing rapidly with new findings and developing technology. Deoxyribonucleic acid (DNA) sequencing according to Sanger is used to search for mutations in candidate genes.[55] The goal is to determine the order of the nucleotide base (adenine, guanine, cytosine and thymine) and to compare this with a reference. Variants found are compared with those reported in databases with data from healthy individuals and characterized according to the effect of the mutation (synonymous/non-synonymous, missense or nonsense mutation). This is a polymerase chain reaction (PCR) based method that works by stepwise prolongation of a specific DNA target sequence, using target-specific primers and a thermostable DNA-polymerase. The DNA sequence is determined through size-based separation of the fragments identified through detection of wavelengths of emitted fluorescence.

Exome sequencing is another method used in the search of genetic variations responsible for different disorders.[56] This method sequences only exons, which limits the study to only known coding regions of genes that affect protein function. This is a high throughput, computerized method in which millions of parallel elongation reactions are investigated at the same time, also with the use of fluorescence, and build up a sequence. The method enables the processing of a larger amount of data than DNA sequencing but the analyses are more

complicated than Sanger sequencing. The aim of the process is the same, however, to find possible mutations.

1.4.3 Environmental factors

Environmental factors increasing the risk of HSCR have been sparsely studied. Vitamin A (retinol) is an essential nutrient and a precursor to retinoic acid (RA). Fu et al have suggested that RA is essential for efficient ENS migration and proposed that some cases of HSCR might be preventable by ensuring adequate maternal vitamin A levels during early gestation.[57] In a chick embryo model by Gasc et al, the combination of phosphoramidon with glucocorticoid (dexamethasone) resulted in a lower frequency of malformations in male embryos but a higher frequency in female embryos, compared with treated only with phosphoramidon. These findings encourage the possible impact of environmental factors, such as maternal stress during pregnancy on the fetal development.[58] Another possible environmental factor may be benzophenone-3, commonly used in personal care products for ultraviolet filter. It has been associated with HSCR in the child of maternal prepregnancy use.[59]

Untreated hypothyroidism in the embryo or the mother during pregnancy may be another environmental factor. In a case report describing congenital hypothyroidism associated with HSCR, Kota et al speculated upon the importance of thyroxin levels for the development of the intestines.[60] This may explain why patients with DS, who have an increased risk of hypothyroidism, also have an increased risk of HSCR.[61]

Another possible environmental factor is maternal medical drug use. In animal models maternal intake of ibuprofen (used for anti-inflammatory effects) or mycophenolate (used for suppressing the immune system) can cause ENS malformations with an HSCR-like pathology, which calls for studies on the maternal intake of drugs during pregnancy and the associated risk of HSCR.[62,63] There have also been vague speculations about the use of selective serotonin re-uptake inhibitors (used for anti-depression) and HSCR.[64]

As a parallel to HSCR and environmental factors, the protozoan parasite Trypanosoma cruzi causes Chagas disease, a disease similar to HSCR.[65] In its chronic phase, Chagas disease is characterized by damage of intramural neurons in the colon. The exact mechanism is still unclear, but a combination of a direct effect of the parasite and an autoimmune response is likely.[66]

1.5 EPIDEMIOLOGY

The birth prevalence of HSCR varies in the literature between 1 in 2,000 to 1 in 12,000 live births, with the highest birth prevalence in Asian populations and the lowest in Hispanics.[67] A large register-based study in Europe reported a birth prevalence of 1.09 in 10,000 live births.[68] This study also reported a small increasing trend although this is probably is due to better registration in the different registers than a true increase. Whether it is preferable to describe incidence or birth prevalence for congenital malformations has been discussed. The

population at risk is unknown due to the unknown number of conceptions that reach the gestational age when the congenital malformations may occur. Since the incidence is calculated on the population at risk, which remains unknown, the calculation on birth prevalence is preferred.[69]

Risk factors and the perinatal characteristics of HSCR have been sparsely studied. Mostly, only small studies are presented in the literature, assessing maternal age, parity and ethnicity, and the results have not been consistent. Goldberg showed an association between maternal age and a risk of HSCR in offspring, which could not be confirmed by Russel et al or Best et al.[70-72] An increased risk of having HSCR for the first born has been reported.[73] Several studies have shown that maternal obesity is associated with an increased risk of structural anomalies in general, but it has not been shown for HSCR specifically.[72,74,75]

Patients with HSCR have been reported to be born at an earlier gestational age than controls.[73] The prevalence of prematurity among HSCR patients was reported to be 4-19.4% (overall prevalence, 14%) in a systemic review based on studies from 2000 to 2013.[76] The reason for being born earlier is still unknown but preterm patients with HSCR have more associated anomalies than full-term patients with HSCR, which may be an explanation.[77]

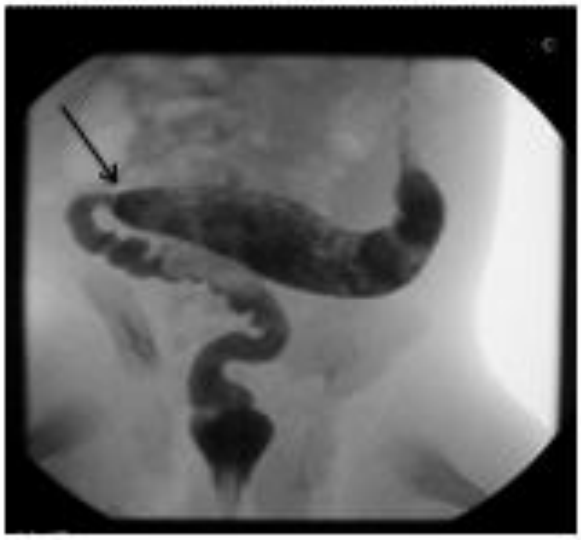
1.6 CLINICAL FEATURES

At two days of age, George had not yet passed meconium. He had vomited and his abdomen was distended. He was admitted to the pediatric surgical clinic. First, he was resuscitated with intravenous fluids and the bowel was decompressed with regular irrigations. Abdominal radiographs showed a bowel obstruction. A couple of days later he was well and could start feeding. Rectal suction biopsies were taken at the ward and a contrast enema showed a suspected transition zone at the recto-sigmoid level. The rectal suction biopsies showed aganglionosis in all three samples; therefore George was planned for surgery.

Most patients (90%) with HSCR present as new-borns with symptoms of neonatal bowel obstruction with delayed abdominal distension (76%), vomiting (69%), passage of meconium (57%), and Hirschsprung-associated enterocolitis (HAEC) (10%), whereas other patients suffering from chronic constipation are diagnosed later in life (10%).[78] The clinical presentation is similar in full-and preterm babies, however, preterm babies have a higher frequency of associated malformations and total colonic aganglionosis (TCA).[76]

The diagnostic procedures comprise rectal suction biopsies, contrast enema and anorectal manometry. Contrast enema shows the transition zone (the area between the dilated bowel and the narrow aganglionic part) in approximately 74% of cases.[79] Anorectal manometry is a reliable non-invasive test showing, in the case of HSCR, absence of the rectoanal inhibitory reflex in response to rectal distension.[80]

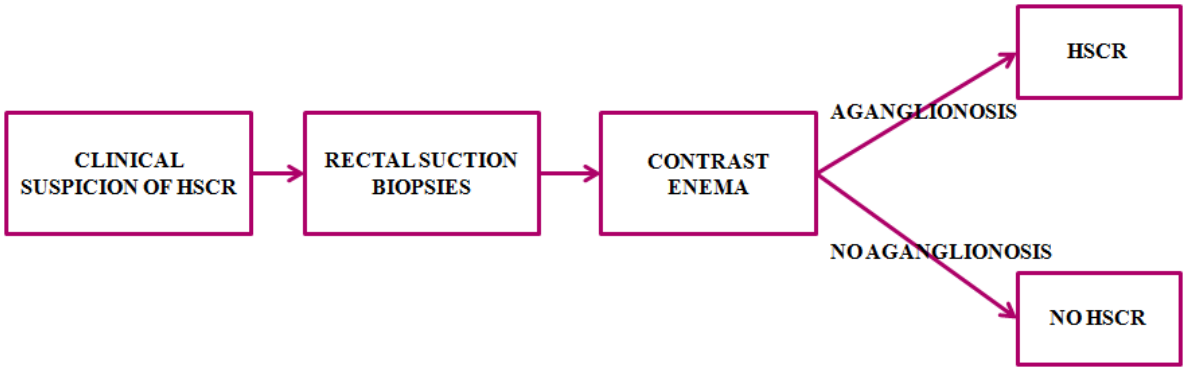
Figure 6. A contrast enema shows the transition zone (arrow) in the sigmoid colon.



The diagnosis is confirmed by rectal suction biopsies. Absence of ganglion cells and hypertrophic nerve bundles (a diameter of >40µm) are typical for HSCR. The presence of ganglion cells excludes HSCR. The extension of aganglionosis is classified into short-segment (limited to rectum and sigmoid colon, 74% of all cases) and long-segment (extended aganglionosis proximally to the sigmoid colon, 18% of all cases). TCA, with or without small bowel aganglionosis, which may involve a very long-segment HSCR (Zuelzers disease), occurs in 8% of cases.[81]

The median age at diagnosis has decreased over the past few decades due to greater awareness of the disease. In 1979, 40% of the patients were diagnosed within the first three months following birth compared with 90% being diagnosed as neonates in 1996.[78,81]

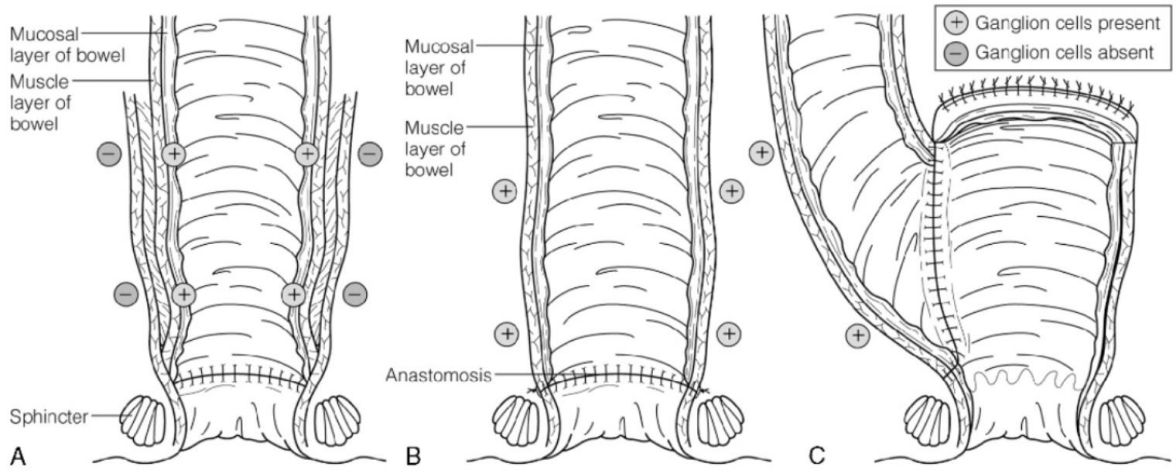
Figure 7. Diagnostic procedures for Hirschsprung disease (HSCR).



1.7 MANAGEMENT

The initial treatment, starting with the suspicion of HSCR, consists of regular bowel irrigations. The treatment of HSCR comprises surgical resection of the aganglionic colon followed by the ganglionic bowel being anastomosed to the anorectum for preservation of the sphincters. Swenson was the first to develop a surgical technique for HSCR, with Duhamel and Soave then modifying it a few years later. [5, 82,83] During the 1980s, one-stage procedures were proposed for uncomplicated cases.[84] The two main techniques performed today are the total transanal endorectal pull-through (TERPT) and the laparoscopic assisted pull-through (LAP), although the Duhamel procedure is also commonly used. There is no difference in length of stay or incidence of postoperative complications between these two methods.[85] In patients with older ages at diagnosis, TCA or with severe enterocolitis, a temporary enterostomy is often preferred as a first step.

Figure 8. Drawings showing the different surgical methods A. Soave procedure, B. Swenson procedure, C. Duhamel procedure. The image is taken from the chapter on Hirschsprung Disease, Pediatric Surgery, 7th edition (2012), edited by Coran et al and published by kind permission of Elsevier Saunders.



1.7.1 Surgical methods

The Swenson procedure, originally described by Swenson et al in 1948, includes transabdominal full-thickness dissection of the colon with coloanal end-to-end anastomosis, as shown in Figure 8B.[86]

The Duhamel procedure comprises a retro-rectal pull-through and an end-to-side anastomosis to the anal canal, as shown in Figure 8C.[82]

The Soave procedure describes a transabdominal endorectal dissection of the colon before pull-through and coloanal end-to-end anastomosis, and is shown in Figure 8A.[83]

LAP, initially described by Georgeson in 1995, is a minimally invasive approach using laparoscopy for colonic biopsies and mobilisation of the colon. It is followed by transanal endorectal dissection of the rectum and coloanal anastomosis.[87]

TERPT includes, as the name implies, submucosal dissection of the rectum and colon endorectally with coloanal end-to-end anastomosis.[88,89] There is also a modified TERPT in which peroperative biopsies are taken from a small umbilical incision at the beginning of the procedure. This enables faster analysis of the peroperative biopsies and thus avoids the risk of missing a patient with TCA.

Sphincterotomy or myectomy have earlier been described as options in the treatment of short-segment HSCR, but are nowadays only occasionally used for persisting late complications such as obstructive outlet constipation or recurrent enterocolitis.[90]

Before one-stage surgery techniques were introduced, most of the patients were initially treated with a stoma. Some of these patients have never had further surgical treatment and are therefore still living with their stoma as a permanent solution. Some patients with persisting problems after pull-through may accordingly receive a stoma as a temporary or permanent solution.[91]

1.8 OUTCOMES

At 10 days old, George had underwent TERPT for HSCR. The surgical procedure was uneventful and he was discharged from hospital three days later.

Two weeks after pull-through, George and his parents were back for a control at the outpatient clinic. The time since surgery had been fine, he had had daily passage of stool and gained weight. At the examination, George had a perianal skin rash and was prescribed local treatment. His abdomen was soft and the anastomosis was calibrated at the rectal examination.

Later on, George was followed-up at the outpatient clinic twice a year. He is still using laxatives due to problems with constipation. At the age of six George was treated with Botulinum toxin due to persistent constipation and has not experienced any soiling or constipation symptoms since.

1.8.1 Mortality

Before the era of possible surgical treatment for HSCR, the mortality rate was very high and only patients with short-segment aganglionosis had any chance of survival. Since the surgical procedure became available, the mortality rate has decreased significantly. Postoperative mortality after the Swenson procedure was reported to be 2.4% between 1947 and 1986.[92] The mortality in HSCR patients undergoing one stage transanal pull-through varies between 0% and 2%.[93,94] Patients with DS, TCA and HAEC seem to have an increased risk of mortality, as well as patients with anastomotic leakage after the pull-through.[28,92,95]

1.8.2 Morbidity

1.8.2.1 Hirschsprung-Associated Enterocolitis

HAEC is the most threatening complication of HSCR since morbidity and mortality are possible outcomes. The pathogenesis remains unknown.[96] It occurs in 5-42% of cases and may develop both before or after surgery for HSCR.[97] Patients with DS have a significantly higher rate of preoperative and postoperative HAEC.[28] Symptoms such as distended abdomen, explosive diarrhea with foul-smelling stool, vomiting and fever are typical, although the symptoms may vary. To promote early diagnosis and treatment, Pastor et al proposed a diagnostic scoring system.[98] The treatment for HAEC is resuscitation, decompression of the gastrointestinal tract and antibiotics.[99]

1.8.2.2 Early complications

The early complications are defined as any complication within 30 days after the pull-through procedure for HSCR. The most frequently occurring complication is perianal skin rash, which may occur in about 46-55% of the cases.[100,101] Complications such as wound infection, bleeding, anastomotic leakage and prolapse of the pulled through segment are less frequently reported. [92,100-102] The overall risk of having an adhesive bowel obstruction after surgery for HSCR has been shown to be about 30%. Although the same study also showed that stoma formation was a risk factor for having a small bowel obstruction.[103] This may indicate that the surgical procedure for HSCR today with one stage performance and an transanal approach may decrease this complication.

1.8.3 To assess outcome

Over the years, many different scoring systems have been employed for the evaluation of bowel function after surgical treatment for HSCR, which makes it difficult to compare the outcomes. The methods used to evaluate bowel function in HSCR patients are the Krickenbeck criteria and the Rintala score (bowel function score) but most commonly are surgeon-reported outcome without a specific score.[104,105] For adults, there are plenty of scoring systems for fecal incontinence such as the Wexner score, St. Mark's incontinence score and the Miller incontinence score.[106-108] The Krickenbeck and Rintala scores use soiling as a sign of fecal incontinence, while fecal incontinence itself may also be sub-divided into incontinence with solid or loose stool, soiling (leakage of fluids) and urgency, as in the bowel function questionnaire by Österberg et al.[109] Another problem with follow-up is the fact that patients may be biased if it is the performing surgeon who is asking the questions. The most objective outcome will probably be achieved if the examiner has not had a current patient-doctor contact. Studies based on patient-reported outcomes have shown that children with HSCR reported more pain and symptoms compared with their parental reports.[110]

1.8.4 Late complications

The most common late complications are functional problems such as fecal incontinence and constipation. The late complications of HSCR have gained significant attention during recent years. The widely held view that the long-term bowel function outcomes of in HSCR are generally favourable has been strongly questioned by several reports.[111,112] In Table 2 the number of late complications among adult patients are presented. However, the long-term effects that are analyzed today are the effects of the early surgical methods.

Table 2. Presented numbers of late complications among adult patients who underwent surgery for Hirschsprung disease.[111-113] Age presented in years with median or mean (range or standard deviation)

Author	Patients studied	Age at follow-up	Main operation	Constipation	Fecal incontinence
Jarvi	92	43 (36-51)	Duhamel	30%	14%
Ieiri	42	33 (19-55)	Duhamel	33%	17%
Heikkinen	100	31(± 7)	Duhamel	1%	9%

Constipation has been reported in 1-50% of patients with HSCR.[95,101,111-114] The definitions of functional constipation are defined in the ROME III classification.[115] The symptoms may be due to stricture, residual aganglionosis, transition zone pull-through, intestinal dysmotility or stool-holding behaviour. Internal anal sphincter achalasia is present in all HSCR patients and while most overcome the inability to relax the internal sphincter, some do not. The obstructive symptoms may also lead to pseudo-incontinence.[116]

Depending on how the bowel function has been assessed, the incidence of fecal incontinence varies between 8-71%.[95,101,111-114] The main reasons for fecal incontinence are sphincter damage causing a deficient sphincter function and/or abnormal sensation after surgical procedures performed mostly through the anal opening, for example TERPT. Stensrud et al performed endosonographic surveys on patients who had either TERPT or LAP for HSCR. They found that patients treated with TERPT had more defects in the internal anal sphincter indicating a risk of fecal incontinence, although the patients in the TERPT group were younger at follow-up than the patients in the LAP group.[117] A meta-analysis comparing 159 patients undergoing LAP and 248 patients undergoing TERPT showed no significant differences between the groups according to postoperative HAEC, fecal incontinence or constipation.[94] The interpretation of these results must be cautious due to the heterogeneity in outcome and follow-up assessment. It must also be remembered that patients with DS and/or TCA are known to have worse long-term functional outcomes.[28,118]

It is of great importance to understand the underlying mechanisms of the long-term complications in order to successfully apply the available management tools. Conservative treatment may be diet changes, biofeedback (training of the pelvic floor muscles) and medication with oral laxatives like as bulking entero-stimulant agents, as well as active bowel management such as transanal irrigation. Non-conservative treatments include injection of botulinum toxin, repeated dilatations, sphincteromyectomy, Malone surgery, permanent stoma, and redo pull-through.[91,119-121]

Other occasional late complications are urinary incontinence and sexual dysfunction as a result of low pelvic dissection during surgery. Controlled long-term follow-up studies designed for this purpose are missing, but small studies have shown micturition disturbances in 10% of patients and sexual dysfunction in 11%.[122] Van den Hondel et al found that 11% of males with HSCR had erectile dysfunction and that 53% of women reported sexual dysfunction.[123]

1.8.5 Quality of life

The world health organisation (WHO) defines quality of life (QoL) as "an individual's perception of their position in life in the context of the culture and value systems in which they live, and in relations to their goals, expectations, standards and concerns".[124] QoL has become an important endpoint in clinical studies and with increased use, different types of measurements have been developed.[125] There are two main approaches to measure QoL: the general health-related generic QoL and the symptom-specific QoL. The generic QoL assesses health and well-being independently of the disease, making it possible to compare QoL of different diseases while the symptom-specific measurement has the advantage of focusing on specific issues relevant to the disease under assessment.

The short-form (36) Health Survey (SF-36) is a generic QoL questionnaire used worldwide. It is a short-form validated survey with 36 questions, translated and validated into Swedish.[126-128] The results are divided into eight subscales; physical functioning, role physical, bodily pain, general health, vitality, social eight unctioning, role emotional and mental health and two summary measures; the physical component score which is defined by physical functioning, role physical, bodily pain and general health, and the mental component score defined by the rest of the subscales. The maximum score for each scale is 100, and the higher the score the better the QoL.

Gastrointestinal quality of life (GIQLI) is a symptom-specific validated 36-item questionnaire for the measurement of symptom-related QoL in a variety of gastrointestinal disorders.[129] It was translated into Swedish and validated in 2009.[130] Each question generates 0 (least desirable) to 4 (most desirable) points, which make the total score range from 0 to 144. Except for a total score, the questionnaire generates scores for five domains: physical role, large bowel function, emotional role, upper gastrointestinal tract function and meteorism. Wigander et al recently translated a new disease specific QoL questionnaire the

Hirschsprung's Disease/Anorectal malformation Quality of life Questionnaire (HAQL) for the evaluation of QoL and fecal incontinence.[131,132]

Since impaired bowel function may impact QoL, it is important to assess it for HSCR outcome.[133] Unfortunately, there are only a few studies assessing QoL in HSCR patients. Järvi et al followed-up patients mostly operated with the Duhamel procedure in childhood. Their QoL, measured with GIQLI, showed only marginally and not significantly lower scores in patients with HSCR. Among the patients with the lowest GIQLI score, low bowel function score tended to predict poor QoL.[111] Gunnarsdóttir et al also evaluated QoL among 47 patients who underwent surgery for HSCR, and who were mostly operated with the Duhamel procedure. They concluded that the overall QoL according to SF-36 was satisfactory but that young females scored significantly lower for general health and mental health when compared with male patients and the norms for female in the Swedish population. Patients with aganglionosis on the right colon also had lower GIQLI score than those with aganglionosis to the left colon.[134]

Other aspects of QoL may not be measured by these instruments such as fertility and the ability to obtain education and work. Patients with HSCR have shown an increased need for special education at school when compared with controls, although when assessing intelligence no differences are apparent.[135]

1.8.6 Risk of other diseases

Patients with HSCR have an increased risk of developing medullary thyroid carcinoma (MTC). The risk is partly attributed to mutations in the *RET* gene shared by HSCR and MTC.[136] A small but interesting study has also shown that HSCR patients may develop inflammatory bowel disease (IBD) later in life.[137]

HSCR has also been associated with tumours of neural origin such as neurofibromatosis.[138] The risk of other diseases is unknown.

1.9 SWEDISH NATIONAL REGISTERS

Since 1947 all residents in Sweden recieve a unique personal identification number after birth or immigration. This enables a linkage between different national registers without personal identification. The comprehensive national health care system represents nearly 100% of all health care produced in Sweden. These factors enable to unique possibilities for studies on large, unselected, and population-based material. This is a summary of the national registers used in Studies IV and V.

1.9.1 The Swedish National Patient Register

The Swedish National Patient Register (NPR) contains prospectively collected information from all hospital admissions in Sweden and is maintained by the Swedish National Board of Health and Welfare. The register was initiated in 1964 and covers all hospitals in Sweden

since 1987. The data includes gender, age, geographical data, surgical procedures, primary and secondary diagnosis and date of admission and discharge. The International Classification of Diseases (ICD) has been modified over the years: ICD-8 in1969-1986; ICD-9 in 1987-1996 and ICD-10 since 1997. From 2001, data on outpatient specialist care was also included in the register. The latest validation of the register showed that the diagnoses are valid in 85-95% of the cases, but the Swedish National Board of Health and Welfare report the inpatient register to lack about 1% of data.[139,140]

1.9.2 The Swedish Medical Birth Register

The Swedish Medical Birth Register (MBR) contains data on all pregnancies and deliveries in Sweden since 1973. The Swedish National Board of Health and Welfare administer the register. Correlation between register data, corresponding original medical records and the validity of the study exposures has been shown to be excellent.[141] The data is collected prospectively from antenatal care clinics, obstetric clinics, and maternity wards and include; maternal age and parity, maternal weight, height, maternal smoking, maternal diseases, duration of pregnancy, single or multiple birth, parity and birth weight.

1.9.3 The Swedish Register of Education

The Swedish Register of Education started in 1985 and registers the highest education level for the inhabitants between 16-74 years of age. The register is national, maintained by Statistics Sweden, and updated annually. The latest evaluation of this national register suggests that it is 85% valid.[142]

1.9.4 The Swedish Income and Taxation Register

The Swedish Income and Taxation Register was started in 1943 and the system for reporting data has improved over the years. The registry is administered by Statistics Sweden and covers all tax-paying citizens. Individual disposal income is the sum of all incomes excluding taxes per individual and year.

2 AIMS OF THE THESIS

The overall aim was to increase the knowledge on different types of outcomes in patients with HSCR.

The specific aims were to:

- identify the molecular background in a family with both HSCR and MS (Study I)

- longitudinally evaluate the functional outcome after LAP for HSCR (Study II)

- assess the bowel function and QoL in adults who underwent surgery for HSCR during childhood (Study III)

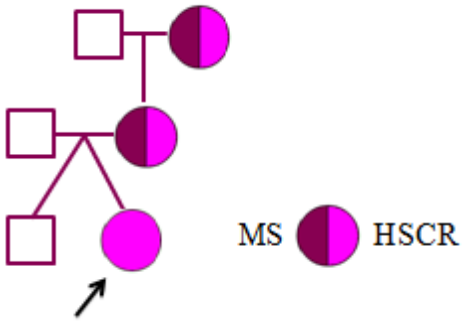
- assess birth prevalence, maternal risk factors and perinatal characteristics of HSCR in Sweden (Study IV) and to assess if HSCR has an impact on educational level and income (Study V)

3 PATIENTS AND METHODS

3.1 STUDY I

This was a genetic study of a family with HSCR and MS. The patients enrolled in this study were three related subjects; a girl with HSCR (proband), her mother and her grandmother, both with HSCR and MS. All patients had undergone surgery for HSCR during childhood. None of them had any associated anomalies. The mother of the proband was diagnosed with MS at the age of 25 years whereas the girl's grandmother was diagnosed with MS at 37 years of age.

Figure 9. Pedigree of the family with three generations with squares indicating males and circles indicating females. Filled symbols are the affected individuals and the arrow indicates the proband. MS: multiple sclerosis, HSCR: Hirschsprung disease.



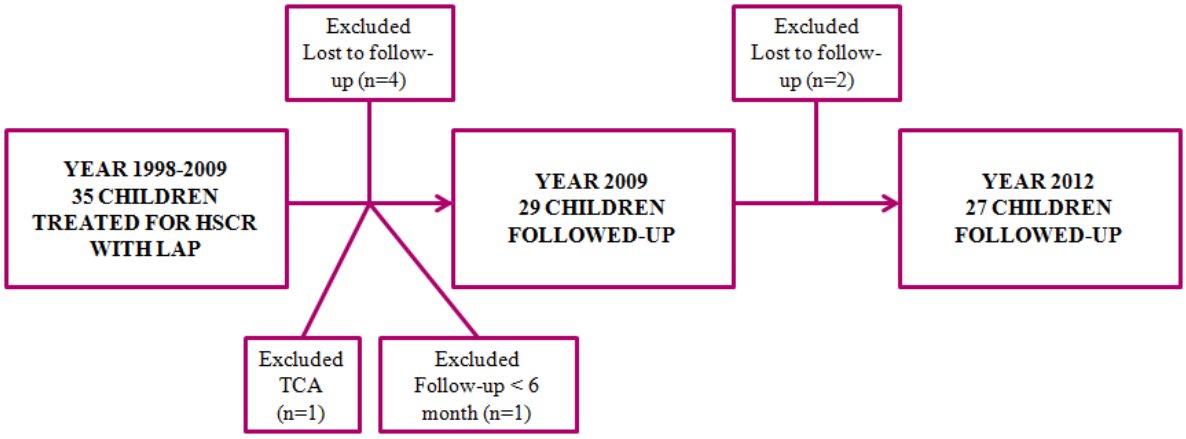
Data on the patients' HSCR and MS phenotypes were collected from the case records. DNA was extracted from ethylenediaminetetraacetic acid (EDTA) preserved blood and isolated according to standard procedures. For the exome sequencing, the library was created with Aglilent Sure Select Human All Exon 50M and Illumina HiSeq2000 was the platform used for sequencing. Postcapture libraries were sequenced as 2x100 base pair end reads. The exome sequencing was performed at the Science for Life Laboratory, Stockholm. After base calling, mapping, variant calling, annotation were performed using an in-house pipeline. Filtration was then preformed before analysis. The analyses mainly focused on the known HSCR genes.

In order to confirm the finding from the exome sequencing, Sanger sequencing of exon 2 of the *EDNRB* gene was performed after amplification by PCR using a standard protocol. The PCR product was purified by Exo I/SAP treatment (Fermentas) prior to direct sequencing using the BigDye Terminator v3.1 kit (Applied Biosystems) and analyzed using the 3730 DNA Analyzer (Applied Biosystems). The Sanger sequencing was performed at the Center for Molecular Medicine, KI, Stockholm.

3.2 STUDY II

This study was a clinical follow-up study which included thirty-five children treated for HSCR with LAP between 1998 and 2009 at the Department of Pediatric Surgery at Karolinska University Hospital. The diagnosis was histopathologically confirmed in all cases. Exclusion criteria were TCA (1 patient), follow-up less than 6 months (1 patient) and lost to follow-up (4 patients). After parental consent, 29 children participated in the study. Data were collected from the case records. Semi-structured interviews using a non-validated questionnaire were performed in 2009 and 2012, respectively, by an independent examiner. At the second round of interviews in 2012, two additional patients were lost to follow-up due to emigration. Consequently, 27 patients eventually completed the study. For the interview protocol, see Appendix A.

Figure 10: Flow chart Study II. HSCR: Hirschsprung disease, LAP: Laparoscopic assisted pull-through TCA: total colonic aganglionosis.

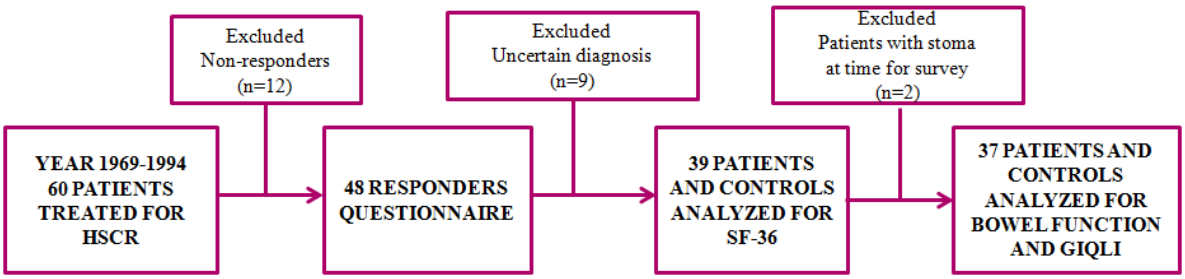


3.3 STUDY III

Study III was conducted as a clinical long term follow-up study. All patients who underwent surgery for HSCR between 1969 and 1994 at St. Göran's Children's Hospital were identified and invited to participate in the study (n=60). Those that accepted received a set of questionnaires (n=48), the response rate was 80%. After histopathological confirmation of the diagnosis, nine patients were excluded and 39 remained (22 males). Demographic data were collected from case records. For the questionnaires, see Appendix B.

One age- and sex-matched control per patient in the HSCR group (n=39) was included. These individuals were randomly selected from the National Swedish Population Register. This control group consisted of 22 males and 17 females and the median age was 25 (19-42) (p=0.738). These controls responded to the same set of questionnaires concerning bowel function [109], urinary function and the SF-36 questionnaires but not to the GIQLI questionnaire. Therefore a different control group, also matched for age and sex, was used for comparison of the GIQLI scores.

Figure 11. Flowchart Study III. HSCR: Hirschsprung disease, SF-36: The Short Form (36) Health Survey, GIQLI: Gastrointestinal Quality of Life Index.



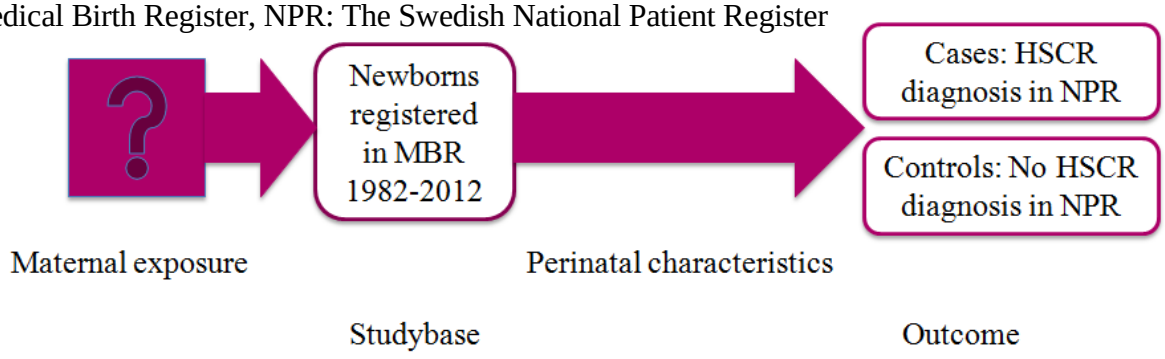
This control group was also randomly selected from the National Swedish Population Register. Since two patients had a stoma at the time of the survey, they were excluded from the analysis of bowel function and GIQLI results. Both the HSCR group and the control group consisted of 20 males and 17 females. The median age in the control group was 28.2 (19.6-42.7) (p=0.709).

3.4 STUDY IV

This study was a national population-based case-control study. The study base included all neonates born in Sweden during the observational period from 1st of January 1982 to 31st of December 2012 registered in the MBR. In the study base, the outcome HSCR or no HSCR was assessed. The outcome HSCR was defined as all cases with an ICD code for HSCR in the NPR (ICD-8: 751.39, ICD-9: 751D, ICD-10: Q431) during the study period (n=816). Since the histopathological diagnosis was not available in the registers, the following inclusion criteria had to be fulfilled in an attempt to certify that the cases were not misclassified as HSCR:

1) HSCR as the main diagnosis and a surgical intervention number specific for HSCR.
2) Admission to a pediatric surgical center at least twice, with a hospital stay of at least four days at least once, and HSCR as the main diagnosis for both hospital stays.
3) One long admission at a pediatric surgical center once and more than one outpatient visit at a pediatric surgical centre with HSCR as the main diagnosis.

Figure 12. Flowchart for Study IV. HSCR: Hirschsprung disease, MBR: The Swedish Medical Birth Register, NPR: The Swedish National Patient Register



Based on these criteria, 216 patients were excluded, resulting in a total of 600 HSCR cases and 590 mothers due to 10 siblings among the cases. For each case, five controls without a history of HSCR were randomly selected using incidence density sampling from the study base and matched for birth year and gender, n=3000. The study exposures were assessed through linkage with the NPR for both cases and their mothers. The definitions and categorization of study exposures are presented in Table 3.

Table 3. Definition and categorization of study exposures. MBR: The Swedish Medical Birth Register, NPR: The Swedish National Patient Register, ICD: International Statistical Classification of Diseases and Related Health Problems, SGA: small for gestational age.

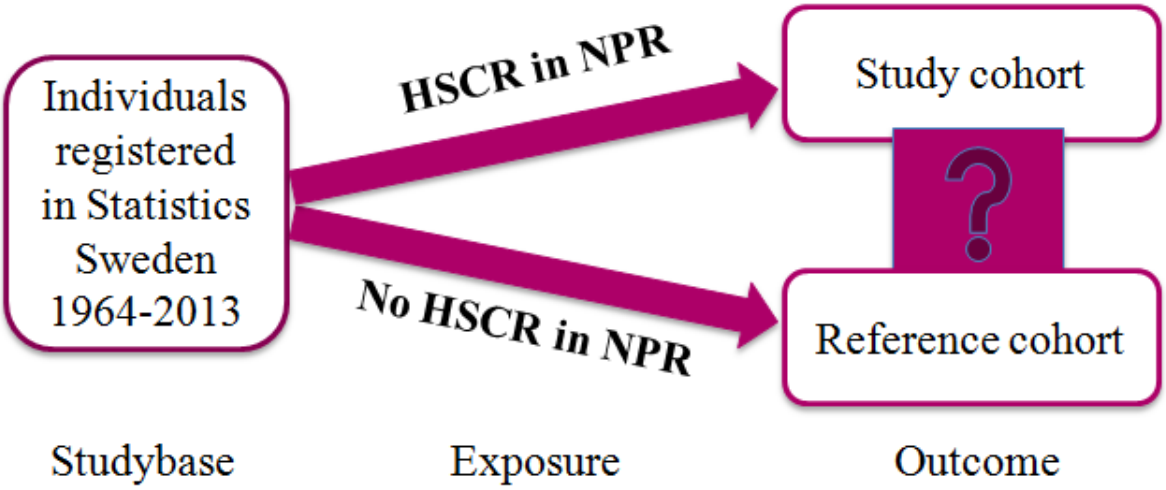
Exposure	Source	Categorization
Maternal risk factors		
Maternal age	MBR	<20 years, 20-24 years, 25-29 years, 30-35 years, >35 years
Maternal smoking	MBR	non-smoker,<10 cigarettes daily, ≥10 cigarettes daily
Maternal parity	MBR	First, second, ≥third
Maternal Body Mass Index	MBR	<18.5 (under weight), 18.5-24.9 (normal weight), 25.0-29.9 (over weight), ≥30.0 (obese)
Maternal diseases	MBR/NPR	ICD-8: 242, 244, 245, 250, 340.99, 563.00-563.10, ICD-9: 242, 244, 245, 250, 340A-341X, 555A-X, 556, ICD-10: E03, E05, E06, E10, E11, E12, E13, E89, G35.9, K50-51.
Perinatal Characteristics		
Delivery mode	MBR	Caesarean section, Vaginal delivery
Gestational age	MBR	<37 gestational weeks (preterm) ≥37 gestational weeks (fullterm)
Birth weight for gestational age	MBR	SGA not SGA
Preeclampsia	MBR/NPR	ICD 8: 637.00-637.04, 637.09-637.10, 637.99 ICD 9: 250 A-H, 648A, 648W ICD-10: E100-149, O240-244, O249 O41.0-O41.9
Congenital malformations	MBR/NPR	ICD-8: 750.00-759.99 ICD-9: 740-759X ICD-10: Q00-Q99

3.5 STUDY V

This was a nationwide, population-based cohort study during the observational period from 1st of January 1964 to 31st of December 2013. The study exposure was HSCR collected from the NPR (ICD-7: 756.31, ICD-8: 751.39, ICD-9: 751D, ICD-10: Q431). A total of 1,267 individuals with these ICD codes were found, and to confirm that they were exposed to HSCR and not misdiagnosed, each individual had to fulfil one of the following inclusion criteria:

1) HSCR as the main diagnosis and a surgical intervention number specific to HSCR.
2) Admission to a pediatric surgical centre at least twice, with a hospital stay of at least four days at least once, and HSCR as the main diagnosis for both hospital stays.
3) One long admission (≥4 days) to a pediatric surgical centre once and more than one outpatient visit to a pediatric surgical centre with HSCR as the main diagnosis.

Figure 13. Flowchart Study V. HSCR: Hirschsprung disease, NPR: The Swedish National Patient Register.



Using these criteria 528 individuals were excluded, resulting in a total of 739 exposed cases. Since the study outcome was the highest educational level and income, we chose to exclude individuals who had died before 2013 (n=16) or were <16 years of age (n=259). Individuals with chromosomal aberrations (n=75) were also excluded. Altogether, after exclusion, a total of 389 cases were included. The unexposed cohort was randomly collected from the Population Register and comprised 10 unexposed individuals for each exposed individual matched for birth year, gender and birth county (n=3,847).

The study outcome of highest education level was collected from The Swedish Educational Register and was categorized into three levels: ≤ 9 years of compulsory school, 2-3 years of upper secondary school, and university education.

The individual disposable income was collected from The Swedish Income and Taxation Register for the year 2013.

3.6 STATISTICS

For Studies II-V, P-values <0.05 were considered statistically significant.

3.6.1 Study II

Categorical data are presented as frequencies/proportions and were analysed with a chi-squared test. Numerical continuous parameters are presented as median and range.

3.6.2 Study III

Categorical variables are presented as frequencies/proportions and were analysed using Fisher's two-tailed exact test. Numerical variables are presented as median and range and were analysed with the Mann-Whitney U test. Occasional missing values within the GIQLI questionnaires were replaced with the median value for that particular question for the whole group. Data were analysed with the software program IBM SPSS Statistics Version 21 (Armonk, NY, USA).

3.6.3 Study IV

Differences in possible correlating factors (gestational age, weight for gestational age, delivery mode) between the HSCR and control groups were analyzed using logistic regression and are presented with odds ratio (OR) estimates, and 95% confidence intervals (CI). Factors possibly directly related to the odds of a child being born with HSCR were analyzed using a conditional logistic regression (clogit in R) stratifying over the matched pairs. Results are presented with ORs and 95% CIs. All the odds of HSCR were evaluated with a univariable approach. Multivariable analysis was considered if the univariable analysis showed that it was appropriate. All statistics were performed in the R program. [143]

3.6.4 Study V

The association between exposed and unexposed individuals was analyzed with the R program. [143] Categorical data are presented as frequencies/proportions and were analyzed using Fisher's two-tailed exact test. Numerical continuous data are presented as median and range and the two-sided Mann-Whitney U test was used for analysis. Ordinal regression of highest educational level was conducted using three levels of education: ≤ 9 years of compulsory school, 2-3 years of upper secondary school, and university education. A linear regression model was conducted for effect of exposure on income, adjusted for age and gender.

3.7 ETHICS

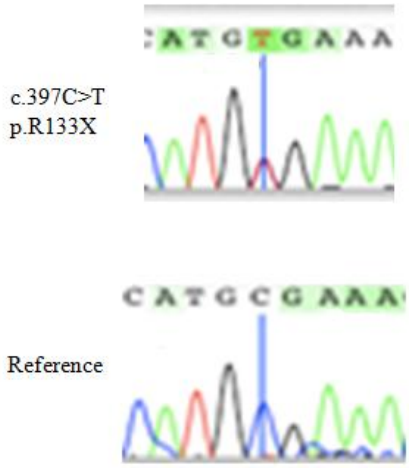
The Regional Ethics Review Board at Karolinska Institutet approved all the studies in this thesis.

4 RESULTS

4.1 STUDY I

A novel heterozygote nonsense mutation was found in the EDNRB gene (c.C397T, p.R133X, ref NM_000115), changing an arginine at position 133 into a premature stop codon in the proband and her grandmother. The mutation was confirmed by Sanger sequencing in all three affected family members.

Figure 14. Results from the Sanger sequencing.



4.2 STUDY II

There were 23 males and 4 females in the study group. Median age at presentation was 4 days (0 days-6 years). Twenty patients (74%) presented with neonatal bowel obstructions followed by five with constipation (19%) and two with HAEC (7%). The extent of aganglionosis according to histopathology was recto-sigmoid in 25 of the patients (93%) and long-segment in two of them (7%). The median age at surgery was 104 days (29 days-8 years). Two patients had postoperative complications, one had anal stricture and one underwent a second operation due to perforation of the rectum at LAP. Associated malformations including chromosomal aberrations were detected in 10 of the children, of whom three patients had DS. The patients followed-up in Study II were of a median age of 4 years (2-16) at the first interview and 7 years (5-19) at the second. The results of the bowel function are shown in Table 4.

Table 4. Functional outcome. Text in bold indicates statistically significance. NS: non significant.

Variables	First follow-up n (%)	Second follow-up n (%)	P-value
Soiling (loose stool)	18 (67%)	16 (59%)	NS
Soiling (solid stool)	16 (59%)	15 (56%)	NS
Constipation	11 (41%)	4 (14%)	**0.023**
Use of laxatives	13 (48%)	8 (30%)	NS

4.3 STUDY III

There were 22 males and 17 females in the study group. The extent of aganglionosis according to histopathology was recto-sigmoid in 37 (95%) of the patients and TCA in two (5%) of them. The median age at surgery was 1 year (1-17). Associated malformations including chromosomal aberrations, were detected in five of the patients of whom one patient had DS. Most of the patients had undergone a Soave procedure, (n=29; 74%) followed by Duhamel, which was performed in five (13%) of the patients, sphincteromyectomy (two patients), ileostomy (one patient), sigmoid colostomy (one patient) and an unknown procedure (one patient). A multi-staged procedure, with a preoperative stoma, was performed in five (19%) of the patients. Seven patients had postoperative complications within one month after surgery. Secondary surgery was needed for four patients and 14 (36%) had dilatations requiring general anaesthesia. The median age at follow-up in the study group was 28 years (20-43).

4.3.1 Results of general questions

There was no significant difference in the body mass index (BMI) between the groups. Patients in the study group reported that their bowel function problems had affected their choice of occupation significantly more than the control group (p=0.006) and had also affected their social relations (p=0.013). Only two patients in the study group had a current contact with medical care.

4.3.2 Results of the bowel function

The results are presented in Table 5. Patients in the study group had significantly more incontinence symptoms such as urgency, a need to rush to the toilet and a higher Miller incontinence score. Patients in the study group also had significantly more constipation symptoms such as flatulence, strain at defecation, and the need of repeated defecations to empty the rectum. In the study group, 29 (78%) out of 37 patients had at least one constipation parameter compared with 13 (35%) individuals in the control group. The number of patients having a Miller incontinence score more than five was seven (19%) compared with none in the control group.

4.3.3 Results of SF-36

The median physical component score was 54 (29-62) in the study group and 56 (30-65) in the control group. The median mental component score was 51 (15-59) in the study group and 49 (16-59) in the control group. Altogether, there was no significant difference in the SF-36 between the two groups.

Table 5. Bowel function in patients with Hirschsprung disease (HSCR) and controls. Data are presented as frequencies or median (range). Text in bold indicates statistically significant P-values.

	HSCR (n=37)		Controls (n=37)		P-value
	Yes	Missing	Yes	Missing	
Constipation parameters					
Use of laxatives	4	1	1	0	0.199
Use of enemas	4	1	0	0	0.054
Bloating	17	1	9	0	0.052
Problems with flatulence	24	1	12	0	**0.005**
Supporting around anus at defecation	1	4	1	0	1.000
Need to strain at defecation	11	4	4	1	**0.042**
Several defecations for emptying	16	5	6	0	**0.004**
Incontinence parameters					
Use of loperamid or equivalent	5	2	2	0	0.254
Need to rush to toilet	17	2	8	0	**0.025**
Use of pad daytime	2	4	0	0	0.219
Deferring time loose stool (min)	15 (0-20)	4	15 (2-20)	4	0.653
Deferring time solid stool (min)	20 (0-20)	3	20 (4-20)	4	0.706
Miller incontinence score (0-18)	0.5 (0-15)	0	0 (0-4)	0	**0.050**

4.3.4 Results of GIQLI

The total GIQLI score was significantly lower in the study group when compared with the control group. A summary of the GIQLI score is shown in Table 6. Males in the study group had lower scores on the large bowel function, compared with the control group; 18 (12-24) versus 22 (8-24), p=0.006. Female participants also showed significantly lower scores than controls; 39 (22-44) versus 42 (19-44), p=0.027 for the physical role.

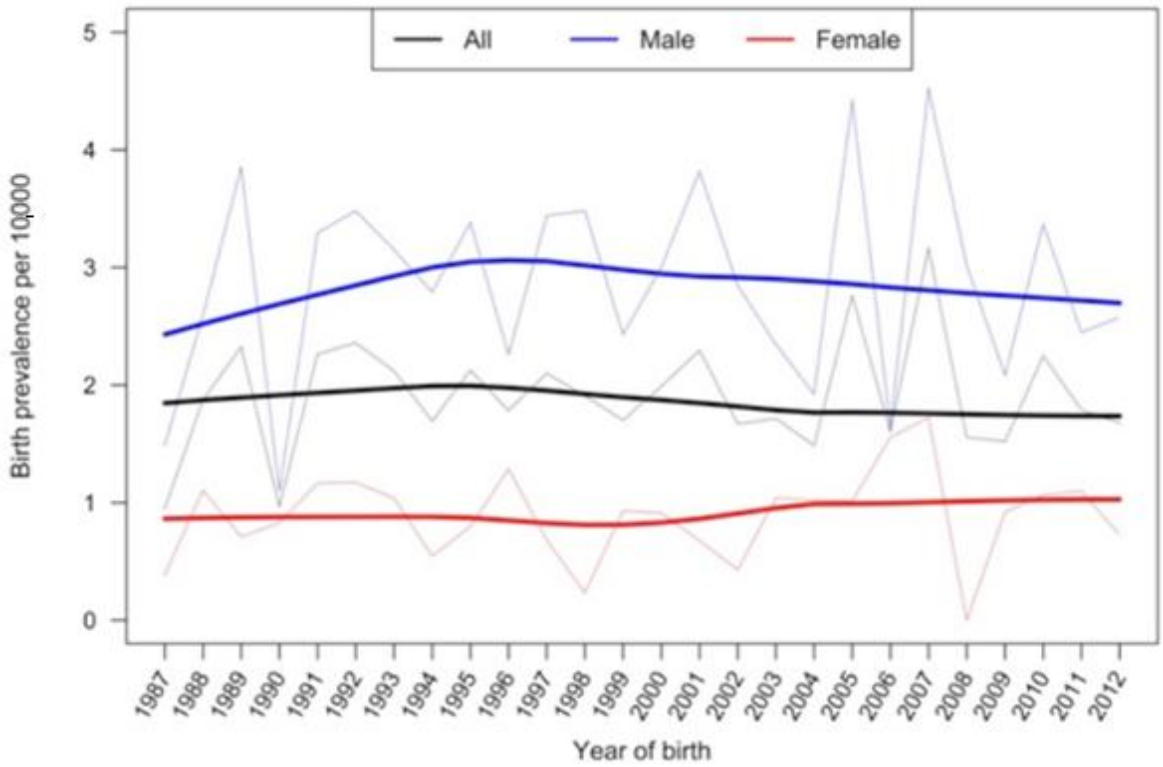
Table 6. Gastrointestinal quality of life scores for the Hirschsprung disease (HSCR) group and controls. Data presented as median (range). Text in bold indicates statistically significant P-values.

Scale	HSCR (n=37)	Controls (n=37)	P-value
Physical role	39 (15-44)	43 (19-44)	**0.009**
Large bowel function	18 (6-24)	21 (8-24)	**0.002**
Emotional role	25 (11-31)	25 (2-32)	0.888
Upper gastrointestinal tract function	26 (12-32)	29 (14-32)	0.064
Meteorism	7 (3-12)	9 (4-12)	**0.041**
Total	116 (59-139)	125 (52-141)	**0.026**

4.4 STUDY IV

The birth prevalence of HSCR in Sweden was 1.91/10,000 between 1987 and 2012 (Figure 15).

Figure 15. Total birth prevalence and male/female birth prevalence. The middle lines are a sliding estimate of average prevalence.



Maternal obesity was associated with an increased risk of the child having HSCR (OR 1.74; CI 1.25-2.44) as well as maternal parity of three or more children (OR 1.25; CI 1.00-1.56). More data on maternal risk factors are shown in Table 7. Children with HSCR were born at an earlier gestational age (OR 1.60; CI 1.18-2.17) than controls, Table 8.

Table 7. Maternal characteristics with univariable, unadjusted odds ratios (OR) and 95% confidence intervals (CI). Text in bold indicates statistical significance.

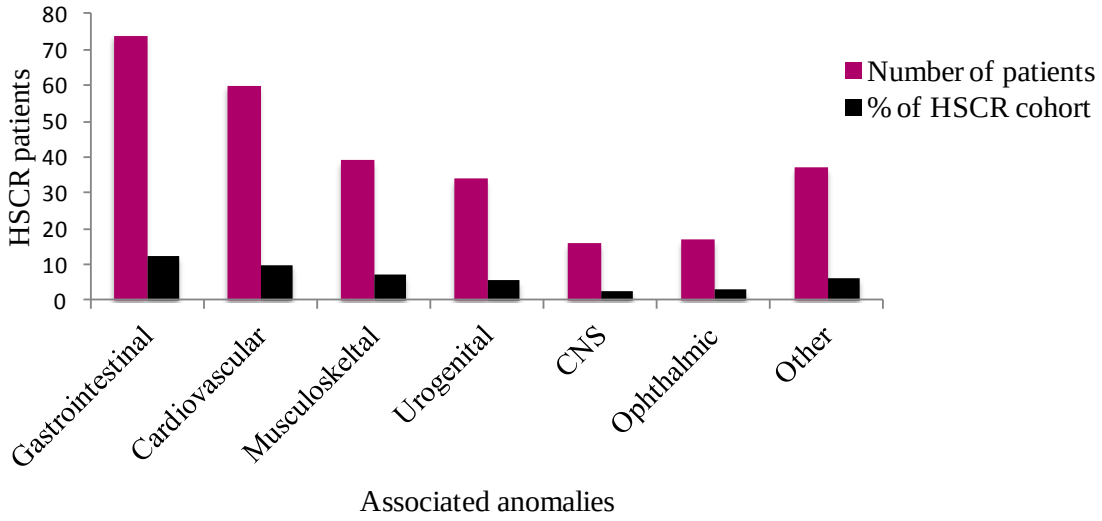
Exposure	Cases (n=600)	Controls (n=3,000)	OR (95% CI)
Maternal age (years)			
<20	9	68	0.65 (0.32-1.33)
20-24	102	520	0.97 (0.75-1.26)
25-29	203	1,005	1
30-34	170	898	0.94 (0.75-1.17)
≥35	116	509	1.13 (0.88-1.46)
Maternal smoking (cigarettes daily)			
None	469	2,369	1
1-9	62	277	1.12 (0.83-1.51)
≥10	18	151	0.62 (0.38-1.03)
Missing data	51	203	
Parity			
1	233	1,249	1
2	215	1,100	1.05 (0.86-1.28)
≥3	152	651	**1.25 (1.00-1.56)**
Maternal Body Mass Index			
Under weight <18.5	9	80	0.62 (0.31-1.26)
Normal weight 18.5-24.9	279	1,533	1
Over weight 25.0-29.9	113	514	1.24 (0.96-1.58)
Obese ≥30.0	57	193	**1.74 (1.25-2.44)**
Missing data	142	680	
Maternal diseases			
Diabetes	11	58	0.95 (0.49-1.82)
Inflammatory Bowel Disease	0	3	-
Multiple Sclerosis	1	3	0.6 (0.06-5.77)
Thyroid diseases	26	101	1.3 (0.84-2.03)

Table 8. Perinatal characteristics with univariable, unadjusted odds ratios (OR) with 95% confidence intervals (CI). Text in bold indicates statistical significance.

Exposure	Cases (n=600)	Controls (n=3,000)	OR (95% CI)
Gestational age (weeks)			
<37	60	196	**1.60 (1.18–2.17)**
≥37	537	2,793	1
Missing data	3	11	
Small for gestational age (SGA)			
SGA	15	56	1.32 (0.74–2.36)
Not SGA	557	2,752	1
Missing data	9	97	
Delivery mode			
Cesarean section	88	430	1.03 (0.80–1.32)
Vaginal delivery	493	2,475	1
Missing data	19	95	

There were associated malformations in 34.5% of the cases, including chromosomal anomalies. Excluding those cases with only chromosomal anomalies, 191 had associated malformations. In total, 59 (9.8%) of the cases had DS and 18 (3%) had other chromosomal anomalies. The distribution of congenital malformations is shown in Figure 16.

Figure 16. Congenital malformations among the cases. CNS: central nervous system.



4.5 STUDY V

The study comprised 389 exposed (294 male) and 3847 unexposed (2906 male) cases and the median age of the cohort was 25 (16-49) years. The highest educational level did not differ between the groups (p=0.327). Ordinal regression showed that the unexposed cohort had 1.19 higher odds (CI 0.97-1.46) of having a higher educational level compared with the exposed cohort. Median individual disposable income was 142,200 (0-817,200) Swedish krona (SEK) in the exposed group and 159,000 (0-3418,900) SEK in the non-exposed group (p=0.615). Results are summarized in Tables 9 and 10. Figure 17 shows the distribution of income in the cohort.

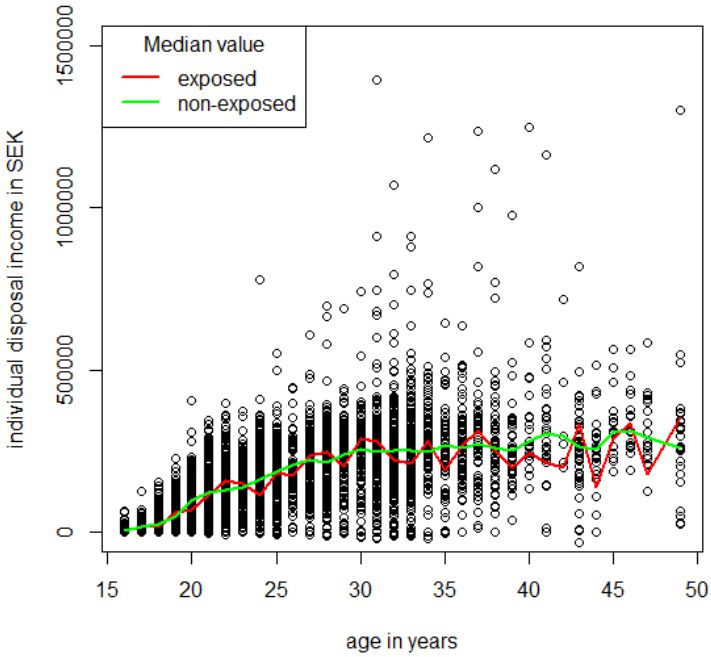
Table 9. The table shows the highest educational level in the study cohorts. There were no statistically significant differences between the groups (p= 0.327).

Educational level	Exposed (n=389)	Unexposed (n=3,847)
Compulsory school ≤9 years	100 (26%)	838 (22%)
Upper secondary school, 2-3 years	170 (44%)	1,742 (45%)
University education	98 (25%)	1,062 (28%)
Data missing	21 (5%)	205 (5%)

Table 10. Data showing the median (range) individual disposable yearly income of the cohort in Swedish krona.

Age (years)	Exposed (n=372)	Unexposed (n=3,566)
16-24	57,900 (0-406,500)	53,100 (0-397,800)
25-30	207,300 (0-580,300)	204,200 (0-780,000)
31-40	250,300 (0-668,100)	253,800 (0-3418,900)
40-44	232,500 (86-817,200)	274,400 (0-1302,500)

Figure 17. Plotted individual disposable income and median value according to age at time for the study. There was no statistically significant difference between the exposed and unexposed (p=0.615).



5 DISCUSSION

5.1 DISCUSSION OF FINDINGS

5.1.1 Genetics

In Study I the family with HSCR and MS was interesting from an etiological point of view, as it is the first description of an association between HSCR and MS, even though HSCR is a congenital malformation and MS is a late onset disorder. In the three subjects, HSCR was confirmed by histopathology on the resected distal colon and MS was diagnosed based on the McDonald criteria in the mother and grandmother.[144] We showed a novel heterozygote non-sense mutation in the *EDNRB* gene in all three subjects. Mutations in the *EDNRB* gene occur in approximately 5% of all cases of HSCR and the mutations are mainly inherited from unaffected parents, and usually associated with short-segment aganglionosis.[53] The *EDNRB* gene is coding for a subtype of G protein-coupled receptors, which play an essential role in the development of both enteric neurons and epidermal melanocytes.[37,38] There are reports on mutations in the *EDNRB* gene in humans with neurological symptoms, all associated with WS4.[145,146] An association between HSCR and demyelinating disorders has earlier been related to mutations in the *SOX10* gene.[147] Cantrell et al described that interaction between the *SOX10* and *EDNRB* genes influences penetrance and severity of the aganglionosis in a $sox10^{Dom}$ mouse model of HSCR.[148] The etiology of MS remains still unknown but it is a complex disorder, that is affected by several minor risk alleles mainly in the HLA-regions.[149] An association between a mutation in the *EDNRB* gene and MS has not been described previously, and it cannot be ruled out that this association may be a coincidence. The found mutation probably causes HSCR in the family but whether the mutation increases the susceptibility of MS is unknown. We also assessed the possibility of an association between HSCR and MS in Study IV, regarding MS as a maternal risk factor, but could not confirm any additional cases.

5.1.2 Functional outcome and QoL

5.1.2.1 Fecal incontinence/soiling

To evaluate long-term complications after surgical treatment for HSCR is an important issue. For the adolescent in Study II, soiling was reported in 59-67% of cases at the first follow-up and in 56-59% three years later. Study III assessed the fecal incontinence (Miller incontinence score higher than five) at 19% in adults with HSCR and 54% of the patients reported at least one symptom of fecal incontinence. These results are comparable with results from other Nordic studies.[101,111] A recently published study of patients having only TERPT surgery showed that 75% were socially continent but that they reported significantly increased impaired bowel function compared with the control group.[150] There are other follow-up studies, which report less soiling/fecal incontinence, but the different results could

be attributed to the lack of an independent examiner.[151] The underlying reason for these sequels are not assessed in these studies and neither is the effect of optimal treatment.

5.1.2.2 Constipation

Study II shows a significant decrease in constipation over time (41-14%) with less usage of laxatives with time. Several studies, as in Study II, have shown progressively improved continence with time as well as constipation.[111,152,153] However, there are also studies suggesting the opposite development.[152] In Study III there were significantly higher constipation parameters in the HSCR group compared with controls, but the use of laxatives was relatively low. Approximately 78% of patients in the HSCR group reported some symptom of constipation, which is high when compared with other studies.[111,150]

5.1.2.3 QoL

In Studies II and III, patients who underwent surgery for HSCR had a significant risk of fecal incontinence and constipation a long time after surgery. Since fecal continence has been suggested as an important predictor of normal QoL in patients with HSCR one of the aims in Study III was to evaluate whether this affected QoL in our study.[154] The conclusion was that there was no impact on the generic QoL, but a significant decrease of symptom-specific QoL. When subanalysing the data there was no difference between age or gender in the total score for either the generic or symptom-specific evaluation. Gunnarsdottír et al showed a good overall QoL, but also that female gender was predictable for a worse total score of generic QoL. Patients with an aganglionic segment reaching the right colon also had a significantly lower total symptom-specific score.[134] In a study of ARM, including young HSCR patients, children reported better QoL but worse bowel function compared with adolescents.[132] Another study on QoL and patients having TERPT surgery for HSCR has shown that the children's QoL was equal to their controls while adults exhibited lower scores on emotions and limitation of personal and sexual relationships.[150] Assessing a symptom-specific evaluation of QoL with GIQLI, earlier studies have shown that individuals who score lower than 105 have constant, on-going gastrointestinal symptoms.[129,155] In our material, nine individuals in the HSCR group had a score below 105. Study III showed a lower QoL in the symptom-specific assessment but looking at the total, patients with HSCR as a group have a normal rate indicating a normal/ ordinary QoL.

5.1.3 Epidemiology

Studies IV and V showed unique data as there is a lack of register studies on larger HSCR cohorts. The birth prevalence in Study IV of 1.91/10,000 or 1 of 5,000 live births, is concurrent with other incidence data and did not change over the study period.[67]

The maternal risk factors presented in Study IV show that obesity may be a maternal risk factor, or at least a risk indicator for the child to have HSCR. Similar studies have shown that maternal obesity is associated with congenital malformations, but not specifically with HSCR.[72, 74,75,156] Two known risk factors for birth defects that may confound this association

are blood folate levels and prepregnancy diabetes. The blood folate level among the cases' mothers is not available from the registers and is therefore unknown. The rate of diabetes among the mothers was analyzed and no differences could be demonstrated between cases and controls. Also, parity of three or more children increases the risk of having a child with HSCR. When subanalysing for gender, boys show the same pattern, but not girls, perhaps due to the small sample size and lack of power. This result is opposite to what Ryan et al described, namely, that being first born increass the risk of having HSCR.[73] Goldberg showed an association between maternal age and the risk of HSCR in offspring, which could not be confirmed in our study or by Russel et al and Best.[68,70,71]

Study IV also showed that HSCR patients were born at a lower gestational age than controls, thus confirming earlier studies.[73] Preterm birth may have many causes, for example, preeclampsia or congenital malformations. Maternal preeclampsia was not overrepresented among the cases' mothers in this study. Downey et al studied preterm patients with HSCR and concluded that they had more associated anomalies than full-term patients with HSCR.[77] The rate of associated malformations, 34.5%, reported in this study is fairly high compared with other studies. This could be explained by our broad definition of malformation: at least one diagnosis in any of the registers, which could cause an overrepresentation of the patients with associated malformations. Since a very high frequency of gastrointestinal malformations was found, these malformations were investigated further. Spounge et al have reported associated malformations in 30% of the cases in their cohort study in Vancouver. As in our study, gastrointestinal malformations were the most frequently reported followed by cardiovascular malformations.[157]

Study V is a unique cohort study exploring the impact of HSCR on education-and income. There were no significant differences between the exposed cohort and non-exposed cohorts concerning highest educational level and individual disposable income.

As shown in Studies II and III, as well as in other studies, patients with HSCR are at risk of long-term complications such as fecal incontinence and constipation.[111] There is no current consensus of the impact on QoL of these long-term complications.[134] Van den Hondel et al recently published data showing that 55% of HSCR patients required special education or remedial teaching at the age of eight years. The intelligence level is normal, but the sustained attention is significantly lower compared with controls.[135] Our results showed no difference in graduation level, suggesting that patients with HSCR can concentrate and perform at the same level as their schoolmates. Taking into consideration the risk of type II errors, we would wish to encourage further studies in the field as there are no similar studies in the literature today.

5.2 DISCUSSION OF METHODOLOGY

5.2.1 Follow-up studies

Both Studies II and III are small observational studies and the studies were conducted as follow-up studies of patients who had undergone surgery for HSCR in the Stockholm area.

The population sample is a challenging problem, when studying a relatively rare disease and is a limitation within these performed studies. This problem encourages multi-centre studies with cooperation between clinics although that may serve for other problems like different surgical procedures and other clinical traditions. Since the functional outcomes after surgery for HSCR are probably dependent on different aspects such as surgical technique and skill, extent of aganglionosis, age at surgery, postoperative complications, syndromes, time to follow-up, age, type of evaluation and type of examiner, these aspects will affect the results. In Study II, we assessed only the LAP technique including all types of levels of aganglionosis and ages at surgery. This was a strength in Study II, in addition to the repeated evaluations, as the participants became their own controls. In Study III, the surgical procedures were different as well as the extent of aganglionosis. The age at surgery and age at follow-up was recorded as well as postoperative complications and possible syndromes. For this study the mix of possible effectors of the outcome was a limitation. The more similar the study group, for example the same surgical technique or level of aganglionosis, the more generalizable the results will be. On the other hand, in Study III, the comparison with the control groups was a strength.

The choice of evaluation method will affect the future comparison possibilities with other studies and if the examiner is unbiased or not will probably reveal more or less true outcomes. In Study II, we used a non-validated questionnaire as the management information for the interviews, which reduces the reproducibility of the study. In Study III, the patients participated through a validated questionnaire, which was processed without knowledge of information on the detailed clinical characteristics of every patient.

In Study III, a dropout analysis was performed to assess any possible selection bias between responders (n=48) and nonresponders (n=12), showing no significant differences with respect to age or gender.

5.2.2 Epidemiology

Epidemiology is the study of the distribution and determinants of health-related states or events. There are two main types of epidemiological studies, cohort studies and case-control studies. A cohort study is a longitudinal study of a designated group of individuals over a period of time. They are defined by their exposure and followed-up for the outcome. The main advantages of the cohort study are that temporal relations can be taken into account but the disadvantages are that it may be time and cost consuming. Study V was a prospective cohort study, meaning that the exposure status is assessed at the beginning of the follow-up.

In a case-control study, the patients with a certain outcome, for example HSCR, are identified in a source population. The distribution of exposed/unexposed individuals is then calculated among cases and a randomly selected control group that should mirror the study base. The selection of controls is one of the main concerns when carrying out a case-control study. Study IV was conducted as a case-control study.

No study is without limitations or possible errors. Within epidemiological studies the two main types of errors are systematic errors and random errors (non-systematic errors). The systematic errors affect the internal validity, meaning to what extent the results actually measure what the study is supposed to measure. Internal validity is important for the external validity, how the generalizability of the findings is to other populations. The systematic errors are classified into selection bias, misclassification and confounding.

Selection bias may be introduced if there is a probability of being included in the study base that is related to the exposure and disease. It occurs if the association between the exposure and outcome differs between those who participate and those who do not. If the cases and controls are recruited from the same population, as in the population-based Studies IV and V, the concern for selection bias decreases. Selection bias may, however, have been introduced due to the fact that Study IV was limited to only live births. Since it was not possible from the registers to find all possible conceptions that may have lived through gestation week 5-12, these cases had to be excluded.

Misclassification is introduced when information regarding the exposure or outcome is wrong, which is also called information bias. This can either be differential, in which case it may affect the result to be false weaker or false stronger, or non-differential meaning that the misclassification does not depend on the person's status for the outcome. The potential difference in the risk rate between the groups is then diluted. The introduction of inclusion criteria in an attempt to differentiate the "true cases of HSCR" from other individuals, who for example had been admitted for suspected HSCR but had negative rectal suction biopsies and still received a HSCR diagnosis, may cause information bias. Recall bias is another form of information bias that may be introduced if information on exposure data has been assessed in a retrospective manner. All the data in Studies IV and V were collected prospectively which is why there is no possibility of any recall bias in this thesis.

A confounder is a factor, a second exposure, which is both associated with the primary exposure and the outcome, for example, age and gender. It can cause both an over- and underestimation of the effect of the primary exposure. There are some possibilities to control for confounders such as matching cases and controls, and the usage of randomization or compensation during the data analysis (stratification or regression models).

Random or non-systematic errors are errors that remain after systematic errors have been eliminated. They may occur during all the stages of a study and cannot be corrected for in the statistical analysis since they affect the precision, which is reflected statistically by confidence intervals and P- values. There are two types of random errors: type I and type II. A type I error occurs when the null hypothesis is rejected even though it is in fact true. This is a threat that all studies face and in an attempt to avoid this type of error in this thesis, we had a clear hypothesis and categorized variables in advance. A type II error occurs when a study fails to reject the null hypothesis, when the null hypothesis is in fact false. This can be a result of insufficient statistical power. This type of error is possible in Studies IV and V due to the small number of individuals, although these are the largest study in this field.

5.2.3 Strengths and limitations

Sweden has well recognized population-based medical registers which make it possible to perform powerful studies. All data were prospectively collected, thus avoiding the risk of recall bias. Our study assessed a large number of HSCR cases with cross-linked data on exposures with the same definition in cases as in controls in Study IV and the same definition on exposure and outcome in Study V. However, the studies also have limitations since there was no histopathology register available for linkage to confirm the HSCR diagnosis. This means that it was necessary to base the diagnosis on the ICD code, which was the reason for using additional inclusion criteria to increase the specificity of the study. Going back to the excluded cases, we found that the majority had only been admitted once during the neonatal period, without having surgery, or admitted to a hospital without pediatric surgery services. Another possible limitation is the risk of type II errors due to the limited sample size. Data on HSCR that occurred in stillbirth or termination for fetal anomaly were not possible to retrieve from the registers and may be a limitation in Study IV.

The controls in Study IV were randomly selected from the study base and the cohort in Study V was randomly selected from the population register, thereby decreasing the risk of selection bias. To decrease the risk of confounders, we chose to match the reference population for age and sex in Study V and matched cases with controls in Study IV. Another possible confounder in Study V was the patients with chromosomal aberrations, which were more common in the exposed cohort. Therefore, all individuals with chromosomal aberrations from both cohorts were excluded. However, confounders such as socioeconomic and educational status among the study population's parents were not monitored and may have influenced the results in Study V.

6 CONCLUSIONS

Study I

In the family with HSCR and MS, a novel mutation in the *EDNRB* gene was found. Mutations in the *EDNRB* gene may have an influence on the central and peripheral as well as the ENS. It could, therefore, be a possible molecular link between HSCR and MS although this can only be suggested from our study and needs further investigations.

Study II

The functional outcome after LAP for HSCR over time in the study group was affected, mostly due to soiling and constipation. The study revealed a high rate of soiling at the two interviews, which is problematic. The constipation rate on the other hand, decreased significantly over time.

Study III

The bowel function was impaired in patients who underwent surgery for HSCR during childhood compared with controls in our study, showing symptoms of fecal incontinence and constipation. Surgery for HSCR in childhood had a significant impact on symptom-specific QoL, but not on the generic QoL.

Study IV

The birth prevalence of HSCR in Sweden was 1.91/10,000 and was stable during the observational period. Maternal obesity and parity were shown to be risk factors for children to develop HSCR and affected children were born at a lower gestational age than controls.

Study V

There was no significant impact of HSCR on educational level and income in our study.

7 FUTURE PERSPECTIVES

This thesis has contributed to understanding the mechanism of HSCR, describing a new mutation in Study I and possible risk factors in Study IV. Since clinical studies have their limitations within the field of rare diseases, often with sample size, the epidemiology approach may encourage new data within the field of HSCR. In Sweden the national registers provide unique opportunities for population-based studies making it possible to analyse various aspects of the disease. Epidemiology is one way of finding associations between potential risk factors and the development of the disease. Studies on other possible risk factors for HSCR as well as HSCR as a risk factor for other events in life are necessary.

There are also other interesting areas for the search of etiology within the field of ENS development and differentiation. Ongoing studies on the possibilities of a future stem-cell based therapy for HSCR may lead to a new treatment for the disease.[14,158] The understanding of microbiome in HSCR and how it affects the risk for HAEC is another challenge for the future.[159]

Today, however, surgery is the treatment of choice for HSCR. As shown in Studies II and III, patients with HSCR have fecal incontinence and constipation a long time after surgery. It is important for pediatric surgeons as well as for general surgeons who treat adult patients with HSCR to be aware of the risk of these symptoms. When seeing these individuals in an out-patient situation it is important to ask for specific signs of incontinence and constipation, instead of asking if everything is normal, since patients with congenital malformations in the gastrointestinal tract may never experience a "normal" bowel function. It is also important as a clinician to be aware of the complication's possible impact on QoL, to be able to react in a supporting way. Ideally these patients would be centralized to be able to receive specialized and individualized care and treatment. Centralization would also improve the transition of the patient from the pediatric care to adult care, which occurs in Sweden at 18 years of age (a sensible time for the patient).

For the future it is of great importance to continue to follow these patients over time to understand the natural course after different types of surgery procedures, what problems may occur, which is the best treatment, and how QoL is affected. Therefore, the introduction of a standardized and validated questionnaire for the evaluation of bowel function and QoL for all outpatient visits would be ideal. Also clinical as well as research cooperation between different pediatric surgery units and between pediatric surgeons and general surgeons is of importance to achieve the best care for the patients.

8 APPENDIX

8.1 A. QUESTIONNAIRE STUDY II

Symtomregistrering

Ålder vid uppföljningen _____ år_____mån

Längd:_____ Vikt:_____ BMI:_____

Släkting Hirschsprung:	N	J	Släktrelation:_____		
Släkting IBD:	N	J	Släktrelation:_____		
Ulkningar/kräkningar:		N	J		
Antal avföringar:	1 var tredje dag-3/dag		4-6/dag	7-10/dag	>10/dag
Förstoppning:		N	J		
Bruk laxantia:		N	J	Typ/dos:_____	
Bruk irrigation/lavemang/ACE:		N	J		
Full avföringskontroll:		N	J		
Luftinkontinens: (>1 gång/v)		N	J		
Soiling vid lös avföring: (>1 gång/v)		N	J		
Soiling vid fast avföring: (>1 gång/v)		N	J		
Påverkas barnet av soiling:		N	J		
Bruk av stoppande medicin:		N	J	Typ/dos:_____	
Antal episoder av enterokolit som krävt behandling:			1	2-5	>5
Miktionsproblem:		N	J	Typ:_____	
Medicinering:		N	J	Typ:_____	
Normal skola:		N	J		
Fysisk aktivitet:		N	J		
Kamrater:		N	J		
Normal kost:		N	J		

Avvikelse:_____

8.2 B. QUESTIONNAIRE STUDY III

A. Allmänna frågor

1. Är Du idag yrkesverksam? (möjlighet att kryssa i fler svar):

   - ❑ Ja, arbetar som ……..
   - ❑ Nej, studerar ……..
   - ❑ Nej, är långtidssjukskriven
   - ❑ Nej, uppbär sjukpenning
   - ❑ Nej är pensionerad
   - ❑ Nej, är arbetssökande
   - ❑ Nej, annat……

2. Yrke:

3. Utbildning:
   - ❑ Grundskola/Gymnasium (Linje/program:……)
   - ❑ Högskola/Universitet (Ämne:…….)
   - ❑ Examen:

4. Har Dina ändtarmsbesvär i barnaåren haft någon inverkan på ditt yrkesval?
   - ❑ Ja Om ja, på vilket sätt…….....
   - ❑ Nej

5. Civilstånd:
   - ❑ Gift /Sambo
   - ❑ Ensamstående med fast relation
   - ❑ Ensamstående utan fast relation

6. Syskon:
   - ❑ Ja, Antal:
   - ❑ Nej

7. Om ja, syskonbarn
   - ❑ Ja, antal…….
   - ❑ Nej

8. Finns någon annan i din släkt som har Hirschsprungs sjukdom?
   - ❑ Ja
   - ❑ Nej

9. Om ja, hur är denna/dessa person(er) släkt med dig?

10. Fritidsintressen
    - ❑ Under tonåren:……..
    - ❑ Nuvarande:…….

11. Sysslar du men någon sport?
    - ❑ Ja Om ja; vilken?
    - ❑ Nej.

12. Vikt: kg

13. Längd: cm

14. Röker Du?
    - ❑ Ja, paket/v sedan år
    - ❑ Nej / Har rökt tidigare Fr.o.m. till

15. Har sjukdomen påverkat din fritid?
    - ❑ Ja Om ja, hur?
    - ❑ Nej

16. Har sjukdomen påverkat ditt kamratliv?
- ❑ Ja Om ja, hur?
- ❑ Nej

17. Har sjukdomen påverkat nära relationer med dina föräldrar?
- ❑ Ja Om ja, hur?
- ❑ Nej

18. Har sjukdomen påverkat nära relationer med dina syskon?
- ❑ Ja Om ja, hur?
- ❑ Nej

19. Har sjukdomen påverkat nära relationer med dina vänner?
- ❑ Ja Om ja, hur?
- ❑ Nej

20. Har du blivit mobbad någon gång?
- ❑ Ja
- ❑ Nej

21. Har du någon gång mobbat andra?
- ❑ Ja
- ❑ Nej

22. Har du fått information om din Hirschsprungs sjukdom från sjukvården?
- ❑ Ja
- ❑ Nej

23. Om ja, hur gammal var du när du fick informationen? År

24. Kommer du ihåg vad du fick reda på? (*fri text)*

25. Har du fått information om Hirschsprungs sjukdom från dina föräldrar?
- ❑ Ja
- ❑ Nej

26. Kommer du ihåg vad du fått reda på av dina föräldrar? *(fri text)*

27. Tycker du att den information du fick var tillräcklig?
- ❑ Ja
- ❑ Nej

28. Om nej, kan du berätta varför? (fri text)

29. Hur upplevde du efterkontrollerna hos läkaren? (Positivt / Negativt)

30. Hur upplevde du informationen kring operationen?(Positivt / Negativt)

31. När har du blivit opererad?

32. Blev det några komplikationer efter någon operation?
- ❑ Ja om ja, vilken?
- ❑ Nej

33. Har du regelbunden kontakt med läkare nu?
- ❑ Ja
- ❑ Nej

34. Skulle du vilja ha mer kontroller hos läkare?
- ❑ Ja
- ❑ Nej

35. Hur tycker du att sjukvården ska hantera patienter med Hirschsprungs sjukdom vad det gäller läkarkontroller?
- ❑ >4/år
- ❑ >1g/år
- ❑ <1g /år
- ❑ bara efter op

36. Hur tycker du det har varit att delta i denna undersökning?
VAS-skala (Mycket jobbigt-Lite *jobbigt-Helt ok- Inga problem)* + kommentarer (*fri text)*

37. Har du ytterligare synpunkter som du vill förmedla?

B. Tarmfunktion

1. Har du stomi?
- ❑ Nej
- ❑ Ja, ileostomi (hoppa över fråga 11-36)
- ❑ Ja, colostomi (hoppa över fråga 11-36)
- ❑ Ja, oklart vilken sort (hoppa över fråga 11-36)

2. Hur ofta har Du avföring/behöver du tömma stomipåsen?
- ❑ Mer än 2 ggr/dag
- ❑ 2 ggr/dag
- ❑ 1 gång/dag
- ❑ Ungefär varannan dag
- ❑ Ungefär 2 ggr/vecka
- ❑ Ungefär 1 gång/vecka
- ❑ Mer sällan än 1 gång/vecka

3. Använder Du piller, droppar, pulver eller vätska för att hålla tarmen igång?
- ❑ Ja, i så fall vad? ……
- ❑ Nej

4. Använder Du lavemang?
- ❑ Ja, via ändtarmen ……..ggr per vecka
- ❑ Ja, via ”appendicostomi eller knapp” …….ggr per vecka
- ❑ Nej

5. Använder Du mikrolavemang (Toilax, Microlax etc)?
- ❑ Ja, i så fall hur ofta? ……..
- ❑ Nej

6. Använder Du någon form av stoppande medicin (imodium, loperamid el dyl)
- ❑ Ja, i så fall hur ofta? ……..
- ❑ Nej

7. Hur är avföringens konsistens?
- ❑ Lös, dvs ej formad
- ❑ Normal, dvs mjuk och formad
- ❑ Hård
- ❑ Växlande mellan lös och hard

8. Besväras Du av smärtor i buken/bäckenet?
- ❑ Ja, i så fall hur ofta/när kommer de och var i magen sitter de?
  …….
  …….
- ❑ Nej

9. Besväras Du av uppblåshet buken?
   - ❑ Ja
   - ❑ Nej

10. Besväras Du av mycket gaser?
   - ❑ Ja
   - ❑ Nej

11. Måste Du hjälpa till med fingret eller annat föremål för att få ut avföringen?
   - ❑ Ja
   - ❑ Nej

12. Måste Du stödja med fingrarna kring ändtarmen eller i slidan för att få ut avföringen?
   - ❑ Ja
   - ❑ Nej

13. Måste Du krysta hårt för att få ut avföringen?
   - ❑ Ja
   - ❑ Nej

14. Behöver Du sitta länge för att tömma tarmen?
   - ❑ Nej
   - ❑ Ja, cirka 10 minuter
   - ❑ Ja, cirka 20 minuter
   - ❑ Ja mer än 20 minuter

15. Försök uppskatta under hur stor del av tarmtömningen som Du behöver krysta.

    Ingen del Halva Hela

16. Tycker Du att det är svårt att få tarmen att kännas tom efter toalettbesök?
   - ❑ Ja
   - ❑ Nej

17. Måste Du gå flera gånger på toaletten samma dag för att det ska kännas tomt?
   - ❑ Ja
   - ❑ Nej

18. Har Du ofrivilligt läckage av gaser?
   - ❑ Nej
   - ❑ Ja, enstaka gånger
   - ❑ Ja, minst 1 gång/vecka
   - ❑ Ja, dagligen

19. Har Du ofrivilligt läckage av lös avföring?
   - ❑ Nej
   - ❑ Ja, enstaka gånger
   - ❑ Ja, minst 1 gång/vecka
   - ❑ Ja, dagligen

20. Har Du ofrivilligt läckage av fast avföring?
   - ❑ Nej
   - ❑ Ja, enstaka gånger
   - ❑ Ja, minst 1 gång/vecka
   - ❑ Ja, dagligen

21. Om Du besväras av avföringsläckage vilken beskrivning passar bäst in på de läckage Du upplevt?
   - ❑ Plötsligt har det inträffat ett läckage utan att jag har känt det?
   - ❑ Jag får trängningar till avföring men kan ej hålla emot
   - ❑ Läckage av avföringsfärgat slem och nedsmutsning av underkläder

22. Bär Du någon form av skydd mot avföringsläckage nattetid?
- ❑ Ja
- ❑ Nej

23. Bär Du någon form av skydd mot avföringsläckage dagtid?
- ❑ Ja
- ❑ Nej

24. Har Du problem med nedsmutsning av trosor/kalsonger?
- ❑ Ja
- ❑ Nej

25. Har Du besvär med klåda kring ändtarmen?
- ❑ Ja
- ❑ Nej

26. Känner Du när avföringen kommer?
- ❑ Ja
- ❑ Nej

27. Kan Du skillnad på om det är gas eller avföring i tarmen?
- ❑ Ja
- ❑ Nej

28. Måste Du snabbt till en toalett när Du känner att Du behöver?
- ❑ Ja
- ❑ Nej

29. Från det att Du fört känner behov av att tömma tarmen hur, hur länge kan du hålla Dig?
Vid **lös** avföring ----------
0 5 10 15 20 minuter

30. Från det att Du fört känner behov av att tömma tarmen hur, hur länge kan du hålla Dig?
Vid **fast** avföring ----------
0 5 10 15 20 minuter

31. Besväras Du av smärtor i eller kring ändtarmen?
- ❑ Ja
- ❑ Nej

32. Har Du smärtor i tarmen/bäckenet/buken i samband med avföring?
- ❑ Ja
- ❑ Nej

33. Har Du smärtor i tarmen/bäckenet/buken efter avföring?
- ❑ Ja
- ❑ Nej

34. Besväras Du av blödning från tarmen eller tarmöppningen?
- ❑ Ja
- ❑ Nej

35. Försök att uppskatta ungefär hur många gånger Du har avföring under en ”vanlig” vecka
……..ggr

36. Försök att uppskatta ungefär hur många gånger Du skulle ha avföring under en vecka ifall du inte tog några lavemang eller mediciner för att reglera avföringsfrekvensen
……..ggr

37. Är Din tarmfunktion besvärande för Ditt allmänna välbefinnande?
- ❑ Ja
- ❑ Nej

38. Är Din tarmfunktion besvärande för Ditt umgängesliv?
- ❑ Ja
- ❑ Nej

39. Behöver Du hålla diet på grund av Dina tarmbesvär
- ❑ Ja, i så fall vad undviker Du att äta?
  ……
- ❑ Nej

40. Är Dina besvär med tarmen så svåra att de hindrar Dig från att resa på semester?
- ❑ Ja
- ❑ Nej

C. Frågor rörande urinvägsfunktion

1. Kan Du hålla tätt för urin vid hosta, nysning, skratt eller tunga lyft?
- ❑ Ja
- ❑ Nej

2. Tycker Du att Du har svårt att tömma urinblåsan?
- ❑ Ja
- ❑ Nej

D. Livskvalitet

1. Hur ofta har du de senaste två veckorna haft buksmärtor?
- ❑ Hela tiden
- ❑ För det mesta
- ❑ Ibland
- ❑ Sällan
- ❑ Aldrig

2. Hur ofta har du under de senaste två veckorna haft en fyllnadskänsla i övre delen av buken?
- ❑ Hela tiden
- ❑ För det mesta
- ❑ Ibland
- ❑ Sällan
- ❑ Aldrig

3. Hur ofta har du under de senaste två veckorna besvärats av uppblåsthet eller känsla av att du haft för mycket gaser i magen?
- ❑ Hela tiden
- ❑ För det mesta
- ❑ Ibland
- ❑ Sällan
- ❑ Aldrig

4. Hur ofta har du under de senaste två veckorna besvärats av gasavgång?
- ❑ Hela tiden
- ❑ För det mesta
- ❑ Ibland
- ❑ Sällan
- ❑ Aldrig

5. Hur ofta har du under de senaste två veckorna besvärats av rapningar eller uppstötningar?
- ❑ Hela tiden
- ❑ För det mesta
- ❑ Ibland
- ❑ Sällan
- ❑ Aldrig

6. Hur ofta har du under de senaste tve veckorna haft påfallande mag- eller tarmljud?
   - ❑ Hela tiden
   - ❑ För det mesta
   - ❑ Ibland
   - ❑ Sällan
   - ❑ Aldrig

7. Hur ofta har du under de senaste två veckorna besvärats av talrika avföringar?
   - ❑ Hela tiden
   - ❑ För det mesta
   - ❑ Ibland
   - ❑ Sällan
   - ❑ Aldrig

8. Hur ofta har du känt nöje och glädje av att äta de senaste två veckorna?
   - ❑ Hela tiden
   - ❑ För det mesta
   - ❑ Ibland
   - ❑ Sällan
   - ❑ Aldrig

9. Hur ofta har du på grund av din sjukdom avstått från mat du annars gärna äter?
   - ❑ Hela tiden
   - ❑ För det mesta
   - ❑ Ibland
   - ❑ Sällan
   - ❑ Aldrig

10. Hur har du under de senaste två veckorna kunnat hantera vardagsstress?
    - ❑ Mycket dåligt
    - ❑ Dåligt
    - ❑ Acceptabelt
    - ❑ Bra
    - ❑ Mycket bra

11. Hur ofta har du under den senaste två veckorna varit ledsen över att du är sjuk?
    - ❑ Hela tiden
    - ❑ För det mesta
    - ❑ Ibland
    - ❑ Sällan
    - ❑ Aldrig

12. Hur ofta har du under de senaste två veckorna känt dig nervös eller orolig på grund av din sjukdom?
    - ❑ Hela tiden
    - ❑ För det mesta
    - ❑ Ibland
    - ❑ Sällan
    - ❑ Aldrig

13. Hur ofta har du under de senaste två veckorna känt dig allmänt tillfreds med livet?
    - ❑ Hela tiden
    - ❑ För det mesta
    - ❑ Ibland
    - ❑ Sällan
    - ❑ Aldrig

14. Hur ofta har du under de senaste två veckorna känt dig frustrerad över din sjukdom?
    - ❑ Hela tiden
    - ❑ För det mesta
    - ❑ Ibland
    - ❑ Sällan
    - ❑ Aldrig

15. Hur ofta har du under de senaste två veckorna känt dig trött eller hängig?
- ❑ Hela tiden
- ❑ För det mesta
- ❑ Ibland
- ❑ Sällan
- ❑ Aldrig

16. Hur ofta har du under de senaste två veckorna känt att du inte mått bra?
- ❑ Hela tiden
- ❑ För det mesta
- ❑ Ibland
- ❑ Sällan
- ❑ Aldrig

17. Hur ofta har du under den senaste veckan (1 vecka) vaknat på natten?
- ❑ Varje natt
- ❑ 5-6 nätter
- ❑ 3-4 nätter
- ❑ 1-2 nätter
- ❑ Aldrig

18. Hur mycket har din sjukdom lett till att ditt utseende förändrats negativt?
- ❑ Väldigt mycket
- ❑ Mycket
- ❑ Måttligt
- ❑ Lite
- ❑ Inte alls

19. Hur mycket har din allmänna styrka avtagit på grund av din sjukdom?
- ❑ Väldigt mycket
- ❑ Mycket
- ❑ Måttligt
- ❑ Lite
- ❑ Inte alls

20. Hur mycket har du till följd av din sjukdom förlorat din uthållighet?
- ❑ Väldigt mycket
- ❑ Mycket
- ❑ Måttligt
- ❑ Lite
- ❑ Inte alls

21. I vilken utsträckning har du till följd av din sjukdom tappat formen?
- ❑ Väldigt mycket
- ❑ Mycket
- ❑ Måttligt
- ❑ Lite
- ❑ Inte alls

22. Har du kunnat fullgöra dina dagliga aktiviteter (t ex arbete, skola och hushåll) de senaste två veckorna?
- ❑ Hela tiden
- ❑ För det mesta
- ❑ Ibland
- ❑ Sällan
- ❑ Aldrig

23. Har du kunnat utföra dina normala fritidsaktiviteter (sport, hobby osv.) de senaste två veckorna?
- ❑ Hela tiden
- ❑ För det mesta
- ❑ Ibland
- ❑ Sällan
- ❑ Aldrig

24. Har du under de senaste två veckorna känt dig negativt påverkad av din medicinska behandling?
- ❑ Väldigt mycket
- ❑ Mycket
- ❑ Måttligt
- ❑ Lite
- ❑ Inte alls

25. I vilken utsträckning har ditt förhållande till dina närstående påverkats av din sjukdom?
- ❑ Väldigt mycket
- ❑ Mycket
- ❑ Måttligt
- ❑ Lite
- ❑ Inte alls

26. I vilken utsträckning har din sjukdom vållat avbräck i ditt sexualliv?
- ❑ Väldigt mycket
- ❑ Mycket
- ❑ Måttligt
- ❑ Lite
- ❑ Inte alls

27. Har du under de senaste två veckorna de senaste två veckorna besvärats av uppstött föda eller vätska i munnen?
- ❑ Hela tiden
- ❑ För det mesta
- ❑ Ibland
- ❑ Sällan
- ❑ Aldrig

28. Hur ofta har du under de senaste två veckorna känt dig besvärad av att du varit tvungen att äta långsamt
- ❑ Hela tiden
- ❑ För det mesta
- ❑ Ibland
- ❑ Sällan
- ❑ Aldrig

29. Hur ofta har du under de senaste två veckorna besvärats av att du haft svårt att svälja föda?
- ❑ Hela tiden
- ❑ För det mesta
- ❑ Ibland
- ❑ Sällan
- ❑ Aldrig

30. Hur ofta har du under de senaste två veckorna besvärats av att du snabbt måste hinna till toaletten för att ha avföring?
- ❑ Hela tiden
- ❑ För det mesta
- ❑ Ibland
- ❑ Sällan
- ❑ Aldrig

31. Hur ofta har du under de senaste två veckorna besvärats av diarréer?
- ❑ Hela tiden
- ❑ För det mesta
- ❑ Ibland
- ❑ Sällan
- ❑ Aldrig

32. Hur ofta har du under de senaste två veckorna besvärats av förstoppning?
- ❑ Hela tiden
- ❑ För det mesta
- ❑ Ibland
- ❑ Sällan
- ❑ Aldrig

33. Hur ofta har du under de senaste två veckorna känt dig besvärad av illamående?
- ❑ Hela tiden
- ❑ För det mesta
- ❑ Ibland
- ❑ Sällan
- ❑ Aldrig

34. Hur ofta har du under de senaste två veckorna oroat dig över blod i avföringen?
- ❑ Hela tiden
- ❑ För det mesta
- ❑ Ibland
- ❑ Sällan
- ❑ Aldrig

35. Hur ofta har du under de senaste två veckorna besvärats av halsbränna?
- ❑ Hela tiden
- ❑ För det mesta
- ❑ Ibland
- ❑ Sällan
- ❑ Aldrig

36. Hur ofta har du under de senaste två veckorna besvärats av okontrollerad avgång av avföringsavgång?
- ❑ Hela tiden
- ❑ För det mesta
- ❑ Ibland
- ❑ Sällan
- ❑ Aldrig

E. SF-36 Hälsoenkät

Besvara frågorna genom att sätta en ring runt den siffra Du tycker stämmer bäst in på Dig. Om Du är osäker ringa ändå in den siffra som känns riktigast.

1. I allmänhet, skulle Du vilja säga att Din hälsa är:

Utmärkt	1
Mycket god	2
God	3
Någorlunda	4
Dålig	5

2. Jämfört med för ett år sedan, hur skulle Du vilja bedöma Ditt allmänna hälsotillstånd nu?

Mycket bättre än för ett år sedan	1
Något bättre än för ett år sedan	2
Ungefär detsamma	3
Något sämre nu än för ett år sedan	4
Mycket sämre nu än för ett år sedan	5

3. De följande frågorna handlar om aktiviteter som Du kan tänkas utföra under en vanlig dag. Är Du på grund av Ditt hälsotillstånd begränsad i dessa aktiviteter nu? Om så fall, hur mycket?

	Ja, mycket begränsad	**Ja, lite begränsad**	**Nej, inte begränsad**
a. **Ansträngande aktiviteter** (springa, lyfta tunga saker)	1	2	3
b. **Måttligt ansträngande aktiviteter** (flytta bord, dammsuga, gå en skogspromenad)	1	2	3
c. Lyfta eller bära matkassar	1	2	3
d. Gå uppför **flera** trappor	1	2	3
e. Gå uppför **en** trappa	1	2	3
f. Böja Dig eller gå ner på knä	1	2	3
g. Gå **mer än två kilometer**	1	2	3
h. Gå **några hundra meter**	1	2	3
i. Gå **hundra meter**	1	2	3
j. Bada eller klä på dig	1	2	3

4. Under de senaste fyra veckorna, har Du haft något av följande hälsoproblem i Ditt arbete eller med andra regelbundna dagliga aktiviteter som en följd av Ditt kroppsliga hälsotillstånd?

	JA	**NEJ**
a. Skurit ned den **tid** Du normalt ägnat åt arbete eller andra aktiviteter?	1	2
b. **Uträttat mindre** än Du skulle önskat?	1	2
c. Varit hindrad att utföra **vissa** arbetsuppgifter eller andra aktiviteter?	1	2
d. Haft **svårigheter** att utföra Ditt arbete eller andra aktiviteter (t.ex genom att det krävde extra ansträngning)?	1	2

5. Under de senaste fyra veckorna, har Du haft några av följande problem i Ditt arbete eller mer andra regelbundna dagliga aktiviteter som en följd av känslomässiga problem (som t.ex. nedstämdhet eller ängslan)?

	JA	**NEJ**
a. Skurit ned den **tid** Du normalt ägnat åt arbete eller andra aktiviteter?	1	2
b. **Uträttat mindre** än Du skulle önskat?	1	2
c. Inte utfört arbete eller andra aktiviteter så **noggrant** som vanligt?	1	2

6. Under de senaste fyra veckorna, i vilken utsträckning har Ditt kroppsliga hälsotillstånd eller Dina känslomässiga problem stört Ditt vanliga umgänge med anhöriga, vänner, grannar eller andra?

Inte alls	1
Lite	2
Måttligt	3
Mycket	4
Väldigt mycket	5

7. Hur mycket värk eller smärta har du haft under de senaste fyra veckorna?

Ingen	1
Mycket lätt	2
Lätt	3
Måttlig	4
Svårt	5
Mycket svår	6

8. Under de senaste fyra veckorna, hur mycket har värken eller smärtan stört Ditt normala arbete (innefattande både arbete utanför hemmet och hushållssysslor)?

Inte alls	1
Lite	2
Måttligt	3
Mycket	4
Väldigt mycket	5

9. Frågorna här handlar om hur Du känner Dig och hur Du haft det under de senaste fyra veckorna. Ange för varje fråga det svarsalternativ som bäst beskriver hur Du känt Dig. Hur

Hur stor del av tiden under de senaste fyra veckorna …

	Hela Tiden	**Största delen av tiden**	**En hel del av tiden**	**En del av tiden**	**Lite av tiden**	**Inget av tiden**
a. …har Du känt Dig riktigt pigg och stark?	1	2	3	4	5	6
b. …har Du känt Dig mycket nervös?	1	2	3	4	5	6
c. …har Du känt Dig så nedstämd att ingenting kunnat muntra upp dig?	1	2	3	4	5	6
d. …har Du känt Dig lugn och harmonisk?	1	2	3	4	5	6
e. …har Du varit full av energi?	1	2	3	4	5	6
f. …har Du känt Dig dyster och ledsen?	1	2	3	4	5	6
g. …har Du känt Dig utsliten?	1	2	3	4	5	6
h. …har du känt Dig glad och lycklig?	1	2	3	4	5	6
i. …har Du känt Dig trött?	1	2	3	4	5	6

10. Under de senaste fyra veckorna, hur stor del av tiden har Ditt kroppsliga hälsotillstånd eller Dina känslomässiga problem stört Dina möjligheter att umgås (t.ex. hälsa på släkt och vänner etc)?

Hela tiden	1
Största delen av tiden	2
En del av tiden	3
Lite av tiden	4
Inget av tiden	5

11. Välj det svarsalternativ som bäst beskrivet hur mycket vart och ett av följande påståenden STÄMMER eller INTE STÄMMER in på Dig

	Stämmer precis	**Stämmer ganska bra**	**Osäker**	**Stämmer inte särskilt bra**	**Stämmer inte alls**
a. Jag verkar ha lite lättare att bli sjuk än andra människor	1	2	3	4	5
b. Jag är lika frisk som vem som helst av dem jag känner	1	2	3	4	5
c. Jag tror min hälsa kommer att bli sämre	1	2	3	4	5
d. Min hälsa är utmärkt	1	2	3	4	5

F. Fertilitet

1. Har du försökt få barn?
   - ❑ Ja
   - ❑ Nej

2. Har du någongång varit gravid eller gjort någon gravid?
   - ❑ Ja
   - ❑ Nej

3. Har du haft svårt att få barn?
   - ❑ Ja Om ja, har du sökt för svårigheten att få barn?
   - ❑ Nej

4. Har du genomgått någon form av infertilitetsutredning?
   - ❑ Ja
   - ❑ Nej

5. Har du fått behandling i syfte att få barn?
   - ❑ Ja Om ja, vilken typ av behandling?
   - ❑ Nej

6. Har du/har du haft impotensbesvär?
   - ❑ Ja
   - ❑ Nej

7. Har du fått någon behandling mot impotens?
   - ❑ Ja Om ja, under vilka perioder? Hur ofta?
   - ❑ Nej

8. Fungerade behandlingen?
   - ❑ Ja
   - ❑ Nej

G. Frågor enbart gällande kvinnor

1. Hur många vanliga förlossningar har Du genomgått?
   ……stycken

2. Användes tång vid någon förlossning?
   - ❑ Ja, vid ……stycken förlossningar
   - ❑ Nej

3. Användes sugklocka vid någon förlossning?
   - ❑ Ja, vid ……stycken förlossningar
   - ❑ Nej

4. Blev Du sydd i underlivet i samband med någon förlossning?
   - ❑ Ja
   - ❑ Nej

5. Är det känt om Du fick en skada på slutmuskeln vid någon förlossning?
   - ❑ Ja
   - ❑ Nej

6. Har Du skötts av gynekolog på grund av underlivsframfall?
   - ❑ Ja
   - ❑ Nej

7. Har Du opererats på grund av underlivsframfall?
   - ❑ Ja
   - ❑ Nej

9 ACKNOWLEDGEMENTS

Först och främst vill jag tacka alla patienter med Hirschsprungs sjukdom och deras familjer för deltagande i mina studier. Det är min högsta önskan att den här avhandlingen på ett eller annat sätt kommer er till nytta.

Utan hjälp och stöd från många värdefulla människor hade den här avhandlingen inte sett dagens ljus. **Ett stort tack till alla er!!! Till några av er vill jag rikta ett särskilt tack:**

Tomas Wester, min huvudhandledare, för att du ledsagat mig genom den här processen. Utan dig hade det här aldrig blivit av! Dina barnkirurgiska kunskaper och ditt intresse för forskning har tillsammans med ditt sätt att handleda gjort den här tiden väldigt lärorik, trevlig och stimulerande. Det är min förhoppning att vårt samarbete inte slutar här, det finns många aspekter av Hirschsprungs sjukdom kvar att studera!

Agneta Nordenskjöld, min bihandledare, för att du under hela tiden uppmuntrat mig och tagit dig tid trots att det kanske inte alltid har funnits. Ditt gedigna forskningsintresse har verkligen smittat mig. Dina stora erfarenheter av doktorandprocessen och KI har varit till stor hjälp under hela denna tid.

Britt Husberg Sellberg, min bihandledare, för dina breda kliniska erfarenheter som varit till stor hjälp under mina studier. Stort tack också för ditt stora undervisningsintresse och engagemang. Mitt intresse för patienter med långtidsproblem efter operation av Hirschsprungs sjukdom har du verkligen väckt.

Staffan Sahlin, min externa mentor, för att du som en far som oroat dig över om jag har trivts och hur jag har blivit behandlad. Tack också för att jag efter ett möte med dig alltid går med ett stort leende på läpparna efter alla dina historier. Utan dig som världens mest engagerade kursansvariga på kirurgkursen på DS 2007 hade mitt intresse för kirurgin kanske aldrig väckts…

Lisa Örtqvist, vän och kollega, för all hjälp, råd och tips som du delat med dig av under åren. Stort tack också för att du är en så fixig och social person som gjorde att jag bland annat kände mig så välkommen den där våren 2009 när jag gjorde mitt examensarbete. Jag ser fram emot många härliga kliniska/forskningsaktiva/privata år med dig!

Emma Öistämö, vän och kollega, för att du är en fantastik person på så många sätt och för att livet i din närhet alltid bjuder på överraskningar. Utan dig hade den här avhandlingen blivit klar tidigare, men utan så mycket skratt och äventyr.

Anna Svenningsson, för att du bidragit med mycket hjälp med epidemiologin. Tack för att du är en sån klippa och glädjespridare.

Philip Frykman, for accepting me into your exciting collaboration about HAEC and for inviting me to Cedars-Sinai. I am so happy that you introduced us to your multi center study that day in May 2009, which became the start of this PhD project. Thank you for your enthusiasm, for never giving up and for attending my dissertation.

PJ Svensson, för dina kloka råd och uppmuntring.

Jenny Oddsberg, för hjälpen med den gedigna planeringen av de epidemiologiska studierna.

Olof Akre, för att du svarat på alla mina epidemiologiska frågor och funderingar i tid och otid.

Christina Nyström, Johanna Winberg, Ellen Markljung, för all hjälp på lab!

Johan Danielson, för ditt arbete med kontroller till delstudie III.

Daniel Nilsson, för ditt arbete med exomsekvensering och att du delat med dig av dina kunskaper.

Eva Hagel, för lärorikt statistiskt samarbete.

DS kirurg och urolog klinik, för att ni gör DS till en fantastisk arbetsplats! Speciellt tack till verksamhetschef **Johanna Albert**, studierektor och schemaläggare **Ylva Falkén**, ST-chef **David Stillström,** prefekt **Erik Näslund** och min kliniska handledare **Klas Pekkari**. Tack också till **Lars Granström**, **Henrik Nilsson** och **Martin Sundelöf** för att ni sedan kirurgkursen visat hur rolig kirurgi verkligen är. Tack också alla som kommit och gått på pigrummet, inkl närliggande rum (**Jennie Engstrand**).

ALB Barnkirurgen, för all uppmuntran och stöd under denna långa process. Tack för att jag fått bli en del av er. Särskilt tack till **Tina Granholm** för att du startade SVK kursen inom barnkirurgi och därmed fick mig på barnkirurgiskt fall.

Astrid Häggblad och Anna Sandberg, Institutionen för kvinnors och barns hälsa, för all er hjälp kring allt administrativt!

Tomas Westers forskargrupp, för trevliga middagar och socialt utbyte.

Doktorandgruppen på ALB, för intressanta och lärorika fredagsmöten.

Familjen Kjellander, framför allt för det senaste årets delade intresse av disputationsprocessen, men också för allt annat!

KI-gänget: Ankan, Maria, Anna, Sofia, Joanna, för att ni förgyllde pluggtiden på KI och att ni fortsätter att förgylla min värld. Tack för alla sjukhusluncher/fikor, middagar, fester, allmänt häng och resor - ni är fantastiska!

Mamma och **Pappa**, för att ni finns! Ni har varit och är fantastiska och stöttande, hjälpande och omhändertagande när jag behöver det. Jag är väldigt lycklig över att ha er som mina föräldrar! Tack också till mina fina bröder **Jonas** och **Victor** med familjer!

Eva och **Thomas**, för all hjälp med barnen genom åren. Utan er hade det här arbetet tagit många år till.

Ebba och **Amanda**, mina underbara döttrar, för att ni alltid visar mig vad som är viktigast, livet med er!

Calle, för att du orkar med mig, jag börjar förstå att det inte är det lättaste. För att du är en fantastisk lyssnare och för att du tar hand om mig när jag behöver det. Jag älskar dig!

Funding for this thesis and for included papers has been provided by the following research foundations: Frimurare Barnahuset, Sällskapet Barnavård och Kronprinsessan Lovisas förening för barnasjukvård.

10 REFERENCES

1. Hirschsprung H. Stuhltragheit Neugeborener in Folge von Dilatation und Hypertrophie des Colons. *Jhrb f Kinderh* 21, 1-7 (1888).
2. Raveenthiran V. Knowledge of ancient Hindu surgeons on Hirschsprung disease: evidence from Sushruta Samhita of circa 1200-600 BC. *J Pediatr Surg* 46, 2204-8 (2011).
3. Ehrenpreis T. Megacolon in the newborn: a clinical and roentgenological study with special regard to the pathogenesis. *Acta Chir Scand* 59, 1071-82 (1946).
4. Whitehouse FR, Kernohan JW. Myenteric plexus in congenital megacolon; study of 11 cases. *Arch Intern Med (Chic)* 82, 75-111 (1948).
5. Swenson O, Rheinlander HF, Diamond I. Hirschsprung's disease; a new concept of the etiology; operative results in 34 patients. *N Engl J Med* 241, 551-6 (1949).
6. Goyal RK, Hirano I. The enteric nervous system. *N Engl J Med* 334, 1106-15 (1996).
7. Le Douarin NM, Teillet MA. The migration of neural crest cells to the wall of the digestive tract in avian embryo. *J Embryol Exp Morphol* 30, 31-48 (1973).
8. Yntema CL, Hammond WS. The origin of intrinsic ganglia of trunk viscera from vagal neural crest in the chick embryo. *J Comp Neurol* 101, 515-41 (1954).
9. Kapur RP. Colonization of the murine hindgut by sacral crest-derived neural precursors: experimental support for an evolutionarily conserved model. *Dev Biol* 227, 146-55 (2000).
10. Wallace AS, Burns AJ. Development of the enteric nervous system, smooth muscle and interstitial cells of Cajal in the human gastrointestinal tract. *Cell Tissue Res* 319, 367-82 (2005).
11. Sasselli V, Pachnis V, Burns AJ. The enteric nervous system. *Dev Biol* 366, 64-73 (2012).
12. Heanue TA, Pachnis V. Enteric nervous system development and Hirschsprung's disease: advances in genetic and stem cell studies. *Nat Rev Neurosci* 8, 466-79 (2007).
13. Le Douarin NM, Teillet MA. Experimental analysis of the migration and differentiation of neuroblasts of the autonomic nervous system and of neurectodermal mesenchymal derivatives, using a biological cell marking technique. *Dev Biol* 41, 162-84 (1974).
14. Fattahi F, Steinbeck JA, Kriks S, et al. Deriving human ENS lineages for cell therapy and drug discovery in Hirschsprung disease. *Nature* 531, 105-9 (2016).
15. Breau MA, Dahmani A, Broders-Bondon F, Thiery JP, Dufour S. Beta1 integrins are required for the invasion of the caecum and proximal hindgut by enteric neural crest cells. *Development* 136, 2791-801 (2009).
16. Soret R, Mennetrey M, Bergeron KF, et al. A collagen VI-dependent pathogenic mechanism for Hirschsprung's disease. *J Clin Invest* 125, 4483-96 (2015).
17. Costa M, Brookes SJ. The enteric nervous system. *Am J Gastroenterol* 89, 129-37 (1994).
18. Gershon MD. The enteric nervous system: a second brain. *Hosp Pract (1995)* 34, 31-2, 35-8, 41-2, 46-7, 51-2 (1999).
19. Furness JB. The enteric nervous system and neurogastroenterology. *Nat Rev Gastroenterol Hepatol* 9, 286-94 (2012).
20. Brazelton TB. A child-oriented approach to toilet training. *Pediatrics* 29, 121-8 (1962).

21. Frenckner B, Euler CV. Influence of pudendal block on the function of the anal sphincters. *Gut* 16, 482-9 (1975).
22. Lawson JO, Nixon HH. Anal canal pressures in the diagnosis of Hirschsprung's disease. *J Pediatr Surg* 2, 544-52 (1967).
23. Nirasawa Y YJ, Ikawa H, Morikawa Y and Katsumata K. Hirschsprung's Disease: Catecholamine Content, Alpha-Adrenoceptors, and the Effect of Electrical Stimulation in Aganglionic colon. *J Pediatr Surg* 21, 136-42 (1986).
24. Puri P, Ohshiro K, Wester T. Hirschsprung's disease: a search for etiology. *Semin Pediatr Surg* 7, 140-7 (1998).
25. Badner JA, Sieber WK, Garver KL, Chakravarti A. A genetic study of Hirschsprung disease. *Am J Hum Genet* 46, 568-80 (1990).
26. Moore SW. The contribution of associated congenital anomalies in understanding Hirschsprung's disease. *Pediatr Surg Int* 22, 305-15 (2006).
27. Stoll C, Dott B, Alembik Y, Roth MP. Associated congenital anomalies among cases with Down syndrome. *Eur J Med Genet* 58, 674-80 (2015).
28. Friedmacher F, Puri P. Hirschsprung's disease associated with Down syndrome: a meta-analysis of incidence, functional outcomes and mortality. *Pediatr Surg Int* 29, 937-46 (2013).
29. Lyonnet S, Bolino A, Pelet A, et al. A gene for Hirschsprung disease maps to the proximal long arm of chromosome 10. *Nat Genet* 4, 346-50 (1993).
30. Angrist M, Kauffman E, Slaugenhaupt SA, et al. A gene for Hirschsprung disease (megacolon) in the pericentromeric region of human chromosome 10. *Nat Genet* 4, 351-6 (1993).
31. Attie T, Pelet A, Edery P, et al. Diversity of RET proto-oncogene mutations in familial and sporadic Hirschsprung disease. *Hum Mol Genet* 4, 1381-6 (1995).
32. Natarajan D, Marcos-Gutierrez C, Pachnis V, de Graaff E. Requirement of signalling by receptor tyrosine kinase RET for the directed migration of enteric nervous system progenitor cells during mammalian embryogenesis. *Development* 129, 5151-60 (2002).
33. Bordeaux MC, Forcet C, Granger L, et al. The RET proto-oncogene induces apoptosis: a novel mechanism for Hirschsprung disease. *EMBO J* 19, 4056-63 (2000).
34. Hearn CJ, Murphy M, Newgreen D. GDNF and ET-3 differentially modulate the numbers of avian enteric neural crest cells and enteric neurons in vitro. *Dev Biol* 197, 93-105 (1998).
35. Young HM, Hearn CJ, Farlie PG, Canty AJ, Thomas PQ, Newgreen DF. GDNF is a chemoattractant for enteric neural cells. *Dev Biol* 229, 503-16 (2001).
36. Angrist M, Bolk S, Halushka M, Lapchak PA, Chakravarti A. Germline mutations in glial cell line-derived neurotrophic factor (GDNF) and RET in a Hirschsprung disease patient. *Nat Genet* 14, 341-4 (1996).
37. Hosoda K, Hammer RE, Richardson JA, et al. Targeted and natural (piebald-lethal) mutations of endothelin-B receptor gene produce megacolon associated with spotted coat color in mice. *Cell* 79, 1267-76 (1994).
38. Baynash AG, Hosoda K, Giaid A, et al. Interaction of endothelin-3 with endothelin-B receptor is essential for development of epidermal melanocytes and enteric neurons. *Cell* 79, 1277-85 (1994).
39. Kusafuka T, Wang Y, Puri P. Mutation analysis of the RET, the endothelin-B receptor, and the endothelin-3 genes in sporadic cases of Hirschsprung's disease. *J Pediatr Surg* 32, 501-4 (1997).
40. Southard-Smith EM, Kos L, Pavan WJ. Sox10 mutation disrupts neural crest development in Dom Hirschsprung mouse model. *Nat Genet* 18, 60-4 (1998).

41. Kapur RP. Early death of neural crest cells is responsible for total enteric aganglionosis in Sox10(Dom)/Sox10(Dom) mouse embryos. *Pediatr Dev Pathol* 2, 559-69 (1999).
42. Pingault V, Bondurand N, Kuhlbrodt K, et al. SOX10 mutations in patients with Waardenburg-Hirschsprung disease. *Nat Genet* 18, 171-3 (1998).
43. Benailly HK, Lapierre JM, Laudier B, et al. PMX2B, a new candidate gene for Hirschsprung's disease. *Clin Genet* 64, 204-9 (2003).
44. Pattyn A, Morin X, Cremer H, Goridis C, Brunet JF. The homeobox gene Phox2b is essential for the development of autonomic neural crest derivatives. *Nature* 399, 366-70 (1999).
45. Fernandez RM, Mathieu Y, Luzon-Toro B, et al. Contributions of PHOX2B in the pathogenesis of Hirschsprung disease. *PLoS One* 8, e54043 (2013).
46. Van de Putte T, Maruhashi M, Francis A, et al. Mice lacking ZFHX1B, the gene that codes for Smad-interacting protein-1, reveal a role for multiple neural crest cell defects in the etiology of Hirschsprung disease-mental retardation syndrome. *Am J Hum Genet* 72, 465-70 (2003).
47. Stanchina L, Van de Putte T, Goossens M, Huylebroeck D, Bondurand N. Genetic interaction between Sox10 and Zfhx1b during enteric nervous system development. *Dev Biol* 341, 416-28 (2010).
48. Yamada Y, Nomura N, Yamada K, et al. The spectrum of ZEB2 mutations causing the Mowat-Wilson syndrome in Japanese populations. *Am J Med Genet A* 164A, 1899-908 (2014).
49. Li Y, Kido T, Garcia-Barcelo MM, Tam PK, Tabatabai ZL, Lau YF. SRY interference of normal regulation of the RET gene suggests a potential role of the Y-chromosome gene in sexual dimorphism in Hirschsprung disease. *Hum Mol Genet* 24, 685-97 (2015).
50. Ramalho-Santos M, Melton DA, McMahon AP. Hedgehog signals regulate multiple aspects of gastrointestinal development. *Development* 127, 2763-72 (2000).
51. Nagy N, Barad C, Graham HK, et al. Sonic hedgehog controls enteric nervous system development by patterning the extracellular matrix. *Development* 143, 264-75 (2016).
52. Liu JA, Lai FP, Gui HS, et al. Identification of GLI Mutations in Patients With Hirschsprung Disease That Disrupt Enteric Nervous System Development in Mice. *Gastroenterology* 149, 1837-48 e5 (2015).
53. Tam PK, Garcia-Barcelo M. Genetic basis of Hirschsprung's disease. *Pediatr Surg Int* 25, 543-58 (2009).
54. Garcia-Barcelo MM, Tang CS, Ngan ES, et al. Genome-wide association study identifies NRG1 as a susceptibility locus for Hirschsprung's disease. *Proc Natl Acad Sci U S A* 106, 2694-9 (2009).
55. Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci U S A* 74, 5463-7 (1977).
56. Ng SB, Turner EH, Robertson PD, et al. Targeted capture and massively parallel sequencing of 12 human exomes. *Nature* 461, 272-6 (2009).
57. Fu M, Sato Y, Lyons-Warren A, et al. Vitamin A facilitates enteric nervous system precursor migration by reducing Pten accumulation. *Development* 137, 631-40 (2010).
58. Gasc JM, Clemessy M, Corvol P, Kempf H. A chicken model of pharmacologically-induced Hirschsprung disease reveals an unexpected role of glucocorticoids in enteric aganglionosis. *Biol Open* 4, 666-71 (2015).
59. Huo W, Cai P, Chen M, et al. The relationship between prenatal exposure to BP-3 and Hirschsprung's disease. *Chemosphere* 144, 1091-7 (2016).

60. Kota SK, Modi KD, Rao MM. Hirschsprungs disease with congenital hypothyroidism. *Indian Pediatr* 49, 245-6 (2012).
61. Purdy IB, Singh N, Brown WL, Vangala S, Devaskar UP. Revisiting early hypothyroidism screening in infants with Down syndrome. *J Perinatol* 34, 936-40 (2014).
62. Lake JI, Tusheva OA, Graham BL, Heuckeroth RO. Hirschsprung-like disease is exacerbated by reduced de novo GMP synthesis. *J Clin Invest* 123, 4875-87 (2013).
63. Schill EM, Lake JI, Tusheva OA, et al. Ibuprofen slows migration and inhibits bowel colonization by enteric nervous system precursors in zebrafish, chick and mouse. *Dev Biol* 409, 473-88 (2016).
64. Nielsen SW, Qvist N. [Citalopram taken during pregnancy and the child born with Hirschsprung's disease]. *Ugeskr Laeger* 175, V03130178 (2013).
65. Ferreira-Santos R. Megacolon and megarectum in Chagas' disease. *Proc R Soc Med* 54, 1047-53 (1961).
66. Pinazo MJ, Canas E, Elizalde JI, et al. Diagnosis, management and treatment of chronic Chagas' gastrointestinal disease in areas where Trypanosoma cruzi infection is not endemic. *Gastroenterol Hepatol* 33, 191-200 (2010).
67. Holschneider AM Prem P, editors. Hirschsprung's disease and Allied Disorder. 3rd ed. Berlin, Heidelberg: Springer Science & Business Media; 2008.
68. Best KE, Addor MC, Arriola L, et al. Hirschsprung's disease prevalence in Europe: a register based study. *Birth Defects Res A Clin Mol Teratol* 100, 695-702 (2014).
69. Mason CA, Kirby RS, Sever LE, Langlois PH. Prevalence is the preferred measure of frequency of birth defects. *Birth Defects Res A Clin Mol Teratol* 73, 690-2 (2005).
70. Goldberg EL. An epidemiological study of Hirschsprung's disease. *Int J Epidemiol* 13, 479-85 (1984).
71. Russell MB, Russell CA, Niebuhr E. An epidemiological study of Hirschsprung's disease and additional anomalies. *Acta Paediatr* 83, 68-71 (1994).
72. Block SR, Watkins SM, Salemi JL, et al. Maternal pre-pregnancy body mass index and risk of selected birth defects: evidence of a dose-response relationship. *Paediatr Perinat Epidemiol* 27, 521-31 (2013).
73. Ryan ET, Ecker JL, Christakis NA, Folkman J. Hirschsprung's disease: associated abnormalities and demography. *J Pediatr Surg* 27, 76-81 (1992).
74. Stothard KJ, Tennant PW, Bell R, Rankin J. Maternal overweight and obesity and the risk of congenital anomalies: a systematic review and meta-analysis. *Jama* 301, 636-50 (2009).
75. Rankin J, Tennant PW, Stothard KJ, Bythell M, Summerbell CD, Bell R. Maternal body mass index and congenital anomaly risk: a cohort study. *Int J Obes (Lond)* 34, 1371-80 (2010).
76. Duess JW, Hofmann AD, Puri P. Prevalence of Hirschsprung's disease in premature infants: a systematic review. *Pediatr Surg Int* 30, 791-5 (2014).
77. Downey EC, Hughes E, Putnam AR, Baskin HJ, Rollins MD. Hirschsprung disease in the premature newborn: a population based study and 40-year single center experience. *J Pediatr Surg* 50, 123-5 (2015).
78. Singh SJ, Croaker GD, Manglick P, et al. Hirschsprung's disease: the Australian Paediatric Surveillance Unit's experience. *Pediatr Surg Int* 19, 247-50 (2003).
79. Frongia G, Gunther P, Schenk JP, et al. Contrast Enema for Hirschsprung Disease Investigation: Diagnostic Accuracy and Validity for Subsequent Diagnostic and Surgical Planning. *Eur J Pediatr Surg*, 26:207-14 (2015).
80. Huang Y, Zheng S, Xiao X. Preliminary evaluation of anorectal manometry in diagnosing Hirschsprung's disease in neonates. *Pediatr Surg Int* 25, 41-5 (2009).

81. Kleinhaus S, Boley SJ, Sheran M, Sieber WK. Hirschsprung's disease -- a survey of the members of the Surgical Section of the American Academy of Pediatrics. *J Pediatr Surg* 14, 588-97 (1979).
82. Duhamel B. A new operation for the treatment of Hirschsprung's disease. *Arch Dis Child* 35, 38-9 (1960).
83. Soave F. HIRSCHSPRUNG'S DISEASE: A NEW SURGICAL TECHNIQUE. *Arch Dis Child* 39, 116-24 (1964).
84. Carcassonne M, Guys JM, Morrison-Lacombe G, Kreitmann B. Management of Hirschsprung's disease: curative surgery before 3 months of age. *J Pediatr Surg* 24, 1032-4 (1989).
85. Guerra J, Wayne C, Musambe T, Nasr A. Laparoscopic-assisted transanal pull-through (LATP) versus complete transanal pull-through (CTP) in the surgical management of Hirschsprung's disease. *J Pediatr Surg*, (2016).
86. Swenson O, Bill AH, Jr. Resection of rectum and rectosigmoid with preservation of the sphincter for benign spastic lesions producing megacolon; an experimental study. *Surgery* 24, 212-20 (1948).
87. Georgeson KE, Fuenfer MM, Hardin WD. Primary laparoscopic pull-through for Hirschsprung's disease in infants and children. *J Pediatr Surg* 30, 1017-21 (1995).
88. De la Torre-Mondragon L, Ortega-Salgado JA. Transanal endorectal pull-through for Hirschsprung's disease. *J Pediatr Surg* 33, 1283-6 (1998).
89. Langer JC, Minkes RK, Mazziotti MV, Skinner MA, Winthrop AL. Transanal one-stage Soave procedure for infants with Hirschsprung's disease. *J Pediatr Surg* 34, 148-51 (1999).
90. Wildhaber BE, Pakarinen M, Rintala RJ, Coran AG, Teitelbaum DH. Posterior myotomy/myectomy for persistent stooling problems in Hirschsprung's disease. *J Pediatr Surg* 39, 920-6 (2004).
91. Ralls MW, Coran AG, Teitelbaum DH. Reoperative surgery for Hirschsprung disease. *Semin Pediatr Surg* 21, 354-63 (2012).
92. Sherman JO, Snyder ME, Weitzman JJ, et al. A 40-year multinational retrospective study of 880 Swenson procedures. *J Pediatr Surg* 24, 833-8 (1989).
93. Tander B, Rizalar R, Cihan AO, Ayyildiz SH, Ariturk E, Bernay F. Is there a hidden mortality after one-stage transanal endorectal pull-through for patients with Hirschsprung's disease? *Pediatr Surg Int* 23, 81-6 (2007).
94. Thomson D, Allin B, Long AM, Bradnock T, Walker G, Knight M. Laparoscopic assistance for primary transanal pull-through in Hirschsprung's disease: a systematic review and meta-analysis. *BMJ Open* 5, e006063 (2015).
95. Rescorla FJ, Morrison AM, Engles D, West KW, Grosfeld JL. Hirschsprung's disease. Evaluation of mortality and long-term function in 260 cases. *Arch Surg* 127, 934-41 (1992).
96. Melendez E, Goldstein AM, Sagar P, Badizadegan K. Case records of the Massachusetts General Hospital. Case 3-2012. A newborn boy with vomiting, diarrhea, and abdominal distention. *N Engl J Med* 366, 361-72 (2012).
97. Demehri FR, Halaweish IF, Coran AG, Teitelbaum DH. Hirschsprung-associated enterocolitis: pathogenesis, treatment and prevention. *Pediatr Surg Int* 29, 873-81 (2013).
98. Pastor AC, Osman F, Teitelbaum DH, Caty MG, Langer JC. Development of a standardized definition for Hirschsprung's-associated enterocolitis: a Delphi analysis. *J Pediatr Surg* 44, 251-6 (2009).
99. Frykman PK, Short SS. Hirschsprung-associated enterocolitis: prevention and therapy. *Semin Pediatr Surg* 21, 328-35 (2012).
100. Wester T, Rintala RJ. Early outcome of transanal endorectal pull-through with a short muscle cuff during the neonatal period. *J Pediatr Surg* 39, 157-60; (2004).

101. Stensrud KJ, Emblem R, Bjornland K. Functional outcome after operation for Hirschsprung disease--transanal vs transabdominal approach. *J Pediatr Surg* 45, 1640-4 (2010).
102. Kim AC, Langer JC, Pastor AC, et al. Endorectal pull-through for Hirschsprung's disease-a multicenter, long-term comparison of results: transanal vs transabdominal approach. *J Pediatr Surg* 45, 1213-20 (2010).
103. Fredriksson F, Christofferson RH, Lilja HE. Adhesive small bowel obstruction after laparotomy during infancy. *Br J Surg* 103, 284-9 (2016).
104. Holschneider A, Hutson J, Pena A, et al. Preliminary report on the International Conference for the Development of Standards for the Treatment of Anorectal Malformations. *J Pediatr Surg* 40, 1521-6 (2005).
105. Rintala RJ, Lindahl H. Is normal bowel function possible after repair of intermediate and high anorectal malformations? *J Pediatr Surg* 30, 491-4 (1995).
106. Jorge JM, Wexner SD. Etiology and management of fecal incontinence. *Dis Colon Rectum* 36, 77-97 (1993).
107. Vaizey CJ, Carapeti E, Cahill JA, Kamm MA. Prospective comparison of faecal incontinence grading systems. *Gut* 44, 77-80 (1999).
108. Miller R, Bartolo DC, Locke-Edmunds JC, Mortensen NJ. Prospective study of conservative and operative treatment for faecal incontinence. *Br J Surg* 75, 101-5 (1988).
109. Osterberg A, Graf W, Karlbom U, Pahlman L. Evaluation of a questionnaire in the assessment of patients with faecal incontinence and constipation. *Scand J Gastroenterol* 31, 575-80 (1996).
110. Hartman EE, Oort FJ, Aronson DC, et al. Children With Anorectal Malformations, Hirschsprung Disease, and Their Siblings: Proxy Reports and Self-Reports. *J Pediatr Gastroenterol Nutr* 61, 630-5 (2015).
111. Jarvi K, Laitakari EM, Koivusalo A, Rintala RJ, Pakarinen MP. Bowel function and gastrointestinal quality of life among adults operated for Hirschsprung disease during childhood: a population-based study. *Ann Surg* 252, 977-81 (2010).
112. Ieiri S, Nakatsuji T, Akiyoshi J, et al. Long-term outcomes and the quality of life of Hirschsprung disease in adolescents who have reached 18 years or older-a 47-year single-institute experience. *J Pediatr Surg* 45, 2398-402 (2010).
113. Heikkinen, Rintala RJ, Louhimo I. Bowel function and quality of life in adult patients with operated Hirschsprung's disease. *Pediatr Surg Int* 10, 342-4 (1995).
114. Yanchar NL, Soucy P. Long-term outcome after Hirschsprung's disease: patients' perspectives. *J Pediatr Surg* 34, 1152-60 (1999).
115. Drossman DA, Dumitrascu DL. Rome III: New standard for functional gastrointestinal disorders. *J Gastrointestin Liver Dis* 15, 237-41 (2006).
116. Levitt MA, Martin CA, Olesevich M, Bauer CL, Jackson LE, Pena A. Hirschsprung disease and fecal incontinence: diagnostic and management strategies. *J Pediatr Surg* 44, 271-7 (2009).
117. Stensrud KJ, Emblem R, Bjornland K. Anal endosonography and bowel function in patients undergoing different types of endorectal pull-through procedures for Hirschsprung disease. *J Pediatr Surg* 50, 1341-6 (2015).
118. Hukkinen M, Koivusalo A, Merras-Salmio L, Rintala RJ, Pakarinen MP. Postoperative outcome and survival in relation to small intestinal involvement of total colonic aganglionosis. *J Pediatr Surg* 50, 1859-64 (2015).
119. Wester T, Granstrom AL. Botulinum toxin is efficient to treat obstructive symptoms in children with Hirschsprung disease. *Pediatr Surg Int* 31, 255-9 (2015).
120. Malone PS, Ransley PG, Kiely EM. Preliminary report: the antegrade continence enema. *Lancet* 336, 1217-8 (1990).

121. Ralls MW, Freeman JJ, Rabah R, et al. Redo pullthrough for Hirschsprung disease: a single surgical group's experience. *J Pediatr Surg* 49, 1394-9 (2014).
122. Moore SW, Albertyn R, Cywes S. Clinical outcome and long-term quality of life after surgical correction of Hirschsprung's disease. *J Pediatr Surg* 31, 1496-502 (1996).
123. van den Hondel D, Sloots CE, Bolt JM, Wijnen RM, de Blaauw I, H IJ. Psychosexual Well-Being after Childhood Surgery for Anorectal Malformation or Hirschsprung's Disease. *J Sex Med* 12, 1616-25 (2015).
124. Group W. Development of the WHOQOL:rationale and current status. *International Journal of Mental Health* 23, 24-56 (1994).
125. Editorial. Quality of life and clinical trials. *Lancet* 346, 1-2 (1995).
126. Ware JE, Jr. SF-36 health survey update. *Spine (Phila Pa 1976)* 25, 3130-9 (2000).
127. Sullivan M, Karlsson J. The Swedish SF-36 Health Survey III. Evaluation of criterion-based validity: results from normative population. *J Clin Epidemiol* 51, 1105-13 (1998).
128. Persson LO, Karlsson J, Bengtsson C, Steen B, Sullivan M. The Swedish SF-36 Health Survey II. Evaluation of clinical validity: results from population studies of elderly and women in Gothenborg. *J Clin Epidemiol* 51, 1095-103 (1998).
129. Eypasch E, Williams JI, Wood-Dauphinee S, et al. Gastrointestinal Quality of Life Index: development, validation and application of a new instrument. *Br J Surg* 82, 216-22 (1995).
130. Sandblom G, Videhult P, Karlson BM, et al. Validation of Gastrointestinal Quality of Life Index in Swedish for assessing the impact of gallstones on health-related quality of life. *Value Health* 12, 181-4 (2009).
131. Wigander H, Frenckner B, Wester T, Nisell M, Ojmyr-Joelsson M. Translation and cultural adaptation of the Hirschsprung's Disease/Anorectal Malformation Quality of life Questionnaire (HAQL) into Swedish. *Pediatr Surg Int* 30, 401-6 (2014).
132. Hartman EE, Oort FJ, Aronson DC, Sprangers MA. Quality of life and disease-specific functioning of patients with anorectal malformations or Hirschsprung's disease: a review. *Arch Dis Child* 96, 398-406 (2011).
133. Diseth TH, Egeland T, Emblem R. Effects of anal invasive treatment and incontinence on mental health and psychosocial functioning of adolescents with Hirschsprung's disease and low anorectal anomalies. *J Pediatr Surg* 33, 468-75 (1998).
134. Gunnarsdottir A, Sandblom G, Arnbjornsson E, Larsson LT. Quality of life in adults operated on for Hirschsprung disease in childhood. *J Pediatr Gastroenterol Nutr* 51, 160-6 (2010).
135. van den Hondel D, Aarsen FK, Wijnen RM, Sloots CE, H IJ. Children with congenital colorectal malformations often require special education or remedial teaching, despite normal intelligence. 105, e77-84 (2016).
136. Pakarinen MP, Rintala RJ, Koivusalo A, Heikkinen M, Lindahl H, Pukkala E. Increased incidence of medullary thyroid carcinoma in patients treated for Hirschsprung's disease. *J Pediatr Surg* 40, 1532-4 (2005).
137. Levin DN, Marcon MA, Rintala RJ, Jacobson D, Langer JC. Inflammatory bowel disease manifesting after surgical treatment for Hirschsprung disease. *J Pediatr Gastroenterol Nutr* 55, 272-7 (2012).
138. Clausen N, Andersson P, Tommerup N. Familial occurrence of neuroblastoma, von Recklinghausen's neurofibromatosis, Hirschsprung's agangliosis and jaw-winking syndrome. *Acta Paediatr Scand* 78, 736-41 (1989).

139. Ludvigsson JF, Andersson E, Ekbom A, et al. External review and validation of the Swedish national inpatient register. *BMC Public Health* 11, 450 (2011).
140. Socialstyrelsen. Slutenvårdsstatistik (Internet). Stockholm: Socialstyrelsen website. http://socialstyrelsen.se/statistik/statistiskefteramne/slutenvard. Accessed March 29, 2016.
141. Cnattingius S, Ericson A, Gunnarskog J, Kallen B. A quality study of a medical birth registry. *Scand J Soc Med* 18, 143-8 (1990).
142. Statistiska central byrån. Evaluering av utbildningsregistret. (Internet). Stockholm, SCBwebsite.http:77www.scb.se/statistik/_publikationer/BE9999_2006A01_BR_BE96ST0604.pdf. Accessed March 29, 2016.
143. R Core Team. Vienna A. R: A language and environment for statistical computing. R Foundation for statistical computing. (2015).
144. Polman CH, Reingold SC, Banwell B, et al. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. *Ann Neurol* 69, 292-302 (2011).
145. Read AP, Newton VE. Waardenburg syndrome. *J Med Genet* 34, 656-65 (1997).
146. Pingault V, Ente D, Dastot-Le Moal F, Goossens M, Marlin S, Bondurand N. Review and update of mutations causing Waardenburg syndrome. *Hum Mutat* 31, 391-406 (2010).
147. Parthey K, Kornhuber M, Kunze C, et al. SOX10 mutation with peripheral amyelination and developmental disturbance of axons. *Muscle Nerve* 45, 284-90 (2012).
148. Cantrell VA, Owens SE, Chandler RL, et al. Interactions between Sox10 and EdnrB modulate penetrance and severity of aganglionosis in the Sox10Dom mouse model of Hirschsprung disease. *Hum Mol Genet* 13, 2289-301 (2004).
149. Fogdell-Hahn A, Ligers A, Gronning M, Hillert J, Olerup O. Multiple sclerosis: a modifying influence of HLA class I genes in an HLA class II associated autoimmune disease. *Tissue Antigens* 55, 140-8 (2000).
150. Neuvonen MI, Kyrklund K, Rintala RJ, Pakarinen MP. Bowel Function and Quality of Life After Transanal Endorectal Pull-through for Hirschsprung Disease: Controlled Outcomes up to Adulthood. *Ann Surg* (2016).
151. Fujiwara N, Kaneyama K, Okazaki T, et al. A comparative study of laparoscopy-assisted pull-through and open pull-through for Hirschsprung's disease with special reference to postoperative fecal continence. *J Pediatr Surg* 42, 2071-4 (2007).
152. Aworanti OM, McDowell DT, Martin IM, Quinn F. Does Functional Outcome Improve with Time Postsurgery for Hirschsprung Disease? *Eur J Pediatr Surg* 26, 192-9 (2015).
153. Conway SJ, Craigie RJ, Cooper LH, et al. Early adult outcome of the Duhamel procedure for left-sided Hirschsprung disease--a prospective serial assessment study. *J Pediatr Surg* 42, 1429-32 (2007).
154. Mills JL, Konkin DE, Milner R, Penner JG, Langer M, Webber EM. Long-term bowel function and quality of life in children with Hirschsprung's disease. *J Pediatr Surg* 43, 899-905 (2008).
155. Koivusalo A, Pakarinen MP, Turunen P, Saarikoski H, Lindahl H, Rintala RJ. Health-related quality of life in adult patients with esophageal atresia--a questionnaire study. *J Pediatr Surg* 40, 307-12 (2005).
156. Correa A, Marcinkevage J. Prepregnancy obesity and the risk of birth defects: an update. *Nutr Rev* 71 Suppl 1, S68-77 (2013).
157. Spouge D, Baird PA. Hirschsprung disease in a large birth cohort. *Teratology* 32, 171-7 (1985).

158. Zhou Y, Besner G. Transplantation of amniotic fluid-derived neural stem cells as a potential novel therapy for Hirschsprung's disease. *J Pediatr Surg* 51, 87-91 (2016).
159. Frykman PK, Nordenskjold A, Kawaguchi A, et al. Characterization of Bacterial and Fungal Microbiome in Children with Hirschsprung Disease with and without a History of Enterocolitis: A Multicenter Study. *PLoS One* 10, e0124172 (2015).