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Hirschsprung's disease

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Hirschsprung's disease (HSCR) is characterized by absence of the enteric nervous system in a variable portion of the distal gut. Affected infants usually present in the days after birth with bowel obstruction. Despite surgical advances, long-term outcomes remain variable. In the last 2 decades, great advances have been made in understanding the genes and molecular biological mechanisms that underlie the disease. In addition, our understanding of normal enteric nervous system development and how motility develops in the developing fetus and infant has also increased. This review aims to draw these strands together to explain the developmental and biological basis of HSCR, and how this knowledge may be used in future to aid children with HSCR.

Hirschsprung's disease (HSCR) is the commonest congenital gut motility disorder and is characterized by a lack of ganglion cells (aganglionosis) in a variable length of distal gut. Affected infants usually present shortly after birth with signs of distal intestinal obstruction that are invariably fatal if left untreated. Current definitive treatment involves surgery to resect the aganglionic bowel segment and "pull-through" and anastomosis of normally innervated (ganglionic) gut close to the anal margin. Although broadly successful in the majority of patients, challenges are encountered in the management of children with more extensive aganglionosis and those who experience repeated bouts of enterocolitis.[1] Furthermore, in the long term up to 75% of children will have some form of continence or constipation problem, and 10% have symptoms sufficiently severe to warrant a permanent colostomy. Clearly, an understanding of the biological and developmental basis of aganglionosis is extremely relevant in understanding the reasons for such variability in biological presentation and also in formulating novel treatments for children with HSCR in future. It has long been noted that HSCR can be familial and also associated with a range of syndrome conditions. This review will therefore address the underlying developmental and biological basis of HSCR with particular emphasis on its' genetic basis.

Incidence and associated anomalies

Demographic studies have shown a remarkably constant incidence of HSCR of approximately 1 in 5000 in both hemispheres, although most epidemiologic studies have been confined to the Caucasian Diaspora, and thus there may be as yet undefined interracial differences. Evidence for this comes from a Californian survey in which the authors found significant interracial differences in incidence of HSCR: 1:10,000 births in Hispanic subjects, 1:6667 in white subjects, 1:4761 in black subjects, 1:3571 in Asian subjects.[2] Differing levels of consanguinity in different populations may explain some of the differences, but the authors of recent genetic studies concerning frequencies of HSCR-associated mutations point to different frequencies in different ethnic populations.[3]

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Table 1 Additional anomalies in Hirschsprung's disease

Anomaly	Example
Neural crest-related anomalies	
Sensorineural deafness	Congenital central hypoventilation syndrome
	Isolated sensorineural deafness
	Waardenburg syndrome
Cardiovascular skeletal and limb anomalies	DiGeorge syndrome
	Postaxial polydactyly and heart defect
	CRASH syndrome (X–linked aqueductal stenosis)
	Congenital muscular dystrophy
Cleft palate	Goldberg Sphrintzen syndrome
	DiGeorge syndrome
Systemic anomalies	Neurofibromatosis type 1
	Multiple endocrine neoplasia type 2A
	Multiple endocrine neoplasia type 2B
	Smith-Lemli Opitz syndrome
	Dysautonomias
Other anomalies	
Trisomy 21	
Microcephaly	
Mental retardation	
Inguinal hernia	
Small bowel atresia	
Duodenal atresia	
Genital reproductive tract	
Undescended testes	
Regional anomalies	
Rectal stenosis	
Anal stenosis	
Imperforate anus	
Colonic atresia	

In the most common, "classical" form of HSCR, aganglionosis is restricted to the rectosigmoid region and is referred to as "short segment" disease. This variant accounts for more than 80% of cases. In the remaining cases, colonic aganglionosis is more extensive and may involve distal small intestine. Total enteric aganglionosis is both rare and associated with high morbidity and mortality. There is a strong male gender bias, with male patients being affected 2 to 4 times more commonly than female ones, although this bias is lost in children with more extensive aganglionosis.

Important clues as to which genes are involved in HSCR have come from the study of the pattern of associated malformations that occur in 4% to 35% of cases (Table 1). Knowledge of associated anomalies is also important in the course of genetic counseling and because of potentially deleterious known associations—medullary thyroid carcinoma as part of multiple endocrine neoplasia syndrome Type 2 B (MEN2B) is perhaps the best example. One of the commonest associated malformations is Down's syndrome (trisomy 21), which carries a 100-fold greater risk for HSCR than the normal population.[4]

As discussed in this article, the enteric nervous system is of neural crest origin, and hence HSCR is regarded as a neurocristopathy. Therefore, it is unsurprising that it is associated with other neurocristopathies because factors affecting the migration of enteric neuroblasts may well affect the migration, differentiation, or survival of other neural crest derived cells, for example, Shah-Waardenburg (WS4).

The role of the enteric nervous system in determining gut motility

Gut motility is a complex process mediated by interaction between intestinal smooth muscle (SM), "pacemaker" cells (interstitial cells of Cajal; ICC), and the enteric nervous system (ENS). Unlike in the heart, intestinal SM cells are unable to generate rhythmic electrical slow waves. In the last 2 decades it has been established that ICC are responsible for slow-wave activity in muscle that can propagate to adjacent muscle.[5,6] Although ICC-generated slow waves result in some contractile activity and a tendency for intestinal contents to be propagated in a craniocaudal direction, the ENS is essential for more widespread coordination plus modulation of amplitude and frequency of SM contraction to generate the 2 main types of contractions in the gut: segmentation and peristaltic waves. Both occur in the absence of extrinsic innervation but require an intact myenteric plexus. Colonic motility is quite distinct from small intestinal motility, and regionalization of contractions in different regions of the colon occurs. ICC-mediated slow-wave activity causes colonic contractions when the depolarization is of sufficient amplitude. At the end of the gastrointestinal tract sits the internal sphincter, a specialized thickening of circular SM within the distal rectum. It maintains a state of tonic contraction thus maintaining continence in association with the external sphincter. Distension of the rectum, typically with feces, results in an ENS-dependent reflexive relaxation of the sphincter (rectoanal inhibitory reflex). To achieve these functions, the ENS is extensive and contains a diverse range of neuronal phenotypes characterized by neurotransmitters and morphology; see Hao and Young for review.[7] The critical role of the ENS is illustrated by the obstruction that occurs in children with HSCR (in which there is congenital absence of the distal ENS): colonic mass movements are unable to propagate through the aganglionic segment that remains in a tonic state. Furthermore, the presence of feces in the rectum fails to elicit relaxation in the aganglionic anal sphincter, which contributes to the obstructive picture seen clinically.

Onset of gut motility in the fetus, normal, and premature neonates

There is a remarkable paucity of data on the ontogeny of human gut motility that reflects the inherent difficulties in studying the developing human. By late gestational age, fetal swallowing results in ingestion of amniotic fluid that is propagated through the gut.[8] Painstaking antenatal ultrasonographic studies of fetal gut motility demonstrate fetal gastric emptying occurring at 24 weeks and assuming more mature patterns by term.[9] Small intestinal peristalsis is rarely observed before 29 weeks and subjective observation suggests active waves of small intestinal peristalsis are infrequently seen before 35 weeks of gestation.[10] Similarly, ultrasound studies on human fetal internal sphincter development suggest that rhythmic contractions commence in the third trimester.[11] Preterm infants appear to manifest similar patterns of onset of gastrointestinal motility, exhibiting markedly delayed gastrointestinal transit times when compared with adults. In the small intestine of preterm children, disorganized peristalsis is seen before the third trimester, with migrating motor complexes being observed in human small intestine after 33 weeks of gestation.[12]

There is a marked lack of data on colonic motility in human preterms. Some evidence can be gleaned from animal studies in that, in common with humans, intestinal contents are propagated through the bowel before birth. The authors of recent studies suggest that effective colonic contractions do occur but that these are not mediated by the ENS.[13,14] Taken together, in both animals and humans although the main components regulating gut motility are present by 14 weeks of gestation, it seems likely that the ENS is relatively quiescent until late in gestation and gut motility is controlled by other factors, such as ICC. This explains our failure to detect HSCR antenatally as if the colonic ENS is not functional until birth no bowel dilatation will be detected on ultrasound. This is also seen clinically, in that invariably the abdominal distension is progressive after birth rather than being clinically detectable at the moment of birth.

Neural crest origin of the enteric nervous system and the pathogenesis of Hirschsprung's disease

Neural crest ablation studies and chick-quail chimaera experiments have shown that ENS neurons and glia are derived from the vagal segment of the neural crest.[15,16] Vagally derived neural crest cells (NCCs) migrate along the course of the vagus nerves, enter the foregut mesenchyme, and spread in a craniocaudal direction throughout the gastrointestinal tract. In humans the process takes 7 weeks, with neural crest derivatives entering the foregut at 5 weeks, reaching the distal ileum by 7 weeks, the midcolon by 8 weeks, but taking a further 4 weeks to reach the distal rectum.[17,18] This slowing in rate of colonization of the distal gut is caused by growth of the bowel rather than a reduction in velocity of migration. In mammals, there is an additional sacral contribution to the colonic ENS but migration of sacral crest cells follows vagal neural crest colonization and these cells in isolation are insufficient in isolation to rescue the HSCR phenotype.[19]

The vagal sourced NCCs in the distal rectum migrate further than any other cells during embryogenesis. It is therefore not surprising that factors affecting proliferation, survival, migration, or differentiation of NCCs results in aganglionosis of the distal gut. Although important advances have been made in identifying the complex genetic picture in HSCR, unraveling the biological mechanisms that prevail in normal neural crest colonization of the gut, and how this goes wrong in HSCR has been a formidable technical challenge because of the inaccessibility of the developing bowel and the lack of reliable in vivo markers.

Mathematical modeling coupled with experimental manipulation of chick-quail chimaeras of gut explants suggest that cell proliferation at the vanguard of migrating NCC drives colonization of aganglionic gut with HSCR, resulting from discordance between the rate of cell proliferation and elongation by growth of the developing gut.[20,21] In recent years, the use of the green fluorescent protein gene as a reporter of NCC expression in explanted mammalian and avian embryonic gut cultures, or in translucent zebrafish, in combination with time-lapse photography has allowed the pattern of neural crest migration and ENS formation to be better understood. Furthermore, experimental manipulation of the embryonic gut environment and gene expression has allowed insights into the pathogenesis of aganglionosis. What has emerged is a complex spatiotemporal interaction between migrating cells, developing neurons, and the gut.

Assumptions about the actions of genes made from isolated neural crest cells in vitro have been found wanting as cells respond to the same cues differently according to their location in the gut and gestational age. Chains of immature neuroblasts migrate through the developing gut and leave a scaffold that subsequent cells follow. This migration has been shown to be directionally driven by noncanonical Wnt signaling, causing contact inhibition;[22] although unpredictable at a single cell level, this seems stereotyped at the organ level. In particular, a single chain of cells appear to extend along the mesenteric border of the cecum well in advance of the rest of the advancing wave of colonization.[23] There is some evidence that migrating cells may be routed along the developing vasculature.[24] Migrating cells have been shown to undergo cell division to increase cell numbers. Furthermore, apoptotic control mechanisms may control the final neuronal density in the gut because inhibition of apoptosis during NCC colonization results in hyperganglionosis.[25] Only a small proportion of migrating cells express neuronal markers and these migrate more slowly.[26] Increasing cell maturation as reflected by expression of neuronal or glial phenotype and subsequent neurotransmitter expression is

associated with loss of migratory ability.[26] Microenvironmental factors in the noninnervated colon, such as overexpression of laminin, have been suspected to be implicated in the pathogenesis of HSCR[27,28] and documented in the aganglionic colon of children with HSCR.[29] In support of these observations, recent gut explant experiments point to age-dependent changes in the gut resulting in restriction of NCC migration into older bowel.[30,31] Clearly in HSCR, several genetic and environmental factors interact to result in failure of colonization of the distal intestine (see the section "Hirschsprung's disease and genes").

A variety of experiments in which small numbers of neural crest-derived cells were cultured in aganglionic bowel has demonstrated that relatively small numbers of these cells can engage in extensive colonization and formation of both neurons and glia expressing a range of phenotypic markers and expressing appropriate neurotransmitters.[32,33] Such observations point to the existence of a reservoir of "stem-cells" within the migrating wave: cells with extensive proliferative and differentiative capacity. As will be discussed later, the existence of these cells may point to a future stem-cell based therapy for HSCR. It should be remembered that at the same time as NCC are migrating and colonizing the gut the gut is maturing in many ways that will impact on future motility. SM and ICC are differentiating from mesenchyme and later in gestation functional connections are forming between neurones, ICC, and SM (for review, consult Burns et al[32]).

Hirschsprung's disease and genes

HSCR is a complex genetic disease with a low, sex-dependent penetrance (frequency of mutation carriers who have HSCR) and variability in the length of the aganglionic segment (for review, see Tam and Garcia-Barcelo[33] and Arnold et al[36]). The genetic diversity observed in HSCR can be attributed to the cascade of molecular and cellular events that take place during the ENS development as outlined above. Disruption of coding sequences resulting in functional changes to gene products of any of the genes responsible for neural crest cell migration, proliferation, differentiation, survival or that alter the permissive environment for NCC migration holds the potential for failure of ENS development resulting in HSCR (Table 2). The HSCR phenotype may result from mutations in single or multiple genes. The existence of individuals with major HSCR-causing mutations who do not manifest the disease underlines the complex multigenic mechanisms of ENS formation and also potentially the role played by environmental factors.

Furthermore, the existence of an overrepresentation of mutations and/or SNPs in gene-receptor complexes, such as ret-GDNF and/or 3rd edn/ENDRB,[34,35] when compared with controls suggests subtle influences of both major gene-receptor complexes in determining HSCR susceptibility. The role of identified genes in shaping the demographic presentation of HSCR is also beginning to be understood. For example, an association between RET and chromosome 21 gene dosage has recently been described.[36] The male preponderance observed in isolated short-segment HSCR may potentially be explained by the recent finding of reduced levels of ECE-1 and endothelin-3 mRNA in normal male mouse bowel versus females.[37] In the same report, comparison of male versus female cultured explanted bowel from heterozygous mice with a RetDN mutation (showing reduced but not absent Ret activity) showed reduced colonization in male mice. Supplementation of male cultured bowel with endothelin-3 peptide resulted in a significant increase in the rate of bowel colonization. Thus, there is increasing evidence that sex-related differences in endothelin-3 expression, on a background of genetic susceptibility, may account for the male overrepresentation in HSCR.

Modifying genes and interaction between signaling pathways

As indicated previously, the successful colonization of the gut by the ENS precursors depends on the network of interacting molecules. Conceivably, there should be a functional and genetic link among these molecules for them to interact. Interaction between pathways requires not only coordination among the pathway members but also with those molecules that mediate their interaction. There is increasing evidence of interactions between genes in apparently different signaling pathways.[33]

Hirschsprung's disease and stem cells

The discovery of a subset of cells with significant proliferative and differentiative capacity within the migrating wave of NCCs has given rise to the hope that these cells could potentially represent enteric nervous system stem cells (ENSC). Stem cells are characterized by their capacity for asymmetric cell division, both self-renewing and producing daughter cells that have the ability to proliferate and form a range of cell types. Putative ENSCs should therefore be demonstrably immortal, clonal, and capable of proliferating to form neurons and glia. That these properties have been demonstrated in mouse NCC strongly supports the existence of ENSCs.[38–43] More recently, human ENSCs have been isolated from children and adults with and without HSCR that can be numerically expanded in vitro and transplanted into animal models of HSCR where they have proliferated and formed neurons and glia.[38,44,45] Furthermore, neuronal function has been demonstrated.[14]

These results appear promising for future clinical applications. Nevertheless significant obstacles remain. The behavior of human ENSCs in the more mature environment of the neonatal gut needs to be assessed and a robust reproducible and safe form of ENSC transplantation into the right

Table 2 Summary of genes involved in Hirschsprung's disease, associated conditions, and proposed function during enteric nervous system development

Gene	Abbreviation	Mutation frequency	Associated conditions	Function
Receptor tyrosine kinase	Ret	50% familial cases 15%-35% sporadic cases overrepresentation of certain SNPs even in absence of coding mutation (may modulate penetrance of other HSCR genes)	Multiple endocrine neoplasia syndrome type IIA (MEN2A), type IIB (MEN2B), medullary thyroid carcinoma	Expressed by ENCC. Promotes proliferation, migration, survival, and differentiation of ENCC.
Glial cell-line derived neurotrophic factor	GDNF	Rare, <5%, 6 cases reported Cosegregate with ret		Produced by gut mesenchyme, particularly cecum. Ret-ligand-promoting proliferation, migration, survival, and differentiation of ENCC.
Neurturin	NTN	1 familial case reported		Coligand for GDNF. produced by gut mesenchyme
Endothelin B receptor	EDNRB	~5%	Shah–Waardenburg syndrome (WS4)	Expressed by ENCC. maintenance of ENCC in undifferentiated state, expression dependent on Sox10
Endothelin-3	3Rd edn	<5% May be common susceptibility gene— overrepresentation of specific 3rd edn haplotype		EDNRB ligand. produced by gut mesenchyme particularly cecum, time-dependent interaction with EDNRB permits distal gut colonization
Endothelin-converting enzyme	ECE-1	1 case report		Proteolytic conversion of endothelin-3 precursor to active form
SRY-related HMG-box 10	Sox10	Waardenburg-Shah syndrome (WS4)		Expressed by ENCC, maintenance of ENCC in undifferentiated state, cell fate and glial cell differentiation. Activates RET transcription, interacts with EDNRB.
Pairedlike homeobox 2 b	Phox2b	No mutations seen in isolated HSCR	Neuroblastoma congenital central hypoventilation syndrome	Expressed by ENCC. Essential for development of autonomic neural crest derivatives. necessary for ret expression
Zinc finger homeobox 1 b or Smad interacting protein 1	Zfhx1b/sip1	No mutations seen in isolated HSCR	Mowat Wilson syndrome	Expressed by ENCC and derivatives. essential for formation of vagal neural crest cells

ENCC, enteric neural crest cells; HSCR, Hirschsprung's disease; SNP, single nucleotide polymorphisms.

environment developed. Furthermore, long-term studies are necessary to demonstrate the genomic stability of transplanted cells to assess potential tumor risk. In addition, techniques that permit in vivo tracking of transplanted ENSC by the use of such technologies as green fluorescent protein labeling or stable integration of nanoparticles, such as superparamagnetic iron oxide nanoparticles within animal models is essential to understand the potential of ENSCs to migrate beyond the bowel.

In conclusion, the last 2 decades have yielded huge advances in our understanding of the developmental and biological basis of Hirschsprung's disease and gut motility in general. Significant challenges remain but increasing understanding of this subject may lead to prediction of HSCR risk and potentially to new treatments and improved outcomes for this condition.

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