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Review

Hirschsprung's disease: clinical dysmorphology, genes, micro-RNAs, and future perspectives

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On the occasion of the 100th anniversary of Dr. Harald Hirschsprung's death, there is a worldwide significant research effort toward identifying and understanding the role of genes and biochemical pathways involved in the pathogenesis as well as the use of new therapies for the disease harboring his name (Hirschsprung disease, HSCR). HSCR (aganglionic megacolon) is a frequent diagnostic and clinical challenge in perinatology and pediatric surgery, and a major cause of neonatal intestinal obstruction. HSCR is characterized by the absence of ganglia of the enteric nervous system, mostly in the distal gastrointestinal tract. This review focuses on current understanding of genes and pathways associated with HSCR and summarizes recent knowledge related to micro RNAs (miRNAs) and HSCR pathogenesis. While commonly sporadic, Mendelian patterns of inheritance have been described in syndromic cases with HSCR. Although only half of the patients with HSCR have mutations in specific genes related to early embryonic development, recent pathway-based analysis suggests that gene modules with common functions may be associated with HSCR in different populations. This comprehensive profile of functional gene modules may serve as a useful resource for future developmental, biochemical, and genetic studies providing insights into the complex nature of HSCR.

The enteric nervous system (ENS) is recognized as a distinct third portion of the autonomic nervous system, which also includes the sympathetic and parasympathetic systems (1). The ENS is involved in peristalsis and, singularly, other spontaneous movements still persist following its isolation from all nervous inputs (2–4). The interstitial cells of Cajal are crucial in mediating nervous impulse onto smooth muscle cells acting as the intrinsic pacemaker of the bowel, while the ENS controls the continuous influence of the sympathetic and parasympathetic systems. The cholinergic (postganglionic) parasympathetic neurons increase peristalsis, secretions, and vasodilation, while the noradrenergic (postganglionic) sympathetic fibers project onto the submucosal and myenteric plexuses, where they play an inhibitory effect on the cholinergic neurons promoting an inhibition of peristalsis and secretions and stimulation of vasoconstriction (5). Parasympathetic fibers reach the gut via vagal nerves to celiac and superior mesenteric plexuses to over the mid-transverse colon, while the rest of the gut is supplied by fibers arising from pelvic splanchnic nerves via the sacral nerves 2–4 going through the pelvic plexus (5).

Hirschprung's disease (HSCR; MIM# 142623), one disorder of the ENS, is a rare congenital developmental disorder of the gastrointestinal tract characterized by a failure of vagal system derived enteric neural crest (NC) cells (ENCC) (neurocristopathy) to fully migrate cranio-caudally during embryonic development and adequately colonize the entire gut, leaving an aganglionic portion of variable length (6–9). Although original studies suggested colonization of the entire length of the human gut by enteric neural precursors is not complete until the 12th week of gestation, more recent studies seem to support complete colonization by the 7th week, which corresponds more closely with data obtained from animal models as well (10). HSCR is named after Dr. Harald Hirschsprung who first described this phenotype at "*The Queen Louise Hospital for Children*" in Copenhagen, Danemark. Aganglionosis is defined as the absence of ganglion cells in the myenteric and submucosal plexuses of the intestinal wall with concomitant hypertrophy of parasympathetic nerve fibers (11,12) (**Figure 1**). When suspected, HSCR is diagnosed by standard histopathological evaluation with or without auxiliary special stains or immunohistochemistry that confirms the diagnosis following biopsy of the distal rectum (**Figure 1a–c**). Expression of calretinin, a vitamin D–dependent calcium-binding protein found in ganglion cells and nerves, has been described as an adjunctive or primary diagnostic test on gut biopsy specimens in HSCR with lack of specific calretinin staining confirming the diagnosis of aganglionosis (**Figure 1d**) (8). Classifying HSCR clinically is not an easy task, because the nervous system colonization failure may be variable or discontinuous (9,13–15). Three phenotypes are usually recognized, including (i) total colonic aganglionosis (TCA), which involves the entire colon which is aganglionic with a potential proximal extension into varying lengths of small bowel (usually no more than 50 cm of

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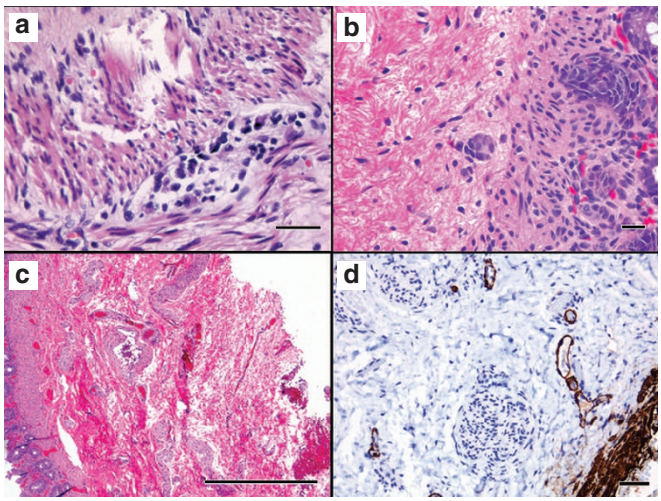

Figure 1. Ganglion cell maturation and Hirschsprung's disease. (**a**) The myenteric plexus of the distal intestinal tract of a baby of 23 wk of gestation highlighting the high nucleus to cytoplasm ratio of the premature ganglion cells (400×, hematoxylin-eosin staining, bar: 400 µm), while panel **b** shows the relatively more mature ganglion cells of a term newborn baby at level of the submucosa of the lower intestinal tract (400×, hematoxylin-eosin staining, bar: 630 µm). (**c**) The lack of ganglion cells and hypertrophy of nerve fibers of a baby born at term (50×, hematoxylin-eosin staining, bar: 50 µm), while panel **d** shows Hirschsprung's disease in a newborn baby confirming the absence of ganglion cells using a monoclonal antibody against calretinin, a calcium-binding protein of 29 kDa and calcium-dependent regulator with positive staining in the perivascular cells of blood vessels (internal control). Moreover, positive calretinin staining may be recognized in the lower right corner showing characteristic dark-brown granular nerve twigs in the muscularis mucosae. No calretinin staining is identified in nerve fibers at the center of the microphotograph (200×, anti-calretinin immunohistochemical staining, avidin-biotin complex, bar: 200 µm).

small bowel proximal to the ileocaecal valve), (ii) total colonic and small bowel aganglionosis, which may involve very long segments of small bowel aganglionosis, and (iii) the more frequent rectal or rectal/sigmoid colonic aganglionosis (RA or RSCA). A debate over the definition and occurrence of several phenotypical entities, including the ultrashort segment and the skip-segmental aganglionosis is ongoing (13,16–22). The majority of treatment remains surgical, while intense efforts are exploring the use of ENS stem cells by means of transplantation (12,23). This review reports on current knowledge about syndromic forms of HSCR, genes and biochemical pathways of HSCR as well as new views regarding pathogenesis of HSCR involving gene modules, microRNAs (miRNAs), and future perspectives.

CLINICAL DYSMORPHOLOGY

HSCR has a population-dependent incidence of 1.5–2.8 per 10,000 births (1.5 in Caucasians and 2.8 in Asians; average 1/5,000 live births) and is a largely variable heterogeneous condition both from the clinical and genetic point of view. There can be varying lengths of aganglionosis, a male preponderance (4:1) for short segment aganglionosis, a familial incidence of 5–20%, but usually appearing as sporadic congenital anomalies (24,25). HSCR occurs as an isolated trait in 70% of the cases, with about 1/3 only occurring in a syndromal setting. Complex genetic susceptibility factors may contribute substantially to the etiology of HSCR and, except for syndromal cases that show various patterns of Mendelian inheritance, it is generally accepted that HSCR follows a multifactorial pattern of inheritance with incomplete penetrance and 5–10% of HSCR cases having additional congenital anomalies (26). These associations suggest that at least some of the susceptibility loci may probably comprise genes with pleiotropic effects (27,28). A synopsis of clinical genetic entities with HSCR as a feature are summarized in **Table 1**. In this section, the most common examples of clinical syndromes associated with HSCR are depicted.

Chromosomal anomalies have been described in up to 12% of HSCR patients, although this rate may be higher because not all patients with HSCR undergo karyotyping (24). More subtle changes can be identified with microarray. Trisomy 21 syndrome or Down syndrome (DS) is found in about 2–10% of the HSCR cases (29). The HSCR risk ratio in DS is greater than the risk given by any single gene mutations associated with HSCR (40-fold) (30). A role for the RET gene in DS-HSCR pathogenesis has been proposed as RET hypomorphic alleles have been found more often in DS-HSCR compared to HSCR or DS alone (31). New experimental evidence shows that zebrafish overexpression of ATP5O (ATP synthase, H^+ transporting, mitochondrial F1 complex, O subunit), a gene found on chromosome 21, leads to enteric hypoganglionosis potentially explaining the association between DS and HSCR (Chauhan, AJHG, Baltimore, 2015, abstract communication).

The human prototype syndrome characterized by mutations in the RET gene is multiple endocrine neoplasia type 2 (MEN2) and familial medullary thyroid carcinoma (FMTC) (32,33). FMTC is part of MEN2. MEN2 is represented by three entities: MEN2A, FMTC, and MEN2B. MEN 2B is suspected when individuals with a Marfanoid habitus have distinctive features including mucosal neuromas of the lips and tongue, enlarged lips, HSCR, and early-onset medullary thyroid cancer. About 40% of affected individuals have diffuse ganglioneuromatosis of the gastrointestinal tract (34–36). RET mutations in MEN2A have been shown to be activating mutations by *in vitro* studies, while haploinsufficiency seems to be the most likely mechanism in sporadic HSCR (37–39). To the best of our knowledge, this data has not yet been translated into clinical guidance for screening of individuals with HSCR in the absence of a family history suggestive of MEN or FMTC.

Waardenburg syndromes (WS) are a group of autosomal dominant (AD) conditions found in 2–5% of congenital deafness cases, characterized clinically by distinctive facies with sensorineural hearing loss and pigmentary anomalies and an incidence of about 1/5,000 live births. Clinically and genetically heterogeneous, WS is classified in four subsets, three following an AD and one an autosomal recessive (AR) inheritance pattern. WS4 is underlined by homozygous mutations in three genes. Approximately 20–30% of cases are due to homozygous or heterozygous mutations within the *EDN3* (endothelin-3) or the *EDNRB* (endothelin receptor type B) genes, whereas approximately 45–55% result from heterozygous mutations within the gene encoding the SOX10 transcription factor,

Table 1. Clinical genetic syndromes

Syndromes	MIM	Gene locus	Etiology	Association strength	Key features
Chromosomal					
Del 10q11 (HSCR1)	142623	10q11	Mutation in RET. 12% of HSCR cases out of 70% isolated trait cases are associated with chromosomal anomaly	High	L-HSCR, ID
Del 13q22	600155	13q22.3	EDNRB	Frequent	S-HSCR, ID, DF, LD
Del 2q22- q23	605802	2q22- q23	Mutations and or deletions of ZFHX1B	Frequent	ID, DF, postnatal LD, epilepsy
Del 1q44-	612337	1q44	mutation in the ZBTB18 gene caused by 3.5 MB deletion of 1q32-q44 region	Low	ID, DF, no speech,
Del 16p11.2	613444	16p11.2	Heterozygous deletion of a 220-kb region or extended 1.7 Mb deletion of chromosome 16p11.2 (approximately 9 genes).	Low	Severe early onset-obesity with developmental delay.
Dup 17q21-q23	613533	17q21.3	Unknown	Rare	DF, SS, microcephaly, hirsutism
Trisomy 21 syndrome	190685	21q22.3	Triplicate state of all or a portion of chromosome 21	Frequent	ID, hearing loss, congenital malformations.
Chromosome 2- Mosaic trisomy syndrome	600430	2q37.3	Caused by mutation in the HDAC4 gene on chromosome 2q37.2	Low	ID, SS, brachymetaphalangia
Single gene disorder					
Van der Woude syndrome	119300	1q32.2	Heterozygous mutation in the gene encoding interferon regulatory factor-6 (IRF6)	Rarely	DF. CL/P
Fronto-nasal dysplasia syndrome	136760	1p13.3	Homozygous mutation in the aristaless-like homeobox-3 gene	Low	DF
Pallister-Hall syndrome	146510	7p14.1	Heterozygous mutation in the GL13 gene	Low	DF, hypothalamic hamartoma, pituitary dysfunction
Waardenburg syndrome type 3 syndrome	148820	2q36.1	Heterozygous or homozygous mutation in the PAX3 gene	Frequent	DF, LD congenital sensorineural hearing loss
Visceral myopathy syndrome	155310	2p13.1	Heterozygous mutation in the ACTG2 gene	Occasional	Abnormal intestinal mobility, intestinal malrotation, malnutrition, gastrointestinal obstruction
MEN2B syndrome	162300	10q11.21	Heterozygous mutation in the RET gene. 95% with specific M918T mutation in exon 16 of the RET gene	Occasional	GR, marfanoid habitus, pheochromocytoma, aggressive medullary thyroid carcinoma
MEN2A syndrome	171400	10q11.21	Heterozygous mutation in the RET oncogene	Frequently	Medullary thyroid carcinoma, parathyroid adenomas, pheochromocytoma
Piebaldism syndrome	172800	4p12; 8q11.21	Heterozygous mutation in KIT proto-oncogene and sometimes in the gene encoding the zinc finger transcription factor SNAI2	Rare	Hypopigmentation, depigmentation and heterochromia of iris
Currarino syndrome	176450	7q36.3	Mutation in the HLXB9 homeobox gene	Low	Sickle-shaped sacrum, pre-sacral mass and anorectal malformation.
Rubinstein-Taybi syndrome	180849	16p13.3	Mutation in the gene encoding the transcriptional coactivator CREB-binding protein CREBBP	Low	ID, DF, LD, microcephaly,
Alveolar capillary dysplasia with misalignment of pulmonary veins syndrome	265380	16q24.1	Heterozygous mutation in the FOXF1 gene.	Rare	Respiratory, congenital cardiovascular, gastrointestinal genitourinary, and musculoskeletal system anomalies.
Werner mesomelic dysplasia syndrome	188740	7q36.3	Heterozygous mutation in an SHH regulatory element (ZRS) that resides in intron 5 of the LMBR1 gene	Rare	DF, LD

Table 1. Continued on next page

Table 1. Continued

Syndromes	MIM	Gene locus	Etiology	Association strength	Key features
Haddad syndrome	209880	4p13, 5p13.2, 10q11.21, 11p14.1, 12q23.2, 20q13.32	Mostly caused by heterozygous mutation in the PHOX2B gene. Rarely caused by mutations in RET, GDNF, EDN3, BDNF, CCHS and ASCL1	Frequent	abnormal control of respiration, neuroblastoma and ganglioneuroma
Bardet-Biedl syndrome	209900	11q13.2	Mutations in BBS1 gene	Occasional	DF, Obesity, hypogonadism, kidney dysfunction, behavioral problems
Riley-Day syndrome	223900	9q31.3	Mutation in IKBKAP gene	Rare	Autonomic nervous system anomalies
Mowat- Wilson syndrome	235730	2q22.3	*De novo* heterozygous mutation in ZEB2 gene	High	ID, DF, microcephaly, global developmental delay
Kauffman-McKusick syndrome	236700	20p12.2	Homozygous or compound heterozygous mutation in the MKKS gene	Occasional	DF, genitourinary malformations, hydrometrocolpos
Mabry syndrome	239300	1p36.11	Homozygous or compound heterozygous mutation in the PIGV gene	Occasional	ID, Various neurological abnormalities (seizures, hypotonia) variable degrees of brachytelephalangy
Cartilage- hair hypoplasia syndrome	250250	9p13.3	Mutations in the RMRP gene	Occasional	Short limb dwarfism, metaphyseal dysplasia immunodeficiency
Fukuyama congenital muscular dystrophy	253800	9q31.2	Homozygous or compound heterozygous mutation in gene encoding fukutin	Rare	ID,DF, Congenital muscular dystrophy, seizures, hydrocephalus
Multicore myopathy syndrome	255320	19q13.2	Homozygous or compound heterozygous mutation in the RYR1 gene	Rare	delayed motor development, generalized muscle weakness and amyotrophy
Osteopetrosis, autosomal recessive 1 syndrome	259700	11q13.2	Subnormal osteoclast function caused by homozygous or compound heterozygous mutation in the TCIRG1 subunit of the vacuolar proton pump	Rare	DF, respiratory and feeding problems, anal stenosis, blindness, deafness.
Persistent Müllerian duct syndrome	261550	12q13.13; 19p13.3	Heterozygous mutation in the gene encoding anti-mullerian hormone (AMH) or in the AMH receptor	Low	Inguinal hernias, uterus and fallopian tubes; bilateral cryptorchidism
Smith-Lemli-Opitz syndrome	270400	11q13.4	Homozygous or compound heterozygous mutation in gene encoding DHCR7	Occasional	ID, DF, LD, Microcephaly, hypospadias,
Spondylo-Epi-Metaphyseal Dysplasia with Joint Laxity (SEMDJL) syndrome	271640	1p36.33	Homozygous or compound heterozygous mutation in the B2GALT6 gene	Low	Vertebral abnormalities, ligamentous laxity, progressive severe kyphoscoliosis, respiratory compromise
Shah- Waardenburg syndrome	277580	13q22.3	Hetero or homozygous mutation in EDNRB	High	Auditory-Pigmentary anomalies.
Creatine transporter syndrome	300036	Xq28	x-linked recessive		Muscle weakness
Chronic idiopathic intestinal pseudo-obstruction syndrome	300048	Xq28	Mutation or duplication in the gene encoding filamin A	Occasional	Severe intestinal obstruction
Lesh- Nyhan syndrome	300322	Xq26.2-q26.3	Mutation in the HPRT gene enclosing hypoxanthine guanine phosphoribosyl- transferase	Low	ID, spastic cerebral palsy, choreoathetosis, uric acid urinary stones
Osteopathia striata with cranial sclerosis syndrome	300373	Xq11.2	mutation in the WTX gene (AMERI) on chromosome Xq11	Low	Osteopathia striata with cranial schlerosis
Hydrocephalus due to congenital Stenosis of Aqueduct of Sylvius syndrome	307000	Xq28	Mutation in gene encoding L1CAM	Occasional	ID, DF, Hydrocephalus, spastic paraparesis
Dermotrichic syndrome	308205	Xp22.12-p22.11	Mutation in the MBTPS2 gene	Occasional	Alopecia; ichthyosis; mental retardation; seizures

Table 1. Continued on next page

Table 1. Continued

Syndromes	MIM	Gene locus	Etiology	Association strength	Key features
Lenz Microphthalmia syndrome	309800	Xq28	Mutation in the NAA10 gene	Rare	Unilateral or bilateral microphthalmia or anophthalmia, and defects in the skeletal and genitourinary systems.
Goldberg-Shprintzen syndrome	609460	10q22.1	Mutation in the KIAA1279 gene	Frequent	HSCR, DF, gyral abnormalities of the brain, microcephaly, urogenital anomalies
Pitt-Hopkins syndrome	610954	18q21.2	Haplo-insufficiency of TCF4 transcription factor gene	Rare	ID, DF, intermittent hyperventilation with apnea
Jeune syndrome	208500	15q13	SRTD1 mapped to chromosome 15q13	Rare	Asphyxiating thoracic dystrophy
Susceptibility loci					
HSCR1 syndrome	142623	10q11.21;	RET gene	Frequent	absence of ganglion cells, anorectal or colonic malformation
HSCR2	600155	13q22.3	EDNRB- Isolated HSCR		
HSCR3	613711	5p13.2	GDNF gene, uncertain	Frequent	Colonic aganglionosis, malrotation of the gut
HSCR4	613712	20q13.32	Variations in EDN3, WS4B, HSCR4	Frequent	Absence of intrinsic ganglion cells in the myenteric and submucosal plexus of the gastrointestinal tract
HSCR5	600156	9q31	Uncertain, possible IKBKAP	Frequent	Absence of intrinsic ganglion cells in the myenteric and submucosal plexus of the gastrointestinal tract
HSCR6	606874	3p21	Uncertain	Frequent	Absence of intrinsic ganglion cells in the myenteric and submucosal plexus of the gastrointestinal tract
HSCR7	606875	19q12	Uncertain	Frequent	Absence of intrinsic ganglion cells in the myenteric and submucosal plexus of the gastrointestinal tract
HSCR8	608462	16q23	Suggested CDH13 and PLCG2	Frequent	Absence of intrinsic ganglion cells in the myenteric and submucosal plexus of the gastrointestinal tract
HSCR9	611644	4q31.3- q32.3	Uncertain	Frequent	Absence of intrinsic ganglion cells in the myenteric and submucosal plexus of the gastrointestinal tract
Molecularly unresolved phenotypes					
Aarskog syndrome	100050		Evidence of autosomal dominant inheritance	Low	DF, LD, SS, hypertelorism
Golden hair syndrome	164210	14q32	Unknown	Occasional	DF, cardiac anomalies, vertebral and central nervous system defects
Toriello-Carey syndrome	217980		Multiple congenital, unknown	Low	ID, DF, LD, agenesis of corpus callosum, cardiac defects
Black locks with albinism and deafness syndrome	227010		Unknown	Rare	Pigmentary disorder with hearing loss
Fryns syndrome	229850		Congenital, autosomal recessive	Low	DF, LD, diaphragmatic defects, absence of lung lobulation
Gastroschisis syndrome	230750		Unknown	Rare	Abdominal wall defects, herniation of abdominal contents and not covered by membrane.
HSCR syndromes with Limb anomalies	235740; 235750; 235760		Unknown	Mandatory	DF, deafness, hypertelorism, unilateral renal agenesis

Table 1. Continued on next page

Table 1. Continued

Syndromes	MIM	Gene locus	Etiology	Association strength	Key features
Visceral neuropathy syndrome	243180		Uncertain	Low	Intestinal obstruction, short small intestine, pyloric hypertrophy, absence of ongoing peristalsis
Intestinal pseudo-obstruction with patent ductus arteriosus and natal teeth syndrome	243185		Possible autosomal or X-linked recessive inheritance	Rare	Intestinal pseudo-obstruction, no passage of meconium, cardiac failure and vomiting of bile
Mutchinick syndrome	249630		Unknown	Rare	ID, DF, LD, heart and renal malformations
Clayton Smith syndrome	258840		Unknown	Rare	DF, Ichthyosis, failure to thrive
Pierre Robbin syndrome	261800	17q24.3-q25.1	Unknown	Rare	DF, Mandibular hypoplasia, obstructive apnea
Visceral myopathy syndrome	277320	8q23-8q24	Uncertain. Autosomal recessive	Low	Ptosis, opthalmoplegia, and progressive intestinal pseudo-obstruction
Hirschsprung with Type D brachydactyly	306980		X-linked recessive inheritance	Mandatory	Brachydactyly type D
Harrod syndrome	601095		Unknown	Rare	ID, DF, hypogenitalism, failure to thrive, small bowel
Yemite deaf-blind hypopigmentation syndrome	601706		Unknown, autosomal recessive inheritance	Rare	Hearing loss, patchy hypo- and hyperpigmentation, colobomata of the iris and choroidea
HD syndromes with limb anomalies	604211		Unknown	Mandatory	DF, heart defect, laryngeal anomalies, aganglionosis of entire colon
Ramos-Arroyo syndrome	122430		Uncertain, autosomal dominant inheritance		ID, DF, sensorineural deafness, persistent ductus arteriosus
Microgastria-limb reduction defects association syndrome	156810		Uncertain	Low	LD
Bone dysplasia, lethal, holmgren type Syndrome	211120		Uncertain, autosomal recessive inheritance	Low	Respiratory insufficiency, severe bone dysplasia
Widow's peak	314570		Unknown, suggested X-linked dominant inheritance	Low	DF, LD, prominent widows peak, bilateral ptosis
Melhem-Fahl syndrome			Unknown	Rare	Extra ribs and vertebrae, HSCR, anal atresia
Congenital segmental dialatation of the colon				Rare	Abdominal distension, respiratory distress, cyanosis, structural dysmorphism
Duodenal atresia	?		Unknown		
Ectodermal dysplasia Syndrome	?				
H-Family syndrome			Autosomal recessive inheritance	Ichthyosis; MR; aminoaciduria	
Hirschsprung-malrotation syndrome			Unknown	Mandatory	
Hyperphosphatasia syndrome		1p	Unknown	Rarely	ID, anal anomalies, nail hypoplasia
Lissencephaly with HSCR syndrome		12q12	Mutation in TUBA1A gene		
Maternal hyperthermia syndrome			Environmental		
Megacystis syndrome		12q13	Unknown	Rare	Megacolon malrotation
Pseudo-obstruction (gut) and neurogenic bladder syndrome			Mitochondrial, autosomal recessive inheritance		
Rhombence-phalosynapsis			Sporadic with autosomal recessive inheritance	Rare	HSCR anal abnormalities

DF, dysmorphic features; HSCR, Hirschprung's disease; ID, intellectual disability; LD, limb dysmorphic features; SS, short stature.

Table 2. Epidemiology and sex-dependent recurrence risk in Hirschsprung disease

Sex of proband	Sex of sibling	Proband phenotype	Recurrence risk %	Penetrance %	Genetic model
		L-HSCR	17	52	Dominant
	Female	S-HSCR	5	17	Multifactorial or recessive
Male		L-HSCR	13	40	Dominant
	Male	S-HSCR	1	4	Multifactorial or recessive
		L-HSCR	33	52	Dominant
	Female	S-HSCR	5	17	Multifactorial or recessive
Female		L-HSCR	9	40	Dominant
	Male	S-HSCR	3	4	Multifactorial or recessive

Table 3. miRNA studies to date exploring involvement of miRNA in HSCR

Study (Ref.)	Year	Cohort	Method	Expression/association results	Conclusions
Tang *et al.* (129)	2013	miR-141 in 70 HSCR tissues and 60 controls.	RT-PCR, western blot, MTT assay, and flow cytometry	↓miR-141, ↑CD47, ↑CUL3 in HSCR patients ($P < 0.05$)	Aberrant reduction of miR-141 may play an important role in the pathogenesis of HSCR with the inhibiting affection on cell migration and proliferation abilities.
Mi *et al.* (128)	2014	50 HSCR patients, miR-124 and Sox9		↑miRNA in stenotic colon	miR-124 and target gene SOX9 are over expressed in the stenotic colon
Zhou *et al.* (121)	2013	73 pairs of human colon/ rectal tissue specimens, including stenotic HSCR and dilated HSCR vs. normal tissue	qRT-PCR to detect MeCP2 and miR-34b. Western blot	↓ MeCP2 in HSCR tissues. miRNA-34b expression was unaffected	Decreased levels of MeCP2 may play an important role in the pathogenesis of HSCR suppressing proliferation.
Zhu *et al.* (119)	2014	254 HSCR cases vs. 265 controls	SNP Genotyping (rs2910164, rs11614913) qRT-PCR to detect exp levels of miRNA146a and miR-196a2	↓ GG and CC ROBO1 genotypes (rs2910164) of pre-mi-R-146a associated with HSCR ($P < 0.005$ OR, 1.54; 95% CI, 1.06-2.23)	ROBO1 downregulation may affect cell proliferation and migration of NCC
Li *et al.* (120)	2014	88 HSCR patients and 75 controls	qRT-PCR and Western blot	↓ miR-200a & miR141 correlated with ↑ PTEN mRNA and protein	The miR200 family may play a crucial role in the pathogenesis of HSCR by coregulating PTEN.
Lei *et al.* (123)	2014	Colon tissue from 78 HSCR patients, 66 controls	Cell counting kit-8 (cck-8), miR-195	↑miR-195 in HSCR patients ($P < 0.05$)	Aberrant expression of miR-195 may be involved in the pathogenesis of HSCR by downregulating the level of DIEXF.
Sharan *et al.* (122)	2015	14 HSCR; 29 non-HSCR		↓of miR206 in HSCR patients compared to controls ($P < 0.05$)	miR206 suppresses cell immigration and proliferation and silencing of SDPR could rescue the extent of the suppressing effects by miR-206 inhibitor.
Tang *et al.* (125)	2015	69 HSCR (42 S-HSCR, 27 L-HSCR); 49 controls	qRT-PCR; Western blot; Cell proliferation, cell cycle and apoptosis, transwell, dual luciferase reporter assays	↑miR-218-1; ↑ SLIT2; ↓ RET, PLAG1	SLIT2 overexpression inhibited cell migration via binding to its receptor, ROBO1
Zhu *et al.* (124)	2015	80 HSCR; 77 controls	qRT-PCR; Western blot	↓mi-R-192 in HSCR ($P = 0.0001$); ↑NID1	Potential decrease in cell proliferation and migration

HSCR, Hirschprung's disease; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; RT-PCR, Real-Time Polymerase-Chain Reaction.

suggesting further genetic heterogeneity (40,41). To the best of our knowledge, only four EDN3 mutations have been found in families with WS4, including three being homozygous for the mutation and one harboring a heterozygous missense mutation in addition to heterozygous mutations of SOX10 identified in 4 out of 15 patients (40,42,43). There is growing evidence for Alu-mediated deletions in noncoding regions around SOX10 as a recent mechanism involved in WS4 pathogenesis (44).

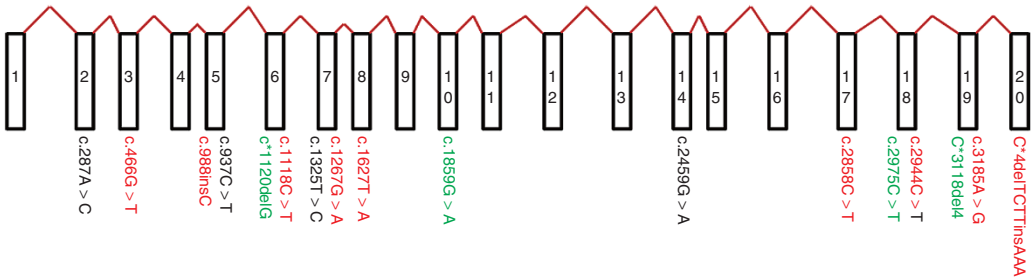

Figure 2. Impact of mutations on Ret gene: unknown/unavailable (black), short segment (red), and long segment (green) (see also text).

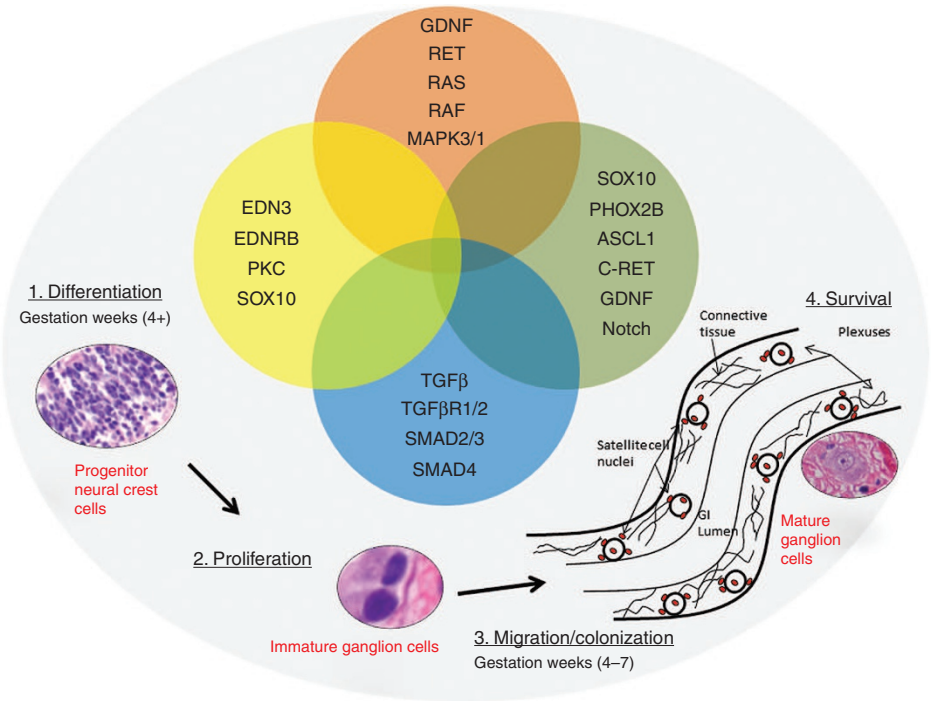

Figure 3. Schematic representation of functionally related-genes that appear to play a key role in the differentiation of NCCs providing a framework of subnetworks related to signal transduction during four stages of embryogenesis in ENS development (see text).

Haddad syndrome (HS; MIM 209880) is a genetic syndrome characterized by congenital central hypoventilation syndrome (CCHS, MIM 209880) and HSCR occurs in about 1/5 of CCHS individuals. There is no sex predilection and patients with HS have more often L-HSCR or TCA (45–47). Mutations in PHOX2B (paired-like homeobox 2b), a transcription factor involved in the development of noradrenergic neurons, has been extensively demonstrated in CCHS, a rare disorder with autonomic nervous system dysregulation and/or tumors of NC origin (48–50). In some cases of syndromic neuroblastoma (MIM 256700) that can present with CCHS or HSCR, heterozygous PHOX2B mutations have also been found (51). Two types of mutations are observed in CCHS: polyalanine repeat expansion mutations (PARM; normal range 22–33 repeats), and nonpolyalanine repeat expansion mutations (NPARM) which typically are out-of-frame deletions/duplications of variable size (1 to 38 nucleotides) (52). It has been shown that individuals harboring a heterozygous 20/27 PARM genotype are at increased risk for HSCR, and nearly all patients with NPARM have HSCR (53–56). Functional studies support PHOX2B as a rare cause of HSCR (33), and a hypomorphic HSCR RET predisposing allele can also be a risk factor for the HSCR phenotype in CCHS and of particular importance are issues related to genetic counseling including germline and somatic mosaicism, as well as late-onset disorder (36).

Goldberg-Shprintzen syndrome (GOSHS; MIM 609460) is an AR multiple congenital anomaly syndrome characterized by moderate intellectual disability, microcephaly, cleft palate, ocular colobomas, and a recognizable pattern of facial dysmorphisms (32,47,57). An abnormal neuronal migration including polymicrogyria has been observed. GOSHS is caused by a homozygous truncating mutation in the *KIAA1279* gene, which encodes KIF-binding protein (KBP) on chromosome 10q21.1, a protein of poorly understood function and it has been suggested that the GOSHS phenotype may result from defects in development of both the ENS and central nervous system (CNS) (58). GOSHS patients usually have truncating homozygous *KIAA1279* mutations (p.Arg90X, p.Ser200X or

p.Arg202IlefsX2) leading to nonsense-mediated mRNA decay and loss of KBP function. KBP expression directly affected neurite growth in the human neuroblastoma SH-SY5Y cell line, in keeping with the central (polymicrogyria) and enteric (HSCR) neuronal developmental defects seen in GOSHS patients, providing the first evidence that an actin-microtubule cross-linking protein may be involved in neuronal development in humans.

Another genetic condition somewhat similar to GOSHSD is Mowat-Wilson syndrome (MIM 235730), an AD disorder with a wide spectrum of multiple congenital anomalies, caused by a *de novo* mutation in the *ZFHX1B* (zinc finger homeobox 1B) on chromosome 2q22 (59–62). Individuals with MOWS generally present with global developmental delay, epilepsy, and congenital anomalies including brain, eye, and heart defects with some patients having HSCR. Heterozygous *de novo* deletions encompassing the *ZFHX1B* gene or truncating mutations within the gene have been found in over 100 MOWS cases (24). The *ZFHX1B* gene encodes Smad-interacting protein-1 (SMADIP1 or SIP1), a transcriptional repressor involved in the TGFβ signaling pathway that is widely expressed during embryonic development. Studies of mutant mice that have lost *ZFHX1B* demonstrate defects of melanocyte and ENS development and loss of vagal NCCs (63). *ZFHX1B* appears to be a susceptibility gene for syndromic rather than isolated HSCR.

In addressing genetic counseling, sporadic HSCR should be considered to be a multigenic and sex-modified trait with a variable pattern of inheritance and an overall 4% recurrence risk in siblings of the proband (relative risk = 200) (24) (**Table 2**). Genetic studies revealed that there is an elevated risk to relatives in terms of heritability in comparison to the general population. There is a higher incidence of HSCR in some individuals of Chinese descent versus other populations and accepted figures include an incidence of 1.0, 1.5, 2.2, 2.8 per 10,000 live births in Hispanics, Caucasian-Americans, African-Americans and Asians, respectively (24,64,65). Furthermore, this recurrence risk is dependent on the sex of both the affected individuals and the relative (**Table 3**). The sex bias exhibited in HSCR depends on the length of the aganglionic segment, and is manifested in the observation that both incidence and penetrance is 2–4-fold higher in males. Another hallmark of this complex disorder is that both penetrance of known mutations and the compatible models of inheritance vary with the presence or absence of associated syndromic features and the highest recurrence risk should be for a male sibling of a female proband with L-HSCR (66). Recurrence risk in genetic conditions with HSCR following Mendelian patterns of inheritance as the few examples mentioned above follows the respective pattern (AD 50%, AR 25%). Taking into account various intricacies of HSCR etiology, this can make genetic counseling complicated. Even when a specific mutation has been identified in a family, predicting whether S- or L-HSCR will develop, or whether HSCR is found in the context of syndromic or nonsyndromic conditions, can often be challenging.

GENES AND PATHWAYS INVOLVED IN SPORADIC HSCR

The complex genetic etiology, which entangles HSCR, is intriguing and linked with mutations in the genes that encode mostly signaling molecules crucial for the proper development of the ENS. The most important genes and pathways that have been investigated include: Glial cell-derived pathway genes (*RET, GDNF, NTN, SOX10, PHOX2b*), Endothelin pathway genes (*EDN3, EDNRB, SOX10*), and TGFβ signaling pathway genes (*ZFHX1B*) (12,48,67,68). These genes seem to perform distinctly with *RET* and *EDNRB* using the receptor tyrosine kinase (RTK) and G-protein-coupled receptor (GPCR) signal-transduction pathways (69). The incidence and severity of intestinal aganglionosis is influenced by potentially multiple interactions between known HSCR associated genes. The mechanism behind these interactions is not yet fully known, but Ret and Ednrb might interact by activating common downstream signaling molecules. Other than genetic interactions, it may be important to emphasize that the incomplete penetrance and variation among families affected with HSCR suggest the involvement of modifier gens.

RET SIGNALING PATHWAY

RET, a proto-oncogene, encoding for a RTK, is the major and most extensively studied gene implicated in HSCR pathogenesis (12,48,70–74). Loss of function mutations seem to be most commonly seen in patients with familial HSCR than sporadic HSCR cases and in individuals with L-HSCR rather than S-HSCR. There are more than 100 unique RET changes in families with HSCR including large deletions encompassing the RET gene, microdeletions and insertions, nonsense, missense, and splicing mutations (75). The first susceptibility locus was mapped to 10q11.2 in a group of multigenerational families segregating HSCR as an incompletely penetrant AD trait, while interstitial deletion at chromosome 10q11 in TCA with intellectual disability made this region a hot spot for biochemical studies (24). Epigenetic changes of *RET* have also been described (76,77). Linkage analysis has shown that in 90% of HSCR families, the colonic phenotype is linked to the RET locus (78–81); however, most familial HSCR cases that show a *RET* locus linkage fail to reveal coding-sequence mutations (82,83). Transmission disequilibrium testing has demonstrated that various *RET* polymorphisms and haplotypes at polymorphic loci are associated with HSCR (84). Functional analysis of HSCR-associated *RET* promoter single-nucleotide polymorphisms (SNPs) shows a reduction of *RET* transcription in the presence of the respectively associated alleles (85). Different SNPs of coding, noncoding regions including conserved enhancer-like sequence in intron 1, and the promoter of *RET* have been identified to increase HSCR susceptibility several fold when compared to control cases in different ethnic groups (86). The *RET RTK* comprises a signal peptide, a CYS-rich region, a transmembrane region, a conserved intracellular TK-catalytic domain, and an extracellular domain (**Figure 2**). *RET* is expressed through the developing nervous system and following activation by glial cell-derived neurotrophic factor (GDNF) family ligands, *RET* mediates signals through a range

of pathways including: RAS/ERK, p38MAPK, NF-κB, PI3/AKT, and JNK, driving cell proliferation, survival, differentiation, migration, and apoptosis, under the support of a glycosyl-phosphatidyl-inositol-linked GDNF coreceptor-α (GFRα1) (78). The development and maintenance of both central and peripheral neurons are linked to specific combinations of proteins that signal through RET, including four related glycosyl-phosphatidyl-inositol-linked coreceptors GFRA1-4 and four soluble growth factor ligands of RET: GDNF, neurturin (NTN), persephin (PSPN), and artemin (ARTN). Interestingly, Tang *et al.* investigated a Han Chinese population with *RET* mutations and noted an HSCR association with *IKBKAP* suggesting population specificity (87,88). *RET* in neural crest development was elucidated by the expression pattern of *RET* during mouse embryogenesis and the phenotype of *Ret* null mice ($Ret^{-/-}$) (89–91). $Ret^{-/-}$ mice demonstrate pyloric stenosis, a dilation of the proximal bowel, and an empty urinary bladder, although early stages of RET-positive vagal NCC migration seem to be RET signaling independent.

GDNF SIGNALING PATHWAY

GDNF acts in conjunction with RET, but is considered a rare susceptibility HSCR gene (<5%) (12,80,92). GDNF and neurturin (NTN) are two structurally related neurotrophic factors that play crucial roles in the control of survival and differentiation of neurons. GDNF family receptor alpha 1 (*GFRA1*) has been shown to interact with *GDNF* and *RET*. GDNF and GFRA1 interact in a heterotetrameric complex with *RTK* and *RET* (12,80). $Gdnf^{-/-}$ and $Gfr\alpha^{-/-}$ mice demonstrate aganglionotic gut with peristaltic failure. $Gdnf^{+/-}$ mice have frequent obstruction of the lower intestine and, in the absence of GDNF signaling, deficits in the initial appearance of NCC can be traced in the gut anlagen. In humans, *GDNF* germline mutations have been found in combination with *RET* mutations (93–96).

ENDOTHELIN SIGNALING PATHWAY (EDNRB)

The endothelins are vasoactive molecules, which make a family of 21 amino acid isopeptides (EDN1, EDN2, and EDN3) with each molecule containing two intra-chain disulphide bonds encoded by a separate gene (97). Mature and active endothelins are produced by the actions of endothelin-converting enzymes (ECE). So far, there are at least four known endothelin receptors, all of which are G protein-coupled receptors whose activation result in elevation of intracellular-free calcium. The final action is the constriction or relaxation of the smooth muscles of the blood vessels, raising or lowering the blood pressure, among other functions. Endothelin receptor type B (EDNRB) is coded by *EDNRB*, which is located on 13q22 and is nonselective with two types of EDNRB arising from the same gene. Mutations in the EDNRB gene are associated with ABCD syndrome (an acronym for albinism, black lock, neuronal migration disorder of the gut, and sensorineural deafness) and some forms of Waardenburg syndrome (40). EDNRB is rapidly desensitized by phosphorylation by the GPCR kinase type 2 once a ligand is bound, followed by internalization via a clathrin-dependent pathway, and transfer to the lysosomal compartment. In ontogenesis, *EDNRB* is expressed in the neural tube before the initiation of NCC migration and continues to be expressed by ENS precursors as they begin to migrate (98). *EDN3* and Endothelin Converting Enzyme 1 (*ECE1*) are expressed by the mesenchyme surrounding the neural tube, along the dorsal migration pathway of the melanoblasts and in the developing gut mesoderm. Enteric EDN3 expression, however, is highest in the embryonic cecum and enhances the proliferation-promoting effects of GDNF on the ENS progenitors and coordinated interaction between RET and EDRB signaling pathways controls the development of the ENS (99) (**Figure 3**). The EDNRB knockout mouse (*Ednrb*-/-) and piebald-lethal mouse are almost completely lacking coat color, do not survive to adulthood, and have megacolon (91,100,101). By comparison, Edn3 (*Edn3*-/-, lethal-spotting) mouse is pigmented over about 1/3 of the body, and only about 15% survive to adulthood. The *Ece*1-deficient mouse lacks enteric neurons and choroidal/epidermal melanocytes, and presents with a phenotype very similar to the *EDNRB* and *EDN3* knockout mice. In humans, HSCR screening for *EDNRB* pathway associated genes has shown mutations of *EDNRB*, *EDN3*, and *ECE1* accounting for about 5% of HSCR cases. A heterozygous *ECE-1* mutation has occurred in a patient with HSCR and accompanied with craniofacial and cardiac defects (102). However, both *EDN3* and *EDNRB* homozygous and heterozygous mutations have been reported in Waardenburg syndrome type 4 and dosage changes may be modifiers of the *EDNRB* pathway.

SOX10

The SOX family of transcription factors is characterized by the presence of a DNA binding high-mobility group domain, and is involved in a wide range of developmental processes (43,45,103–106). SOX10 (Sry bOX10) has a potent transcription activating domain at its C-terminus that functions through two important types of DNA response elements both binding SOX10 monomers and favoring SOX10 dimers with DNA. Transcriptional activation by SOX10 is dependent on cooperating partners that are currently being identified. SOX10 is indeed expressed in NC cells as they leave the neural tube and expression continues during their migration. Although NCC formation may be normal in the absence of SOX10 and does not seem to be required for early migration, SOX10 is crucial for their survival. An *in vitro* study by Kulbrodt *et al.* analyzed the effect of these mutations on SOX10 function and concluded that each mutation likely leads to functional inactivation the protein. The heterozygous Dom mouse with *Sox10* mutation has aganglionosis and hypopigmentation, while the $Sox10^{-/-}$ embryo is lethal. Mutations in *SOX10* that result in the loss of the *trans* activating domain or that disrupt protein–protein interactions with PAX3 result in Waardenburg syndrome types 2 (WS2) without HSCR (71). *SOX10* mutations, likely resulting in haploinsufficiency, are present in some cases with WS4 (43), and are only rarely associated with isolated HSCR, probably not exceeding more than 5% of HSCR cases, while mutations in evolutionarily conserved regulatory elements of

SOX10 have been shown to be the cause of isolated, previously unexplained HSCR.

INTERACTION OF RET-GDNF, EDNRB, AND SOX10 SIGNALING

The complex process of how ENS development leads to adequate function of the gastrointestinal tract is far from being understood. Multiple signaling pathways, briefly summarized above, have been shown to interact in this intricate process (**Figure 3**). EDNRB and SOX10 are components of signaling cascades that are critical to the development of the ENS together with RET-GDNF signaling. Carter *et al.* (66) suggest intriguing relationships between RET candidate genes (HOXB5 and PHOX2B) and *RET* expression. The helix loop helix (HLH) transcription factor *Ascl1* retards the differentiation of myenteric neural cells in the intestine of mouse embryos toward ganglionic differentiation and induces *Ret* expression and neurogenesis in cell cultures of NC stem cells (66). Disruption of the adhesion molecule *L1cam* and the transcription factors *Hoxb5* and *Phox2b* results in the delay or failure of migration of ENCCs to the distal intestine of murine embryos. *Sox10* regulates the expression of both EDNRB and RET receptors, and it also modulates the expression of EDNRB expression in NC-derived cells as they approach the cecum—an area rich for ligands for EDN3 and GDNF. Interestingly, GDNF-induced proliferation is enhanced by EDN3, whereas GDNF-induced migration is inhibited by EDN3. Thus, a complex balance between RET and EDRB activation may allow for significant expansion of the NC stem cell pool in the cecum while affecting migration, which is dependent on cell–cell contact. Additionally, by reducing the attraction of NC cells to GDNF, EDN3 may allow the cells to migrate beyond the cecum. EDN3/EDNRB exhibits a complex interaction with RTK signaling with developmental-stage specific effects. It is intimately involved in RET signaling in the ENS lineage. Haploinsufficiency for SOX10 appears to interfere with the development and survival of ENS precursors due to its role as an important transcriptional regulator of several other genes known to be important in ENS development. SOX10 is also reported to regulate RET expression synergistically with PAX3 and SRY binds to the promoter of the *RET* gene at its both enhancer regions by interacting with transcription factors including PAX3 and NKX2-1 (107,108). SRY can inhibit RET expression generating haploinsufficiency of *RET* suppressing its function in ENS development. **Figure 3** highlights functionally related genes that are associated with HSCR, providing a framework of subnetworks that are related to signal transduction during the formation and migration of ganglion cells in ENS development and genes associated with the GDNF and the EDNRB pathway interact with genes of the SOX10 signaling pathway, which play a key role in the differentiation of NCCs.

SEMAPHORIN (SEMA) SIGNALING

Semaphorins are a group of proteins involved in signaling, characterized by the presence of a semaphorin domain, *SEMA*, at the N terminal (109). Semaphorins have an integral role in the migratory pathway of NCC during ENS development, involved in proliferation, migration, and differentiation (89). Class 3 semaphorin receptors include neuropilins 1 and 2 for binding and plexins, coreceptors for signaling. A cluster of SEMA SNPs was identified by GWAS, with allelic effects independent of RET and *RET* mutations may occur in patients carrying *SEMA3* variants were noted (89,110,111). Not only did these mutations suggest a pathogenic effect on the disease, but the coexistence with RET mutations also substantiated the additive genetic model that has been proposed for the rarer forms of the disease (112,113). Recently, semaphorin 3C/3D signaling has been suggested to be an evolutionarily conserved regulator of the ENS development (114). Luzon-Toro *et al.* studying semaphorin class 3 genes through SNP analysis and by next generation sequencing technologies, associated *SEMA3A* (7p12.1) and *SEMA3D* (7q21.11) in the pathogenesis of a subset of S-HSCR (115). It seems that increased SEMA3A expression is a risk factor for HSCR through the upregulation of the gene in the aganglionic smooth muscle layer of the colon of HSCR individuals. Finally, SEMA3A polymorphisms have been discovered in different ethnic backgrounds (Caucasian, Northeastern Chinese, and Thai) (113).

GENE ONTOLOGY STUDIES AND MIRNAS

A recent international study cohort of 162 S-HSCR trios genotyped and analyzed using the Transmission Disequilibrium Test (TDT) by PLINK software confirmed a strong association of gene ontology (GO) modules related to signal transduction and regulation of ENS formation as well as other processes related to the disease (116). Their results revealed a clear association of GO terms connected to Ras signaling, a pathway known to play a key role in ENS formation. This network of 53 genes provides a current hypothesis in the context of genomic studies for the genetic complexity of HSCR (48,117). Some of the most interesting genes and networks affected that appear to be significantly associated with HSCR based on GO modules include: *CRK*, related to regulation of small GTPase mediated signal transduction and Ras protein signal transduction; *GRB2*, related to Ras protein signal transduction; *ITGB1*, related to cell–cell adhesion, cell migration, cell projections and neuron development; *PLCG1*, related to cell migration; *RPS27AP16*, related to synaptic transmission, cell projection organization and neuron development; *SH3GL3* and *TP53* which in the context of HSCR are related to CNS development. Overall, GO biological processes significantly associated with HSCR strongly support that HSCR is caused in different cell populations by specific genes belonging to the same (or related) GO modules; and these gene modules carry out biological functions that are essential to both neurogenesis and signaling.

MicroRNAs (miRNAs or miRs) are small, noncoding RNA molecules that are about 19–25 nucleotides long that regulate cell differentiation, proliferation, migration, and apoptosis (118–129). MiRs regulate target gene functions by triggering mRNA degradation or translational repression through complementary binding to the 3'-untranslated regions of target

mRNA. However, their role in HSCR is yet to be clearly defined; so, a better understanding of miRNAs during ENCCs development is necessary. In the last couple of years, several miRNA targets have been proposed to play a role into the pathogenesis of HSCR including: SLIT2/ROBO1, MeCP2, NID1, SDPR, CD47/CUL3, SOX9, PTEN, and DIEXF (**Table 3**). New methodologies and techniques are currently discovering new horizons in genetics and molecular biology and this will affect our understanding of this very complex disease.

FUTURE PERSPECTIVES

New avenues for therapies for ENS disorders provided from recent advances in molecular and stem cell biology have led to the development of a unique ENS stem cell field (6,12,23,130) and genomic-wide association studies (GWAS) are an incredible resource with potential identification of candidate genes, which may need to be further investigated in the nearest future (6). The gene hunting has been galvanized by new molecular biology technologies including next-generation sequencing (NGS), conditional and cell-lineage-specific animal models, and stem cell biology providing an astonishing resource for investigating the early human development, etiology and progression of HSCR and defining the interaction of signaling pathways in detail. The platform of patient specific induced pluripotent stem cells will exquisitely dissect disease heterogeneity widening the perspectives introduced by whole-genome sequencing (6). The better risk prediction and development of stratified surgical approaches for some subsets of patients will be key for tailoring a personalized medical approach and bowel transplantation for short-gut syndrome may harbor novel strategies (131). In the future, stem cell-based therapeutic approaches may become available to children with TCA. HSCR remains a complex congenital anomaly and is still mysterious over a hundred years since its first report.

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REFERENCES

1. Kalcheim C, Langley K, Unsicker K. From the neural crest to chromaffin cells: introduction to a session on chromaffin cell development. Ann N Y Acad Sci 2002;971:544–6.
2. Gershon MD. Transplanting the enteric nervous system: a step closer to treatment for aganglionosis. Gut 2007;56:459–61.
3. Gershon MD, Ratcliffe EM. Developmental biology of the enteric nervous system: pathogenesis of Hirschsprung's disease and other congenital dysmotilities. Semin Pediatr Surg 2004;13:224–35.
4. Gershon MD. *The Second Brain: A Groundbreaking New Understanding of Nervous Disorders of the Stomach and Intestine*. New York City, New York: Harper Perennial; 1999.
5. Jacobson S, Marcus EM. *Neuroanatomy for the Neuroscientist*. 2nd edn. Heidelberg, Germany: Springer; 2011.
6. Tam PK. Hirschsprung's disease: a bridge for science and surgery. J Pediatr Surg 2016;51:18–22.
7. Keyzer-Dekker CM, Sloots CE, Schokker-van Linschoten IK, Biermann K, Meeussen C, Doukas M. Effectiveness of rectal suction biopsy in diagnosing Hirschsprung disease. Eur J Pediatr Surg 2016;26:100–5.
8. Takawira C, D'Agostini S, Shenouda S, Persad R, Sergi C. Laboratory procedures update on Hirschsprung disease. J Pediatr Gastroenterol Nutr 2015;60:598–605.
9. Schäppi MG, Staiano A, Milla PJ, et al. A practical guide for the diagnosis of primary enteric nervous system disorders. J Pediatr Gastroenterol Nutr 2013;57:677–86.
10. Fu M, Tam PK, Sham MH, Lui VC. Embryonic development of the ganglion plexuses and the concentric layer structure of human gut: a topographical study. Anat Embryol (Berl) 2004;208:33–41.
11. Sergi C. Hirschsprung's disease: Historical notes and pathological diagnosis on the occasion of the 100(th) anniversary of Dr. Harald Hirschsprung's death. World J Clin Pediatr 2015;4:120–5.
12. Schriemer D, Sribudiani Y, IJpma A, et al. Regulators of gene expression in Enteric Neural Crest Cells are putative Hirschsprung disease genes. Dev Biol 2016;416:255–65.
13. Coe A, Avansino JR, Kapur RP. Distal rectal skip-segment Hirschsprung disease and the potential for false-negative diagnosis. Pediatr Dev Pathol 2016;19:123–31.
14. Moore SW. Total colonic aganglionosis and Hirschsprung's disease: a review. Pediatr Surg Int 2015;31:1–9.
15. Friedmacher F, Puri P. Classification and diagnostic criteria of variants of Hirschsprung's disease. Pediatr Surg Int 2013;29:855–72.
16. Erten EE, Çavuşoğlu YH, Arda N, et al. A rare case of multiple skip segment Hirschsprung's disease in the ileum and colon. Pediatr Surg Int 2014;30:349–51.
17. Raghunath BV, Shankar G, Babu MN, et al. Skip segment Hirschsprung's disease: a case report and novel management technique. Pediatr Surg Int 2014;30:119–22.
18. Moore SW, Sidler D, Schubert PA. Segmental aganglionosis (zonal aganglionosis or "skip" lesions) in Hirschsprungs disease: a report of 2 unusual cases. Pediatr Surg Int 2013;29:495–500.
19. Do MY, Myung SJ, Park HJ, et al. Novel classification and pathogenetic analysis of hypoganglionosis and adult-onset Hirschsprung's disease. Dig Dis Sci 2011;56:1818–27.
20. O'Donnell AM, Puri P. Skip segment Hirschsprung's disease: a systematic review. Pediatr Surg Int 2010;26:1065–9.
21. Ballard ET. Ultrashort segment Hirschsprung's disease: a case report. Pediatr Pathol Lab Med 1996;16:319–25.
22. Neilson IR, Yazbeck S. Ultrashort Hirschsprung's disease: myth or reality. J Pediatr Surg 1990;25:1135–8.
23. Burns AJ, Goldstein AM, Newgreen DF, et al. White paper on guidelines concerning enteric nervous system stem cell therapy for enteric neuropathies. Dev Biol 2016; pii: S0012-1606(16)30111–7. doi: 10.1016/j.ydbio.2016.04.001 (Epub ahead of print).
24. Amiel J, Sproat-Emison E, Garcia-Barcelo M, et al.; Hirschsprung Disease Consortium. Hirschsprung disease, associated syndromes and genetics: a review. J Med Genet 2008;45:1–14.
25. Tam PK, Garcia-Barceló M. Genetic basis of Hirschsprung's disease. Pediatr Surg Int 2009;25:543–58.

26. Amiel J, Lyonnet S. Hirschsprung disease, associated syndromes, and genetics: a review. J Med Genet 2001;38:729–39.
27. Badner JA, Chakravarti A. Waardenburg syndrome and Hirschsprung disease: evidence for pleiotropic effects of a single dominant gene. Am J Med Genet 1990;35:100–4.
28. Reissmann M, Ludwig A. Pleiotropic effects of coat colour-associated mutations in humans, mice and other mammals. Semin Cell Dev Biol 2013;24:576–86.
29. Asim A, Kumar A, Muthuswamy S, Jain S, Agarwal S. "Down syndrome: an insight of the disease". J Biomed Sci 2015;22:41.
30. Arnold S, Pelet A, Amiel J, et al. Interaction between a chromosome 10 RET enhancer and chromosome 21 in the Down syndrome-Hirschsprung disease association. Hum Mutat 2009;30:771–5.
31. de Pontual L, Pelet A, Clement-Ziza M, et al. Epistatic interactions with a common hypomorphic RET allele in syndromic Hirschsprung disease. Hum Mutat 2007;28:790–6.
32. Moore SW. Chromosomal and related Mendelian syndromes associated with Hirschsprung's disease. Pediatr Surg Int 2012;28:1045–58.
33. Moore SW, Zaahl MG. Multiple endocrine neoplasia syndromes, children, Hirschsprung's disease and RET. Pediatr Surg Int 2008;24:521–30.
34. Alberti L, Carniti C, Miranda C, Roccato E, Pierotti MA. RET and NTRK1 proto-oncogenes in human diseases. J Cell Physiol 2003;195:168–86.
35. Alves MM, Sribudiani Y, Brouwer RW, et al. Contribution of rare and common variants determine complex diseases-Hirschsprung disease as a model. Dev Biol 2013;382:320–9.
36. Hansford JR, Mulligan LM. Multiple endocrine neoplasia type 2 and RET: from neoplasia to neurogenesis. J Med Genet 2000;37:817–27.
37. Pelet A, Attie T, Goulet O, et al. De-novo mutations of the RET proto-oncogene in Hirschsprung's disease. Lancet 1994;344:1769–70.
38. Pelet A, de Pontual L, Clément-Ziza M, et al. Homozygosity for a frequent and weakly penetrant predisposing allele at the RET locus in sporadic Hirschsprung disease. J Med Genet 2005;42:e18.
39. Pelet A, Geneste O, Edery P, et al. Various mechanisms cause RET-mediated signaling defects in Hirschsprung's disease. J Clin Invest 1998;101:1415–23.
40. Doubaj Y, Pingault V, Elalaoui SC, et al. A novel mutation in the endothelin B receptor gene in a moroccan family with shah-waardenburg syndrome. Mol Syndromol 2015;6:44–9.
41. Mahmoudi A, Rami M, Khattala K, Elmadi A, Afifi MA, Youssef B. Shah-Waardenburg syndrome. Pan Afr Med J 2013;14:60.
42. Lecerf L, Kavo A, Ruiz-Ferrer M, et al. An impairment of long distance SOX10 regulatory elements underlies isolated Hirschsprung disease. Hum Mutat 2014;35:303–7.
43. Pingault V, Ente D, Dastot-Le Moal F, Goossens M, Marlin S, Bondurand N. Review and update of mutations causing Waardenburg syndrome. Hum Mutat 2010;31:391–406.
44. Bondurand N, Fouquet V, Baral V, et al. Alu-mediated deletion of SOX10 regulatory elements in Waardenburg syndrome type 4. Eur J Hum Genet 2012;20:990–4.
45. Szymońska I, Borgenvik TL, Karlsvik TM, et al. Novel mutation-deletion in the PHOX2B gene of the patient diagnosed with neuroblastoma, Hirschsprung's disease, and congenital central hypoventilation syndrome (NB-HSCR-CCHS) cluster. J Genet Syndr Gene Ther 2015;6:.
46. Majumdar S, Wood P. Congenital central hypoventilation syndrome (CCHS) with Hirschsprung disease (Haddad syndrome): an unusual cause of reduced baseline variability of the fetal heart rate. J Obstet Gynaecol 2009;29:152–3.
47. Shahar E, Shinawi M. Neurocristopathies presenting with neurologic abnormalities associated with Hirschsprung's disease. Pediatr Neurol 2003;28:385–91.
48. Fernández RM, Bleda M, Luzón-Toro B, et al. Pathways systematically associated to Hirschsprung's disease. Orphanet J Rare Dis 2013;8:187.
49. Weese-Mayer DE, Berry-Kravis EM, Ceccherini I, Rand CM. Congenital central hypoventilation syndrome (CCHS) and sudden infant death syndrome (SIDS): kindred disorders of autonomic regulation. Respir Physiol Neurobiol 2008;164:38–48.
50. Gershon MD. NPARM in PHOX2B: why some things just should not be expanded. J Clin Invest 2012;122:3056–8.
51. Trochet D, O'Brien LM, Gozal D, et al. PHOX2B genotype allows for prediction of tumor risk in congenital central hypoventilation syndrome. Am J Hum Genet 2005;76:421–6.
52. Berry-Kravis EM, Zhou L, Rand CM, Weese-Mayer DE. Congenital central hypoventilation syndrome: PHOX2B mutations and phenotype. Am J Respir Crit Care Med 2006;174:1139–44.
53. Heide S, Masliah-Planchon J, Isidor B, et al. Oncologic phenotype of peripheral neuroblastic tumors associated with PHOX2B non-polyalanine repeat expansion mutations. Pediatr Blood Cancer 2016;63:71–7.
54. Shimokaze T, Sasaki A, Meguro T, et al. Genotype-phenotype relationship in Japanese patients with congenital central hypoventilation syndrome. J Hum Genet 2015;60:473–7.
55. Armstrong AE, Weese-Mayer DE, Mian A, et al. Treatment of neuroblastoma in congenital central hypoventilation syndrome with a PHOX2B polyalanine repeat expansion mutation: New twist on a neurocristopathy syndrome. Pediatr Blood Cancer 2015;62:2007–10.
56. Weese-Mayer DE, Rand CM, Berry-Kravis EM, et al. Congenital central hypoventilation syndrome from past to future: model for translational and transitional autonomic medicine. Pediatr Pulmonol 2009;44:521–35.
57. Isidor B, Lefebvre T, Le Vaillant C, et al. Blepharophimosis, short humeri, developmental delay and hirschsprung disease: expanding the phenotypic spectrum of MED12 mutations. Am J Med Genet A 2014;164A:1821–5.
58. Drévillon L, Megarbane A, Demeer B, et al. KBP-cytoskeleton interactions underlie developmental anomalies in Goldberg-Shprintzen syndrome. Hum Mol Genet 2013;22:2387–99.
59. Kilic E, Cetinkaya A, Utine GE, Boduroğlu K. A diagnosis to consider in intellectual disability: Mowat-Wilson syndrome. J Child Neurol 2016;31:913–7.
60. Murray SB, Spangler BB, Helm BM, Vergano SS. Polymicrogyria in a 10-month-old boy with Mowat-Wilson syndrome. Am J Med Genet A 2015;167A:2402–5.
61. Ferris Villanueva E, Guerrero Bautista R, Chica Marchal A. Hirschsprung disease associated with Mowat-Wilson syndrome: report of a case. Nutr Hosp 2015;31:1882–4.
62. Buraniqi E, Moodley M. ZEB2 gene mutation and duplication of 22q11.23 in Mowat-Wilson syndrome. J Child Neurol 2015;30:32–6.
63. Kam MK, Cheung MC, Zhu JJ, et al. Perturbation of Hoxb5 signaling in vagal and trunk neural crest cells causes apoptosis and neurocristopathies in mice. Cell Death Differ 2014;21:278–89.
64. Zhang Z, Jiang Q, Li Q, et al. Genotyping analysis of 3 RET polymorphisms demonstrates low somatic mutation rate in Chinese Hirschsprung disease patients. Int J Clin Exp Pathol 2015;8:5528–34.
65. Xu C, Chen P, Xie H, et al. Associations between CYP2B6 rs707265, rs1042389, rs2054675, and Hirschsprung disease in a Chinese population. Dig Dis Sci 2015;60:1232–5.
66. Carter TC, Kay DM, Browne ML, et al. Hirschsprung's disease and variants in genes that regulate enteric neural crest cell proliferation, migration and differentiation. J Hum Genet 2012;57:485–93.
67. Heanue TA, Pachnis V. Enteric nervous system development and Hirschsprung's disease: advances in genetic and stem cell studies. Nat Rev Neurosci 2007;8:466–79.
68. Griseri P, Vos Y, Giorda R, et al. Complex pathogenesis of Hirschsprung's disease in a patient with hydrocephalus, vesico-ureteral reflux and a balanced translocation t(3;17)(p12;q11). Eur J Hum Genet 2009;17:483–90.
69. Kawai H, Satomi K, Morishita Y, et al. Developmental markers of ganglion cells in the enteric nervous system and their application for evaluation of Hirschsprung disease. Pathol Int 2014;64:432–42.
70. Badner JA, Sieber WK, Garver KL, Chakravarti A. A genetic study of Hirschsprung disease. Am J Hum Genet 1990;46:568–80.
71. Lesueur F, Corbex M, McKay JD, et al. Specific haplotypes of the RET proto-oncogene are over-represented in patients with sporadic papillary thyroid carcinoma. J Med Genet 2002;39:260–5.
72. Seri M, Yin L, Barone V, et al. Frequency of RET mutations in long- and short-segment Hirschsprung disease. Hum Mutat 1997;9:243–9.

73. Pasini B, Ceccherini I, Romeo G. RET mutations in human disease. Trends Genet 1996;12:138–44.
74. Romeo G, Ronchetto P, Luo Y, et al. Point mutations affecting the tyrosine kinase domain of the RET proto-oncogene in Hirschsprung's disease. Nature 1994;367:377–8.
75. Kashuk CS, Stone EA, Grice EA, et al. Phenotype-genotype correlation in Hirschsprung disease is illuminated by comparative analysis of the RET protein sequence. Proc Natl Acad Sci USA 2005;102:8949–54.
76. Vitale G, Dicitore A, Messina E, Sciammarella C, Faggiano A, Colao A. Epigenetics in medullary thyroid cancer: from pathogenesis to targeted therapy. Recent Pat Anticancer Drug Discov 2016;11:275–82.
77. Tang CS, Tang WK, So MT, et al. Fine mapping of the NRG1 Hirschsprung's disease locus. PLoS One 2011;6:e16181.
78. Borrego S, Ruiz-Ferrer M, Fernández RM, Antiñolo G. Hirschsprung's disease as a model of complex genetic etiology. Histol Histopathol 2013;28:1117–36.
79. Sánchez-Mejías A, Fernández RM, López-Alonso M, Antiñolo G, Borrego S. Contribution of RET, NTRK3 and EDN3 to the expression of Hirschsprung disease in a multiplex family. J Med Genet 2009;46:862–4.
80. Fernandez RM, Ruiz-Ferrer M, Lopez-Alonso M, Antiñolo G, Borrego S. Polymorphisms in the genes encoding the 4 RET ligands, GDNF, NTN, ARTN, PSPN, and susceptibility to Hirschsprung disease. J Pediatr Surg 2008;43:2042–7.
81. Borrego S, Ruiz A, Saez ME, et al. RET genotypes comprising specific haplotypes of polymorphic variants predispose to isolated Hirschsprung disease. J Med Genet 2000;37:572–8.
82. Bolk S, Pelet A, Hofstra RM, et al. A human model for multigenic inheritance: phenotypic expression in Hirschsprung disease requires both the RET gene and a new 9q31 locus. Proc Natl Acad Sci USA 2000;97:268–73.
83. Carrasquillo MM, McCallion AS, Puffenberger EG, Kashuk CS, Nouri N, Chakravarti A. Genome-wide association study and mouse model identify interaction between RET and EDNRB pathways in Hirschsprung disease. Nat Genet 2002;32:237–44.
84. Emison ES, McCallion AS, Kashuk CS, et al. A common sex-dependent mutation in a RET enhancer underlies Hirschsprung disease risk. Nature 2005;434:857–63.
85. Zhang XN, Zhou MN, Qiu YQ, Ding SP, Qi M, Li JC. Genetic analysis of RET, EDNRB, and EDN3 genes and three SNPs in MCS + 9.7 in Chinese patients with isolated Hirschsprung disease. Biochem Genet 2007;45:523–7.
86. Emison ES, Garcia-Barcelo M, Grice EA, et al. Differential contributions of rare and common, coding and noncoding Ret mutations to multifactorial Hirschsprung disease liability. Am J Hum Genet 2010;87:60–74.
87. Cheng WW, Tang CS, Gui HS, et al. Depletion of the IKBKAP ortholog in zebrafish leads to hirschsprung disease-like phenotype. World J Gastroenterol 2015;21:2040–6.
88. Tang CS, Sribudiani Y, Miao XP, et al. Fine mapping of the 9q31 Hirschsprung's disease locus. Hum Genet 2010;127:675–83.
89. Jiang Q, Arnold S, Heanue T, et al. Functional loss of semaphorin 3C and/or semaphorin 3D and their epistatic interaction with ret are critical to Hirschsprung disease liability. Am J Hum Genet 2015;96:581–96.
90. Uesaka T, Enomoto H. Neural precursor death is central to the pathogenesis of intestinal aganglionosis in Ret hypomorphic mice. J Neurosci 2010;30:5211–8.
91. Roberts RR, Bornstein JC, Bergner AJ, Young HM. Disturbances of colonic motility in mouse models of Hirschsprung's disease. Am J Physiol Gastrointest Liver Physiol 2008;294:G996–G1008.
92. Gui H, Tang WK, So MT, et al. RET and NRG1 interplay in Hirschsprung disease. Hum Genet 2013;132:591–600.
93. Gabriel SB, Salomon R, Pelet A, et al. Segregation at three loci explains familial and population risk in Hirschsprung disease. Nat Genet 2002;31:89–93.
94. Doray B, Salomon R, Amiel J, et al. Mutation of the RET ligand, neurturin, supports multigenic inheritance in Hirschsprung disease. Hum Mol Genet 1998;7:1449–52.
95. Salomon R, Attié T, Pelet A, et al. Germline mutations of the RET ligand GDNF are not sufficient to cause Hirschsprung disease. Nat Genet 1996;14:345–7.
96. McCallion AS, Emison ES, Kashuk CS, et al. Genomic variation in multigenic traits: Hirschsprung disease. Cold Spring Harb Symp Quant Biol 2003;68:373–81.
97. Stewart DR, von Allmen D. The genetics of Hirschsprung disease. Gastroenterol Clin North Am 2003;32:819–37, vi.
98. Barlow A, de Graaff E, Pachnis V. Enteric nervous system progenitors are coordinately controlled by the G protein-coupled receptor EDNRB and the receptor tyrosine kinase RET. Neuron 2003;40:905–16.
99. Nagy N, Goldstein AM. Endothelin-3 regulates neural crest cell proliferation and differentiation in the hindgut enteric nervous system. Dev Biol 2006;293:203–17.
100. Martucciello G, Ceccherini I, Lerone M, Jasonni V. Pathogenesis of Hirschsprung's disease. J Pediatr Surg 2000;35:1017–25.
101. Stobdan T, Zhou D, Ao-Ieong E, et al. Endothelin receptor B, a candidate gene from human studies at high altitude, improves cardiac tolerance to hypoxia in genetically engineered heterozygote mice. Proc Natl Acad Sci USA 2015;112:10425–30.
102. Yanagisawa H, Yanagisawa M, Kapur RP, et al. Dual genetic pathways of endothelin-mediated intercellular signaling revealed by targeted disruption of endothelin converting enzyme-1 gene. Development 1998;125:825–36.
103. Bondurand N, Sham MH. The role of SOX10 during enteric nervous system development. Dev Biol 2013;382:330–43.
104. Lake JI, Heuckeroth RO. Enteric nervous system development: migration, differentiation, and disease. Am J Physiol Gastrointest Liver Physiol 2013;305:G1–24.
105. Butler Tjaden NE, Trainor PA. The developmental etiology and pathogenesis of Hirschsprung disease. Transl Res 2013;162:1–15.
106. Pusch CM, Sasiadek MM, Blin N. Hirschsprung, RET-SOX and beyond: the challenge of examining non-mendelian traits (Review). Int J Mol Med 2002;10:367–70.
107. 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 2015;24:685–97.
108. Bondurand N, Pingault V, Goerich DE, et al. Interaction among SOX10, PAX3 and MITF, three genes altered in Waardenburg syndrome. Hum Mol Genet 2000;9:1907–17.
109. Abuetabh Y, Tivari S, Chiu B, Sergi C. Semaphorins and their significance in cancer. Austin J Clin Pathol 2014;1:1–5.
110. Luzón-Toro B, Fernández RM, Torroglosa A, et al. Mutational spectrum of semaphorin 3A and semaphorin 3D genes in Spanish Hirschsprung patients. PLoS One 2013;8:e54800.
111. Jiang Q, Turner T, Sosa MX, Rakha A, Arnold S, Chakravarti A. Rapid and efficient human mutation detection using a bench-top next-generation DNA sequencer. Hum Mutat 2012;33:281–9.
112. Wang LL, Zhang Y, Fan Y, et al. SEMA3A rs7804122 polymorphism is associated with Hirschsprung disease in the Northeastern region of China. Birth Defects Res A Clin Mol Teratol 2012;94:91–5.
113. Wang LL, Fan Y, Zhou FH, et al. Semaphorin 3A expression in the colon of Hirschsprung disease. Birth Defects Res A Clin Mol Teratol 2011;91:842–7.
114. Phusantisampan T, Sangkhathat S, Phongdara A, Chiengkriwate P, Patrapinyokul S, Mahasirimongkol S. Association of genetic polymorphisms in the RET-protooncogene and NRG1 with Hirschsprung disease in Thai patients. J Hum Genet 2012;57:286–93.
115. Luzón-Toro B, Espino-Paisán L, Fernández RM, Martín-Sánchez M, Antiñolo G, Borrego S. Next-generation-based targeted sequencing as an efficient tool for the study of the genetic background in Hirschsprung patients. BMC Med Genet 2015;16:89.
116. Liu Y, Lee YF, Ng MK. SNP and gene networks construction and analysis from classification of copy number variations data. BMC Bioinformatics 2011;12 Suppl 5:S4.
117. 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 USA 2009;106:2694–9.

118. Tang W, Cai P, Huo W, et al. Suppressive action of miRNAs to ARP2/3 complex reduces cell migration and proliferation via RAC isoforms in Hirschsprung disease. J Cell Mol Med 2016;20:1266–75.
119. Zhu H, Cai P, Zhu D, et al. A common polymorphism in pre-miR-146a underlies Hirschsprung disease risk in Han Chinese. Exp Mol Pathol 2014;97:511–4.
120. Li H, Tang J, Lei H, et al. Decreased MiR-200a/141 suppress cell migration and proliferation by targeting PTEN in Hirschsprung's disease. Cell Physiol Biochem 2014;34:543–53.
121. Zhou Z, Qin J, Tang J, et al. Down-regulation of MeCP2 in Hirschsprung's disease. J Pediatr Surg 2013;48:2099–105.
122. Sharan A, Zhu H, Xie H, et al. Down-regulation of miR-206 is associated with Hirschsprung disease and suppresses cell migration and proliferation in cell models. Sci Rep 2015;5:9302.
123. Lei H, Tang J, Li H, et al. MiR-195 affects cell migration and cell proliferation by down-regulating DIEXF in Hirschsprung's disease. BMC Gastroenterol 2014;14:123.
124. Zhu D, Xie H, Li H, et al. Nidogen-1 is a common target of microRNAs MiR-192/215 in the pathogenesis of Hirschsprung's disease. J Neurochem 2015;134:39–46.
125. Tang W, Tang J, He J, et al. SLIT2/ROBO1-miR-218-1-RET/PLAG1: a new disease pathway involved in Hirschsprung's disease. J Cell Mol Med 2015;19:1197–207.
126. Rogler LE, Kosmyna B, Moskowitz D, et al. Small RNAs derived from lncRNA RNase MRP have gene-silencing activity relevant to human cartilage-hair hypoplasia. Hum Mol Genet 2014;23:368–82.
127. Tang W, Li H, Tang J, et al. Specific serum microRNA profile in the molecular diagnosis of Hirschsprung's disease. J Cell Mol Med 2014;18:1580–7.
128. Mi J, Chen D, Wu M, Wang W, Gao H. Study of the effect of miR-124 and the SOX9 target gene in Hirschsprung's disease. Mol Med Rep 2014;9:1839–43.
129. Tang W, Qin J, Tang J, et al. Aberrant reduction of MiR-141 increased CD47/CUL3 in Hirschsprung's disease. Cell Physiol Biochem 2013;32:1655–67.
130. 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 2016;409:473–88.
131. Dicken BJ, Sergi C, Rescorla FJ, Breckler F, Sigalet D. Medical management of motility disorders in patients with intestinal failure: a focus on necrotizing enterocolitis, gastroschisis, and intestinal atresia. J Pediatr Surg 2011;46:1618–30.