Hirschsprung Disease

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There are many excellent articles on Hirschsprung disease (HSCR) that provide detailed information about the clinical presentation, epidemiology, genetics, diagnosis, and associated medical problems [1–10]. This chapter summarizes and simplifies the complex HSCR literature. Percentages in the text and tables are estimates, since widely divergent numbers are presented in different manuscripts.

Definition

The enteric nervous system (ENS) is an integrated network of neurons and glia that controls most aspects of intestinal function. This includes intestinal motility, response to luminal and intramural stimuli, regulation of epithelial and immune cell activity, and control of blood flow [11–13]. To perform these tasks, neurons are normally distributed along the entire length of the bowel. When the ENS is absent or defective in any region of the bowel, profound problems with intestinal function occur causing significant morbidity and in some cases death.

Hirschsprung disease (HSCR), the most well-understood intestinal motility disorder, is characterized by the complete absence of enteric neurons (i.e., aganglionosis) in the myenteric and submucosal plexus of the distal bowel. In the absence of ganglion cells, the bowel tonically contracts causing functional intestinal obstruction. Many, but not all, clinical manifestations of HSCR result from tonic contraction of aganglionic bowel.

Nomenclature describing the extent of aganglionosis in HSCR is not consistent. However, most affected individuals have "short-segment" disease, where aganglionosis is restricted to the rectosigmoid region of the colon [14, 15]. "Long-segment" HSCR means that aganglionosis extends proximal to the sigmoid colon and is usually distinguished from "total colonic" aganglionosis. In a small percentage of cases, aganglionosis extends into the small bowel leading to very serious lifelong disability often requiring total parenteral nutrition (Table 26.1) [15, 16]. Although some authors have suggested that clinical presentation varies with the length of aganglionosis [17], others say that clinical symptoms are not related to the extent of disease [18]. From a practical standpoint, it is best to assume that the extent of aganglionosis and the severity and character of symptoms are unrelated.

Table 26.1 Extent of aganglionosis

Short segment	74–89%
Long segment	12–22%
Total colon	4–13%
Total colon and small bowel (partial or total)	3–5%

Clinical Presentation

HSCR is debilitating and can be fatal. Clinical presentation is highly variable and diagnosis requires a high index of suspicion (Table 26.2). Recognizing HSCR is important, since surgical management dramatically reduces disease morbidity and mortality.

In the current era, most people with HSCR are diagnosed by 12 months of age [19–23], but it remains common to diagnose HSCR in older children and HSCR has been diagnosed in adults up to 73 years of age [24]. A case report from 2021 describes a 53-year-old man in Japan with newly diagnosed HSCR [25]. He had constipation since childhood, but lacked other HSCR symptoms, highlighting the variability in symptom character and severity discussed below. HSCR needs to be considered in anyone with severe chronic constipation that began in early infancy, especially if suppositories or enemas are needed for stool passage. However, because

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Table 26.2 Presenting symptoms in HSCR

Symptom	Comment
Abdominal distension	Very common in HSCR or anatomic bowel obstruction
Bilious emesis	Common and suggests HSCR or anatomic obstruction
Constipation	Common in older children with HSCR but also in healthy toddlers and infants
Diarrhea	Foul-smelling, bloody, or "explosive" diarrhea suggests enterocolitis (HAEC)
Delayed meconium	Common in HSCR, but 50% of infants with HSCR do not have delayed meconium
Bowel perforation	Should raise concern for HSCR

constipation is common, affecting up to 35% of all children [26, 27], and HSCR is rare (1/5000 people), recognizing distinct features suggest that HSCR is important for diagnosis. Furthermore, constipation is only one feature of HSCR. Typical presentations for HSCR include:

Neonatal Intestinal Obstruction

Infants present with marked abdominal distension and bilious emesis. Distension may be severe enough to cause respiratory compromise. Obstruction may occur on the first day of life, but children may also initially have apparently normal bowel movements or "mild constipation" and then present acutely with abdominal distension and vomiting at an older age. Because HSCR requires a high index of suspicion for diagnosis, some infants are hospitalized repeatedly for episodes of presumed "gastroenteritis" that were actually a manifestation of HSCR-associated intestinal obstruction or enterocolitis. The clinical distinction is that gastroenteritis may cause severe vomiting, but does not typically cause as much abdominal distension as HSCR. Vomiting associated with infectious enteritis is also usually followed by diarrhea, whereas intestinal obstruction should be accompanied by reduced stool passage. Enterocolitis causes explosive diarrhea and marked abdominal distension (see details below). A distended abdomen occurs in 57–93% of infants with HSCR and bilious emesis occurs in 19–37% [16, 18, 28–30]. Abdominal distension and bilious emesis are also a very common presentation in premature infants with HSCR (96% and 92%, respectively). Note that since the ENS forms during the first trimester of pregnancy, incidence of HSCR is similar in term and preterm infants [31].

Neonatal Bowel Perforation

HSCR presents with bowel perforation about 5% of the time [32, 33] and HSCR causes about 10% of all neonatal bowel perforations [34]. Symptoms may not be specific and include poor feeding, emesis, abdominal distension, constipation, diarrhea, and lethargy. In two series with 55 cases reported [32, 33], only one child with perforation was more than 2 months old. Sixty-two percent of the perforations were in the cecum or ascending colon and 15% were in the appendix. Many of the children with bowel perforation had long-segment disease (34% total colonic aganglionosis, with an additional 23% having aganglionosis proximal to the splenic flexure). Since long-segment HSCR is less common than short-segment disease (Table 26.1), proximal colon perforation in a young infant should dramatically raise concern for long-segment HSCR. In 55% of reported cases, the perforation was proximal to the transition zone in ganglion cell containing bowel. In 13%, the perforation was at the transition zone. In 30%, however, the perforation occurred in aganglionic bowel distal to the transition zone.

Delayed Passage of Meconium

Delayed passage of meconium should suggest the diagnosis of HSCR, but defining HSCR risk in infants with delayed passage of meconium is challenging, because the timing of meconium passage reported for healthy infants is variable. In a study of 979 infants older than 34 week gestational age in the United States, 97% passed meconium by 24 h of life, and 99.8% passed meconium by 36 h of life [35]. Breastfeeding or bottle-feeding did not influence the timing of the first bowel movement, and multivariate analysis demonstrated that only prematurity was a significant predictor of delayed passage of meconium. A similar study in Turkey [36] also demonstrated that 724/743 (97%) passed meconium by 24 h after birth and 740/743 (99.6%) passed meconium by the time that they were 48 h. However, a smaller study in the Netherlands reported that only 56/71 (79%) of term infants passed meconium by 24 h after birth [37], and in a study of 267 healthy infants in Nigeria, only 92% passed their first bowel movement by 48 h after birth [38]. In the Nigerian study, 5% of the infants were preterm, but even if the preterm infants are excluded, the data suggest that at most 97% of the healthy full-term infants studied passed their first bowel movement by the time that they were 48 h. Excluding premature infants from the analysis is important, since prematurity predisposes to delayed passage of meconium. A study of 611 infants reported that only 57% of infants less than 29 week estimated gestational age (EGA), 66% of infants between 29 and 32 week EGA, and 80% of infants between 32 and 37 week EGA [39] passed meconium by the end of their "second calendar day" and 1% of premature infants did not pass meconium until after day of life 9.

Table 26.3 Making sense of HSCR risk for isolated delayed passage of meconium

Symptom in full term infants	Frequency in healthy infants (number used to calculate risk)	Frequency in children who have HSCR	HSCR risk if this is the only symptom
No meconium at 24 h after birth	97% (300/10,000)	1/10,000	1/300[a]
No meconium at 48 h after birth	99.8% (20/10,000)	1/10,000	1/20
No meconium at 48 h after birth	92% (800/10,000)	1/10,000	1/800

[a] *Method of calculation*: 97% means 300 out of 10,000 healthy neonates will not have passed meconium at 24 h after birth. About 1/5000 children have HSCR, but half of those children do pass meconium in the first 24 h after birth, so only 1/10,000 neonates has HSCR and presents with delayed passage of meconium. Thus, if delayed passage of meconium at 24 h after birth is the only HSCR symptom in a full-term infant, then risk of HSCR is about 1/300 instead of the usual population risk of 1/5000. Similar logic applies for the other calculated risk estimates

In children with HSCR , delayed passage of meconium is much more common than in healthy infants. Nonetheless, up to 50% of children with HSCR pass meconium by 48 h after birth [28, 40, 41], so passage of meconium within 48 h of birth does not exclude a diagnosis of HSCR. Table 26.3 defines HSCR risk based on these numbers, and highlights why it is important to consider HSCR symptoms, family history, and associated birth defects or genetic syndromes when deciding who to evaluate for HSCR.

Chronic Severe Constipation

HSCR causes constipation, but constipation unrelated to HSCR is very common (e.g., >25% of healthy children) and HSCR is rare, so constipation alone usually does not indicate HSCR (using the logic above, 1250/5000 children have constipation, so if constipation is the only symptom 1/1250 will have HSCR). "Severe" constipation and constipation beginning within the first few months of life does increase concern for HSCR and the likelihood of disease. For example, in one study, rectal biopsy was performed on all children over a year of age who were referred to a specialty center for consultation and who had constipation refractory to more than 6 months of medical management. Nineteen out of 395 biopsies demonstrated HSCR (5%), a 250-fold increased risk compared to the population prevalence of HSCR (1/5000) [42]. Constipation in isolation also appears to be an uncommon presentation of HSCR in infants. In particular, the wide range of normal bowel movement frequency in healthy infants makes it difficult to use constipation as the only indication to evaluate for HSCR. In a study of 911 healthy children in Turkey [36] between 2 and 12 months of age, mean stool frequency was once a day, but at 2 months of age, stool frequency varied from once a week to eight times per day.

Abdominal Distension Relieved by Rectal Stimulation or Enema

In children with HSCR, rectal exam or other forms of rectal stimulation may cause a sudden "explosive" release of intraluminal contents and relieve abdominal distension. Explosive release of stool and air after rectal exam is a sign of HSCR-associated enterocolitis (HAEC) [43]. This sign uncommon in other conditions and should raise concern about HSCR. Rectal exam is, however, not otherwise useful in identifying children with HSCR. In particular, "anal tone" is not a reliable indicator of disease. Occasionally anal stenosis or sacral teratoma can also be detected by rectal exam, so rectal exam can be valuable in children with intractable constipation and suspected HSCR.

Enterocolitis

Defining when children have enterocolitis presents its' own challenges (see below for symptoms), but enterocolitis is a dangerous and common presentation for HSCR. When enterocolitis occurs, children with HSCR have diarrhea instead of constipation.

Who Should Be Biopsied to Evaluate for Hirschsprung Disease?

Rectal biopsy is the "gold standard" diagnostic test for HSCR (see below). Unless another diagnosis is evident, children with the following clinical presentations *should undergo* rectal biopsy to evaluate for HSCR:

1. Neonates with significant abdominal distension, especially in combination with bilious vomiting or delayed passage of meconium, unless mechanical blockage in the bowel, is demonstrated.
2. Neonates with bowel perforation.

Also *consider* rectal biopsy for HSCR in children with:

1. Neonatal bloody diarrhea. Given the low incidence of infectious enteritis in breastfed or formula-fed neonates, bloody diarrhea in neonates is concerning for HAEC (see

below). Note, however, that many infants have small streaks of blood in the stool without diarrhea or other symptoms of HSCR, and hematochezia alone does not warrant rectal biopsy.
2. Healthy-appearing full-term infants with delayed passage of meconium even in the absence of other symptoms. Given the risks associated with untreated HSCR, I usually recommend biopsy in full term infants who do not pass meconium within 48 h of birth (Table 26.3 suggests that 1/20 will have HSCR). If meconium is first passed at 24 h after birth, rectal biopsy is much less likely to demonstrate HSCR, unless other symptoms of HSCR are present. I do not recommend biopsy for infants who pass meconium at 24 h after birth unless other signs or symptoms suggest HSCR.
3. Young children with constipation refractory to oral medication. Constipation beginning after a year of age is rarely due to HSCR. Constipation that improves dramatically with oral medication is also unlikely to be due to HSCR. The common form of functional constipation that occurs in toddlers may be challenging to treat, usually requiring complete disimpaction and daily maintenance medicine for relief of symptoms, so it can be challenging to know if toddlers are truly "refractory to oral medication." Some children with HSCR have very few symptoms within the first year of life, however, so the absence of neonatal symptoms does not exclude HSCR.

Red Flags (Conditions That Should Raise Suspicion for HSCR)

1. Constipation with episodes of abdominal distension or vomiting. Constipation does not cause vomiting, but many disorders cause both vomiting and reduced bowel movement frequency, including HSCR.
2. Growth failure. This is a common feature of untreated HSCR.
3. Trisomy 21. HSCR occurs in 1–2% of children with Down syndrome, so HSCR should be more readily suspected in children with trisomy 21 [44–46].
4. The presence of additional major anomalies also increases the likelihood of HSCR, but remember that most children with HSCR (>70%) do not have other major medical problems [22, 47, 48]. In particular, congenital anomalies of the kidney and urinary tract (CAKUT) occur in ~20% of children with HSCR and should raise suspicion of HSCR.
5. Family history of HSCR (see section "Epidemiology/Genetics Overview") may dramatically increase HSCR risk.
6. HSCR-associated genetic syndromes also increase risk (see Table 26.4 and added detail below).

Table 26.4 Selected HSCR-associated syndromes

Syndrome name	Genetic defect	Comments
MEN2A = multiple endocrine neoplasia 2A	RET mutation in codons 609, 611, 618, or 620	~2% of children with HSCR may have MEN2A RET mutations
FMTC = familial medullary thyroid carcinoma		20–30% of families with Ret 609, 611, 618, or 620 mutations have members with both FMTC and HSCR
Down syndrome	Trisomy 21	1–2% of children with trisomy 21 have HSCR
		2–10% of children with HSCR have Down's
WS4 = Waardenburg syndrome	WS4A = EDNRB	9% of children with HSCR have WS4
	WS4C = SOX10	Syndrome includes HSCR, deafness, and pigmentary abnormalities
CCHS = congenital central hypoventilation syndrome	PHOX2B	20% of children with CCHS have HSCR
		0.5–1.5% of children with HSCR have CCHS
MWS = Mowat–Wilson syndrome	ZFHX1B	60% of children with MWS have HSCR
		6% of children with HSCR have MWS
		Syndrome includes HSCR, intellectual disability, epilepsy, dysmorphic facial features, and brain and heart defects
Goldberg–Shprintzen megacolon syndrome	KIAA1279	Syndrome includes HSCR, intellectual disability, dysmorphic facial features, and brain and heart defects
CHH = cartilage–hair hypoplasia syndrome	RMRP	Syndrome includes short stature (dwarfism), other skeletal defects (short limbs), fine sparse hair, and immunodeficiency
		~9% of children with CHH have HSCR
		CHH is rare in children with HSCR

Given the diverse presenting symptoms of HSCR, it remains difficult to decide who to evaluate. The more "classic" features of HSCR that are present, the more likely the child has HSCR. Given the high morbidity and mortality in untreated HSCR, evaluation for HSCR should be performed in many children who do not end up having this disease to avoid missing this potentially life-threatening medical problem. My recent review provides additional details [8].

Diagnostic Strategies

HSCR by definition means that affected individuals do not have ganglion cells in the distal bowel. Rectal biopsy is, therefore, required to make the diagnosis and is considered the "gold standard" approach [49]. A number of other strategies for diagnosing HSCR are used, but each has problems.

Rectal Suction Biopsy

Rectal suction biopsy is a simple procedure taking only a few minutes using an instrument designed to take small pieces of the rectal mucosa (e.g., Noblett, Solo-RBT, or rbi2 instrument) to reduce the risk of bowel perforation or hemorrhage [50]. Because there are no sensory nerve endings that respond to cutting in the area of the rectum where the biopsies should be obtained, sedation and pain medicines are not required, but sedation is sometimes used in older children. Biopsies should be obtained at 2–3 cm from the dentate line (i.e., the transition between rectal and squamous mucosa), because there is a physiological submucosal hypoganglionosis in the terminal rectum. From a practical standpoint, however, some authors advocate obtaining biopsies at multiple levels (e.g., 1–3 cm from the dentate line), because precise positioning of the biopsy can be difficult. Biopsy tissue obtained is sectioned, stained, and examined by a pathologist to identify ganglion cells. There is some controversy about the optimal staining method, but hematoxylin and eosin (H&E) and acetylcholinesterase are commonly used techniques [49, 50]. Acetylcholinesterase staining might also help predict the risk of HAEC [51]. Calretinin staining might improve diagnostic accuracy [52, 53], but data are limited. A meta-analysis analyzing data from 993 patients indicated that the mean sensitivity of rectal suction biopsy for HSCR is 93%, and the mean specificity is 98% [54]. A more recent manuscript documents 935 cases of HSCR diagnosed by rectal mucosal biopsy (a total of 19,365 biopsies in 6615 children) with no false-positive or false-negative diagnoses (i.e., 100% sensitivity and specificity) [55]. Serious bleeding and bowel perforation are uncommon with rectal suction biopsy, but can occur. One series of 1340 biopsies [56] reported three bowel perforations (0.2%), one death (0.07%), and three rectal hemorrhage (0.2%) requiring blood transfusion. More recent studies also document low but nonzero rates of serious bleeding or bowel perforation (0 complication in 297 children [57], 0 complication in 88 infants [58], and two episodes of bleeding requiring transfusion (0.7%) plus one episode of rectal perforation and sepsis (0.035%) in 272 children) [59]. The most common problem with rectal suction biopsies, however, is that they are so small that 6–26% are "inadequate", requiring repeat biopsy to make a diagnosis [57, 59, 60]. The more recently introduced rbi2 biopsy instrument appears to give a lower frequency of "inadequate specimens" [58] and may give larger biopsies. It is not yet clear if there are also more complications (bleeding or bowel perforation) using the new instrument, since large cohort studies have not been published. Checking platelets, hemoglobin, and PT/PTT/INR prior to biopsy seems prudent, although I do not know of cases, where bleeding after rectal biopsy was due to coagulopathy.

Anorectal Manometry

This method tests for the rectoanal inhibition reflex (RAIR) using a small balloon attached to a tube inserted into the rectum [54]. The RAIR is reflex relaxation of the internal anal sphincter in response to rectal distension. This reflex is absent in children with HSCR. Sensitivity and specificity of anorectal manometry are 91% and 94%, respectively, but this test is not required to diagnose HSCR [54]. The equipment needed to do anorectal manometry is also expensive, and significant experience is needed to evaluate results in infants less than a year of age, so the test is not widely available. Recently developed high-resolution anorectal manometry does not appear to provide increased sensitivity or specificity for HSCR diagnosis (89% and 83%, respectively, compared to rectal suction biopsy) [61]. In fact, one study reported that 28/111 (25%) children with absent RAIR detected using high-resolution manometry were diagnosed with "internal anal sphincter achalasia" after rectal biopsies showed ENS ganglion cells, making HSCR unlikely [62].

Contrast Enema

This is an X-ray test where images are obtained as contrast is gradually infused into the colon via the anal canal to look for evidence of the distal bowel contraction that occurs in areas of aganglionosis. The region where bowel caliper changes from contracted distal aganglionic bowel and more dilated ganglion cell containing bowel is called the "transition zone". When rectum is narrower than more proximal colon, it suggests HSCR. Although contrast enema may have value in planning the surgical approach to HSCR, the radiographic and anatomic transition from aganglionic to ganglion cell containing bowel may not be in the same location. Note too that in total colonic HSCR, there is no transition zone in the colon, since the entire colon is contracted. Furthermore, the sensitivity (70%) and specificity (50–80%) are considerably lower using contrast enema for HSCR diagnosis than other methods [30, 54]. The role of contrast enema in HSCR diagnosis, therefore, remains a matter of debate, but enema is

also valuable to evaluate for other uncommon anatomic problems (e.g., stricture, sigmoid volvulus, colon cancer, and sacral teratoma).

Full-Thickness Rectal Biopsy

Deeper biopsies can be performed by a surgeon under general anesthesia if the diagnosis remains uncertain after rectal suction biopsy. This method should unambiguously identify enteric neurons if they are present. Rectal biopsy is discussed in much more detail in this excellent review [50].

Epidemiology/Genetics Overview

HSCR is a multigenic disorder, but non-genetic factors may also influence disease occurrence. As of the year 2021, rare damaging protein-altering variants had been reported in at least 35 genes in people with HSCR (*RET*, *GDNF*, *NRTN*, *ARTN*, *PSPN*, *GFRA1*, *EDNRB*, *EDN3 ECE1*, *ZFHX1B*, *SOX10*, *PHOX2B*, *KIAA1279*, *NRG1*, *ERBB2*, *SEMA3C/D*, *IHH*, *GLI1*, *GLI2*, *GLI3*, *L1CAM*, *ITGB4*, *PTK2*, *DENND3*, *NCLN*, *NUP98*, *TBATA*, *VCL*, *BACE2*, *ACSS2*, *ENO3*, *SH3PXD2A*, *UBR4*, *and TITF1 TCF4*; reviewed in Chap. 18) and there are more than 30 HSCR-associated genetic syndromes. Reduced RET kinase activity is the most commonly identified predisposing genetic risk factor for human HSCR, but most predisposing genetic variants for RET are non-coding (e.g., a common intronic SNP reduces RET expression). Copy number variants (especially trisomy 21), miRNA, and epigenetic changes are also implicated in HSCR. For more detailed reviews of molecular and cellular mechanisms that control ENS development and HSCR genetics, please see [3, 7–10, 63–65]. One valuable observation from the clinical perspective is that even when whole genome sequencing is performed, many children with HSCR do not have readily identified genetic changes that predispose to HSCR [66]. This suggests that combinations of genetic and non-genetic factors are responsible for most HSCR cases. Non-genetic risk factors for HSCR have not been defined in humans, but based on animal models, vitamin A deficiency [67], mycophenolate [68], and some medicines such as ibuprofen might increase HSCR occurrence [69].

For short-segment disease, there is an approximately 4:1 male-to-female ratio, but for total colonic aganglionosis, the male-to-female ratio is near 2:1. HSCR has been reported throughout the world in many ethnic groups. There are geographic and racial differences described in HSCR incidence, but these data are difficult to evaluate. Most reports have not been replicated over extended time periods and the difficulty in HSCR diagnosis increases uncertainty in interpreting regional data. Furthermore, it is often not possible to determine from large-scale epidemiological studies, the number of affected individuals who share mutations by common descent, so data may be skewed by families with multiple affected members such as has been described in some Mennonite communities [70]. HSCR incidence per 10,000 live births in California was reported as 1.0, 1.5, 2.1, and 2.8 for Hispanics, Caucasian–Americans, African–Americans, and Asians, respectively [71], even though these racial categories do not correlate well with most human genetic risk variants [72]. Future studies should instead discuss ethnicity, geographic origins, and ancestry instead of these racial categories. HSCR incidence was reported as 1.4 per 10,000 in Denmark, 1.8–2.1 per 10,000 in Japan [15], and 2.3 per 10,000 in British Columbia [73]. Considerably, higher rates of HSCR are reported in some small geographic areas or ethnic groups. For example, HSCR incidence is 2.9 per 10,000 in Tasmania [74], 5.6 per 10,000 for native Alaskans [75], 7.3 per 10,000 in Pohnpei State in the Federated States of Micronesia [76], and 5.6 per 10,000 in Oman [77]. In Oman, rates of consanguinity are reported to be high (75% first or second cousins), but this was not reported in other areas. The European registry (EUROCAT—European Registration of Congenital Anomalies and Twins) also describes striking differences between reporting regions, but ascertainment for HSCR is challenging, and it seems unlikely that the 31 reporting regions use the same ascertainment strategies [22]. Nonetheless, founder effects within populations, nutritional factors, differences in medicine use, or environmental toxins may account for these differences in HSCR incidence.

Recurrence Risk for HSCR in Families is High

Recurrence risk for siblings of children with HSCR is dramatically elevated compared to the general population and varies from 1:3 to 1:100 [6, 78] depending on the sex of the proband and their extent of aganglionosis. Because female sex protects against HSCR and because long-segment disease implies more serious genetic risk than short-segment disease, male siblings of females with long-segment HSCR have a 33% chance of HSCR, while new sisters have only a 9% risk. Siblings of males with long-segment HSCR have a recurrence risk of 17% and 13% in new brothers and sisters, respectively. For a male proband with short-segment HSCR, the risk of recurrence is 5% in male siblings, but only 1% in female siblings. For a female proband with short-segment disease, recurrence risk is 5% and 3% for new male and female siblings, respectively. Risk of HSCR in children whose parents have HSCR is also high. Twenty-two percent of reported familial cases include an affected parent and child [79]. These complex epidemiologic and recurrence risk data are a direct reflection of the non-Mendelian genetic underpinnings of HSCR. While these “average” data are

helpful in discussions with families, better estimates of HSCR recurrence risk might theoretically be obtained using modern molecular genetic techniques if highly penetrant gene defects were identified. From a practical perspective, I tell parents about the elevated HSCR risk in future children and about diverse HSCR presentations, so that they can alert pediatricians if any symptoms suggest HSCR. I recommend that mothers take prenatal vitamins before conception and that they avoid taking medicines or herbal supplements that are not providing clear benefit. Since ENS precursors colonize fetal bowel during the first trimester of pregnancy (weeks 3–8 of gestation) and many women first know that they are pregnant at weeks 6–7 of gestation, changes implemented after pregnancy is recognized are less likely to affect HSCR occurrence.

HSCR-Associated Medical Problems

HSCR is an isolated birth defect in ~70% of affected individuals, but ~30% of children with HSCR have additional birth defects, including the ~12% of children with HSCR who have chromosomal anomalies [22, 41, 48, 73, 80–82]. A very wide range of additional defects have been reported in children with HSCR. The most common defects are congenital heart disease, sensory neural problems (e.g., hearing loss), visual problems, CAKUT, and skeletal anomalies [83]. Many different chromosomal defects have been described in people with HSCR, but trisomy 21 is by far the most common. There are >30 genetic syndromes associated with HSCR (reviewed in [6, 84]). A few HSCR-associated syndromes are summarized in Table 26.4.

Surgical Management

Although Harald Hirschsprung first described children with the disease that now bears his name in 1886 [85], the pathophysiology of HSCR and management strategies remained unknown until the first successful surgical approach was described in 1948 [86]. There are many modifications of the original pull-through surgery, but the most common procedures today are the Swenson, Duhamel, and Suave endorectal techniques with modification of surgical approaches for total colonic HSCR [1, 18, 87]. For each of these procedures, intraoperative biopsies are obtained to determine the extent of aganglionosis. The Swenson procedure involves complete resection of the aganglionic bowel with reanastomosis of ganglion cell containing bowel to a 1–2 cm rectal cuff. In the Duhamel modification, ganglion cell containing bowel is brought through the retrorectal space and anastomosed to a segment of aganglionic rectum using a side-to-side anastomosis. In the Suave procedure as modified by Boley, the rectal mucosa and submucosa are removed and the ganglion cell containing bowel is pulled through a muscular cuff of distal aganglionic bowel and then attached within 1 cm of the anal verge. There are innumerable studies of surgical outcome, but few large-scale systematic comparisons are available [88], so it remains unclear that one procedure is better than another. Over the past two decades, there have been three major changes in surgical management. These include laparoscopic surgery, transanal surgery, and increased use of one-step surgical procedures [16, 89–92]. Systematic reviews and meta-analyses of transanal versus transabdominal surgeries suggests that the children who had transanal endorectal pull-through procedures for HSCR had shorter hospitalization, but reviews differ in conclusions about relative rates of post-operative incontinence, constipation, and enterocolitis [21, 93–95]. A comparison of single versus multistage pull-through surgery suggested that children with single-stage surgery tend to do better, but a subgroup of children who are seriously ill with HSCR may do best with multistep surgery [96]. A meta-analysis of Soave pull-through procedures suggests that children <2.5 months of age had more complications compared to children who had Soave surgery at older ages [97]. Unfortunately, many children continue to have problems after HSCR surgery (see section "Long-Term Outcome" below) and the best way to avoid these problems is not yet defined [8].

Cost for Initial Management

For children with HSCR, initial hospitalization costs average $105,000 (in 2007 dollars, Nashville Tennessee, USA; $139,000 in 2021 dollars) and the hospital stay averaged almost a month [98]. Taking into account HSCR incidence and birth rates, estimated cost for initial care of children with HSCR in the United States is at least $86 million/year (2007 dollars, $114 million in 2021 dollars). This cost estimate does not include the expense of lost work time or other expenses families encounter while caring for an ill child. Estimates also do not include the cost of ongoing care after the initial hospitalization, which in some cases may be significant, especially in children with enterocolitis. For children with aganglionosis extending into the small bowel, long-term parenteral nutrition adds dramatically to cost and disease morbidity. Finding new ways to treat or prevent HSCR remains desirable.

Enterocolitis

HAEC is common, can occur at any time before or after surgery, and is the most frequent cause of death in infants and children with HSCR [8, 99–101]. Death from HAEC occurs,

because HSCR predisposes to bacterial translocation into the bloodstream that leads to sepsis. Nonetheless, recognizing HAEC is difficult, and until recently, there was no standard clinical definition for HAEC. In 2009, a consensus of expert surgeons and gastroenterologists developed a systematic scoring system to identify children with HAEC [43]. Components of the score include "explosive" diarrhea, foul-smelling diarrhea, or bloody diarrhea. Additional components include abdominal distension, explosive discharge of gas and stool with rectal exam, reduced peripheral perfusion, lethargy, and fever. Radiographic findings include multiple air fluid levels, distended loops of bowel, sawtooth and irregular mucosal lining, pneumatosis, and rectosigmoid cutoff sign with the absence of distal air. Laboratory findings include leukocytosis and a left shift. Many of these features are also listed as presenting symptoms for HSCR, because HAEC is common in children with HSCR, especially before surgery.

The reasons that children with HSCR develop HAEC are not clear, but enterocolitis does not occur in children with "severe" functional constipation. Possible predisposing factors for HAEC in children with HSCR include residual partial bowel obstruction, defects in epithelial integrity, reduced blood flow to bowel under pressure, dysbiosis, and abnormalities in the mucosal immune system [8, 101, 102]. Partial obstruction may result from stricture or from intestinal dysmotility leading to increased intraluminal pressure and changes in gut flora [103–105]. Epithelial dysfunction may occur, because enteric neurons and glia support bowel epithelial repair and regulate fluid secretion, in addition to controlling antimicrobial peptide and mucin production [13, 63, 106–120]. Furthermore, aganglionic bowel has a "leaky" epithelial barrier that is permeable to small proteins (and perhaps larger molecules or bacteria) [121] Mechanisms, underlying these observations are complex and often involve interactions between microbes or microbial components, neurons, glia, immune systems cells, and epithelial cells [13, 122–128]. For example, diverse immune system cells respond to ENS neurotransmitters, including vasoactive intestinal peptide (VIP), neuropeptide Y (NPY), calcitonin gene-related peptide (CGRP), acetylcholine, substance P, and serotonin [129]. Enteric glia modulate bowel immunity [119] by producing CXCL10 in response to gamma interferon [130], increasing NGF and NT-3 while reducing IL-18 and IL-β in response to *Bifidobacterium* [131], and by producing brain-derived neurotrophic factor, which reduces TLR4 responses to lipopolysaccharide [132]. In addition, enteric glia secrete GDNF that activates RET on group 3 innate lymphoid cells (ILC3) to induce the production of IL-22. Secreted IL-22 enhances epithelial reactivity and repair to reduce bacterial translocation and bowel inflammation [133]. IL-18 from enteric neurons reduces bacterial invasion by increasing epithelial anti-microbial peptide production [126]. Mucins that reduce microbial invasion are abnormal in HSCR [111, 115, 120, 134]. Macrophages closely interact with the ENS to impact ENS function and respond to ENS signals [13, 125, 127, 135, 136]. Bowel macrophages can reduce or promote inflammation (depending on macrophage phenotype) [137, 138], and can also break down the blood–myenteric plexus barrier, permitting inflammatory cells to damage the ENS leading to chronic dysfunction and dysmotility [139]. Finally, extrinsic innervation may impact risk of enterocolitis, since the absence of mucosal acetylcholinesterase-stained fibers in aganglionic colon predicts increased enterocolitis in human children [51]. There is much more to learn about these gut microbe–epithelial–neuron–glia–extrinsic innervation–immune cell interactions, but these emerging data provide strong support for the hypothesis that enterocolitis results in part from a compromised epithelial and immune cell barrier when the ENS is absent or defective. Some genes mutated in children with HSCR also have ENS-independent immune cell roles, suggesting that shared genetic mechanisms independently impact immune cell function. For example, RET is important for Peyer's patch formation [140] and alters activity of other immune cells [141, 142], while EDNRB is important for spleen development [143]. Collectively, dysmotility, dysbiosis, and dysregulation of immune and epithelial cell function probably explain why HSCR predisposes to HAEC.

Optimal methods to treat or prevent HAEC are not yet known. Current treatment includes bowel rest, nasogastric tube drainage, intravenous fluids, decompression of dilated bowel via rectal dilation and/or rectal irrigation with normal saline, and the use of broad-spectrum antibiotics [102]. When the child is acutely ill with HAEC and markedly distended, simple rectal exam can lead to rapid release of air and stool, restoring blood flow to the bowel. Children with HAEC often feel and act better quickly after rectal exam, rectal irrigation, or rectal tube reduces intra-abdominal pressure. This therapy may also reduce the risk of sepsis, so do not delay rectal decompression. Routine (e.g., daily) rectal irrigation [144] and long-term metronidazole for children at high risk of enterocolitis may further reduce HAEC episodes. Probiotics might reduce HAEC frequency [145], but beneficial effects are not consistently reported [146]. However, probiotics, prebiotics, and dietary effects on enterocolitis have barely been investigated. Two recent observations suggest that these therapies might be effective. First, a prospective study demonstrated that exclusive breastfeeding reduced HAEC risk by 40% in children ($n = 111$, 95% CI 0.44–0.85, $P = 0.003$) with HSCR [105]. Our study in an inbred HSCR mouse model also demonstrated a dramatic (fivefold) change in life expectancy when mouse facility and diet changed [121]. Because HAEC is potentially fatal, it is critical that families understand symptoms of enterocolitis and that plans are in place for prompt treatment should HAEC symptoms arise.

Long-Term Outcome

HSCR is a deadly disease, but outcome with modern surgical methods and improved medical management strategies is dramatically better than in the past. Nonoperative management leads to very high mortality rates (e.g., >50–80%), and reports from the 1970s describe mortality rates of 25–35% [18, 147] even with surgical treatment. HSCR death rates today remain about 2–6% despite modern therapy in large part attributable to enterocolitis [14, 15, 41, 148, 149]. Enterocolitis occurs commonly both before and after surgery for HSCR (25–45% of children) [21, 98, 150, 151]. Long-term outcome even years after surgery also remains less than ideal with only 45–89% having normal bowel function. Many individuals continue to have soiling (4–29%), constipation (3–22%), or permanent stomas (7–10%) [152–154]. Normal bowel function is even less common in children with Down syndrome (34%). Bowel function appears to improve as children get older with "normal" continence in 58% at 5–10 years after surgery, 68% at 10–15 years after surgery, and 89% at 15–20 years after surgery in one study [154]. In this analysis, however, 7% had marked limitation in their social life 5–10 years after surgery, but this problem improved as children became older.

Lessons from Mouse Models

There are many mouse models with distal bowel or total intestinal aganglionosis that mimic human HSCR [3, 63, 155–160]. This includes mice with mutations in *Ret*, *Sox10*, *Ednrb*, *Edn3*, *Ece1*, *Ezh2*, *Phox2b*, *Zfhx1b*, *Sall4*, *Hoxb5*, *Ihh*, *Itgb1*, *Pds5A*, *Pds5B*, *Pax3*, *Raldh2*, *Impdh2*, *Rara*, and *Pax3*. Recent mouse studies also suggest that excess collagen VI may underlie increased HSCR risk in Down syndrome [161]. Overexpression or inactivation of many additional genes also affect ENS structure or function without causing distal bowel aganglionosis, including *Ahr*, *Apoe*, *App*, *Ascl1*, *BMP4*, *C3ar1*, *Card11*, *Cdh2*, *Celsr3*, *Dat*, *Dcc*, *Dmd*, *Erbb2*, *Fzd3*, *Gas1*, *Gfra2*, *Gdnf*, *Gli1*, *Gli3*, *Gnaz*, *Hand2*, *Hlx1*, *Hoxa4*, *Kif26a*, *L1cam*, *Lgi4*, *Lrrk2*, *Mecp2*, *Met*, *Nedl2*, *Net*, *Nlgn3*, *Nog*, *Nos1*, *Nrtn*, *Nt3*, *Ntrk3*, *Pbx3*, *Phactr4*, *Pofut1*, *Pten*, *Raldh1*, *Raldh3*, *Rara*, *Rest*, *Sert*, *Shh*, *Smn*, *Smo*, *Snca*, *Spry2*, *Tbx3*, *Tcof1*, *Tfam*, *Tlr2*, *Tlr4*, *Tlx2*, *Tph2*, *Uchl1* (arranged alphabetically) as well as a wide array of neurotransmitters, neurotransmitter receptors, and proteins that re-uptake or degrade neurotransmitters [13]. These observations in combination with the large number of human genetic variants documented in people with HSCR [10, 66, 162–164] suggest that ongoing problems after pull-through surgery may be manifestations of ENS dysfunction that results from abnormal "wiring", abnormal ENS cell subtype ratios, or abnormal function of specific ENS cell types in regions deemed "normal" based on clinical pathology. A few mouse studies confirm that ENS is abnormal in the proximal bowel of mice with distal bowel aganglionosis [165–167], but much more detailed analyses of ENS structure and function need to be done, especially in human tissue. Finally, in some mouse models, ENS anatomy is nearly normal, but function is profoundly abnormal [168, 169], emphasizing that even sophisticated pathological methods may not provide the information needed to optimize intestinal function. Limited human data support the hypothesis that ENS in the bowel of children with HSCR bowel may not be normal even when "ganglion cells are present" [170–172]. Consistent with this hypothesis, bowel motility problems of the stomach, small bowel, and esophagus appear to be common in humans with HSCR [173–177].

The Future of Hirschsprung Disease

Outcomes for children with HSCR today are quite good, but many challenges remain. The primary problems and opportunities include:

1. *There have been major advances in our understanding of the genetic underpinnings of HSCR, but these findings are not yet routinely incorporated into clinical practice*. Furthermore, there is no consensus about what type of molecular genetic testing, if any, should be performed on children with HSCR. One reasonable argument is that all children with HSCR should be tested for RET mutations that cause MEN2A (but this is still not common practice), since people with MEN2A are at high risk for potentially preventable malignancy. As genetic testing becomes less expensive and the capacity to test for many mutations simultaneously increases, it may become practical to perform more comprehensive analysis that would provide information about the risk of other medical problems. It is important that we develop user-friendly methods to understand the type of complex genetic data that are relevant for children with HSCR.
2. *There are many HSCR-associated medical problems that may be missed if routine screening is not implemented*. One prospective study of 106 consecutive children with HSCR arranged for each child to have a renal ultrasound, cardiac ultrasound, cerebral ultrasound, as well as audiology and ophthalmology assessments [83]. Forty-six children had ophthalmologic issues (mostly refractive errors), 22 had CAKUT, 5 had congenital heart disease, 5 had hearing impairment, and 1 had corpus callosum agenesis. These rates are much higher than prior retrospective reports that did not employ systematic screening. This

suggests that routine screening for HSCR-associated anomalies makes sense, especially for problems not easily identified by history or physical exam.

3. *Enterocolitis remains a common cause of morbidity and the most common cause of mortality in children with HSCR. We need a more complete understanding of factors that predispose to HAEC and new ways to prevent this problem*. Recent studies demonstrate many complex interactions between gut microbes, enteric neurons, glia, epithelial cells, macrophages, and other hematopoietic lineage cells in the bowel wall. These interactions maintain the protective barrier that prevents bacterial translocation from the lumen while preventing excess bowel inflammation. There is undoubtedly much more to be learned about why aganglionosis predisposes to HAEC, but new mechanistic observations allow us to think creatively about novel strategies to treat or prevent HAEC. For example, how do medicines that alter acetylcholine or serotonin signaling affect epithelial or immune cell barrier function? Would strategies to increase GDNF production in enteric glia be helpful, since many factors impact GDNF synthesis [178]. Would probiotics or specialized diets be useful? Are there additional medicines that could reduce HAEC rates? Would a more systematic analysis of pathology at the time of surgery help? The underexplored emerging information about HAEC biology should lead to human clinical trials as new data define mechanisms.
4. *We need improved methods to evaluate and visualize the ENS*. Acousto-optic spectral imaging [179] and optical coherence microscopy [180] permit visualization of the ENS in mice, but the thicker human bowel wall makes it challenging to visualize the ENS without getting closer to the cells of interest. Human ENS can be visualized in vivo using confocal laser endomicroscopy and fluorescent contrast agents once the mucosa is removed or bypassed [181–183]. To take full advantage of this approach, we still need to define normal human ENS anatomy at various ages in defined bowel regions. Then, confocal laser endomicroscopy might make pull-through surgery faster and provide better data about the location of the anatomic transition zone. New imaging data should improve surgical outcomes and reduce postsurgical HAEC rates by enhancing the surgeon's ability to evaluate the density of enteric neurons in the bowel intraoperatively. To begin to address this problem, we developed a new way to make fixed bowel translucent, stain the ENS with antibodies and image via confocal microscopy [184]. Our method cannot be used intraoperatively, but generates detailed images of ENS cells over cm^2 bowel regions without sectioning, permitting three-dimensional relationships to be readily understood. By applying this method to bowel from children with and without HSCR, we hope to define anatomic features that predict good outcomes after pull-through surgery.
5. *We need to determine if there are ways to reduce HSCR occurrence rates or to reduce the extent of aganglionosis in affected individuals*. New data from model systems suggest that many environmental factors, including maternal vitamin A levels, mycophenolic acid, ibuprofen, and other medicines, might impact the likelihood that children develop HSCR [67, 68, 185]. Reports of monozygotic twins discordant for HSCR also suggest that HSCR is not a purely genetic disease [41, 48, 186, 187]. Large-scale epidemiological studies coupled with work in model systems should be pursued to identify maternal medicines, health conditions, or nutritional problems that could be modified to prevent HSCR.
6. *We need to find new ways to replace or repair the damaged ENS to rebuild the ENS when development is abnormal*. Recent exciting studies suggest that stem cell therapy might provide substantial benefit for treating ENS defects [188–192], but many obstacles need to be overcome for stem cell replacement therapy to become a practical treatment strategy. One promising approach transplants gut-derived ENS progenitors to the bowel after in vitro culture [193–195]. These cells integrate into the ENS and form functional enteric neurons and glia. Recent studies also provide a method to convert human embryonic stem cells (hESC) or induced pluripotent stem cells (iPSC) into ENS precursor-like cells. These hESC-derived cells can prevent death in a murine HSCR model after transplantation [196]. This work suggests that autologous stem cell therapy using iPSC might be an alternative to pull-through surgery for HSCR if safety concerns could be addressed (e.g., risk that transplanted cells will become neoplastic). Several other sources of cells are being tested for beneficial effects in HSCR models [188, 197]. As an alternative to stem cell therapy, 5-HT4 agonists and GDNF enemas appear to induce regeneration of the endogenous ENS and might be beneficial in specific settings [121, 198]. Manipulating gut microbes, inflammatory responses, and micronutrients also seem likely to be valuable strategies to shape ENS biology [199].

Summary

Over the past century, dramatic advances have been made in HSCR diagnosis, surgical management, developmental biology, and genetics. Ongoing studies provide new hope that we will be able to reduce HSCR incidence, prevent HAEC, replace missing enteric neurons using stem cells, regenerate

the ENS from endogenous cells, image the ENS intraoperatively, improve surgical techniques, and incorporate genetics into clinical practice.

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