Pathogenesis of Hirschsprung's Disease and its Variants: Recent Progress

By Prem Puri and Toko Shinkai
Dublin, Ireland

The enteric nervous system (ENS) is a complex network of interconnected neurons within the wall of the intestine that controls intestinal motility, regulates mucosal secretion and blood flow, and also modulates sensation from the gut. The cells that form the ENS in mammals are derived primarily from vagal neural crest cells. During the past decade there has been an explosion of information about genes that control the development of neural crest. Molecular-genetic analysis has identified several genes that have a role in the development of Hirschsprung's disease. The major susceptibility gene is RET, which is also involved in multiple endocrine neoplasia type 2. Recently, genetic studies have provided strong evidence in animal models that intestinal neuronal dysplasia (IND) is a real entity. HOX11L1 knockout mice and endothelin B receptor-deficient rats demonstrated abnormalities of the ENS resembling IND type B in humans. These findings support the concept that IND may be linked to a genetic defect.

HIRSCHSPRUNG'S DISEASE (HSCR) IS a relatively common cause of intestinal obstruction in the newborn. It is characterized by absence of ganglion cells in the distal bowel and extending proximally for varying distances. The aganglionosis is confined to rectosigmoid in 75% of patients; the sigmoid, splenic flexure, or transverse colon in 17%; and total colon along with a short segment of terminal ileum in 8% of patients.[1,2] Total intestinal aganglionosis with absence of ganglion cells from duodenum to the rectum is the most rare form of HSCR.[3,4] The incidence of HSCR is estimated to be 1 in 5000 live births.[5,6] The disease is more common in boys, with a male to female ratio of 4:1.[6,7] The male preponderance is less evident in long segment HSCR, where the male-to-female ratio is 1.5-2:1.[6,8,9]

HSCR occurs as an isolated trait in 70% of cases.[10] A chromosomal abnormality is associated with HSCR in 12% of cases, trisomy 21 being by far the most frequent (>90%). Associated congenital anomalies are found in 18% of HSCR patients and include gastrointestinal malformations, cleft palate, cardiac malformations, craniofacial anomalies, and polydactyly.[10] Other chromosomal abnormalities that have been described in association with HSCR include interstitial deletion of distal 13q, partial deletion of 2p, reciprocal translation, and trisomy 18 mosaic.[1] A number of unusual hereditary syndromes have been reported in patients with HSCR. These include Shah-Waardenburg syndrome, multiple endocrine neoplasia (MEN) type 2 syndromes, congenital central hypoventilation syndrome (Ondine's curse), Goldberg-Shprintzen syndrome, Mukusick-Kauffman syndrome, Bardet-Biedl syndrome, Smith-Lemli-Opitz syndrome, Cartilage-hair hypoplasia syndrome, and syndromes with HSCR and distal limbs anomalies.[10]

Intestinal neuronal dysplasia (IND) is the most commonly encountered variant of HSCR.[11-14] It was first described by Meier-Ruge[15] in 1971 as a malformation of the enteric plexus. In 1983, Fadda et al[16] subclassified IND into two clinically and histologically distinct subtypes. Type A occurs in less than 5% of cases, is characterized by congenital aplasia or hypoplasia of the sympathetic innervation, and presents acutely in the neonatal period with episodes of intestinal obstruction, diarrhea, and bloody stools. The clinical picture of type B resembles HSCR disease and is characterized by malformation of the parasympathetic submucous and myenteric plexuses and accounts for over 95% of cases of isolated IND. These children have intractable constipation and grossly slowed intestinal transit time. IND occurring in association with HSCR is of type B.[17,18] The characteristic histologic features of IND B include hypoganglionosis of the submucous and myenteric plexuses, giant ganglia, ectopic ganglion cells, and increased acetylcholinesterase activity in the lamina propria and around submucosal blood vessels.[19-21]

The incidence of isolated IND has varied from 0.3% to 40% of all rectal suction biopsies in different centers.[22-25] IND immediately proximal to a segment of aganglionosis is not uncommon and often presents as persistent obstructive symptoms after a pull-through operation for HSCR.[17] Some investigators have reported that 25% to 35% of patients with HSCR have associated IND.[16,17,26] However, others have rarely encountered IND in associated with HSCR.[22] The uncertainty regarding the incidence of IND has resulted from the considerable confusion regarding the essential diagnostic criteria. Many investigators have raised doubts about the existence of IND as a distinct histopathologic entity.[27-29] It has been suggested that the pathologic changes seen in IND may be part of normal development or may be a secondary phenomenon induced by congenital obstruction and inflammatory disease.[28,30] One strong piece of evidence

From Children's Research Centre, Our Lady's Hospital for Sick Children, University College Dublin, Ireland.
Address reprint requests to Prem Puri, Children's Research Centre, Our Lady's Hospital for Sick Children, Crumlin, Dublin 12, Ireland.

1055-8586/04/1301-0004$30.00/0
doi:10.1053/j.sempedsurg.2003.09.004

that IND is a real entity stems from animal models. Recently, two different *Hox11L1* knockout mouse models have been generated.[31,32] In both cases, homozygous mutant mice were viable, but megacolon developed at the age of 3 to 5 weeks. Histological and immunohistochemical analyses showed hyperplasia of myenteric ganglia, a phenotype similar to that observed in IND. More recently Von Boyen et al[33] reported abnormalities of the enteric nervous system in heterozygous endothelin B receptor (EDNRB)-deficient rats resembling IND in humans. They showed that a heterozygous 301 bp deletion of the EDNRB gene led to abnormalities of the submucous plexus, such as giant ganglia, hyperganglionosis, and hypertrophy of the nerve fiber strands. These findings support the concept that IND may be linked to a genetic defect.

DEVELOPMENT OF THE ENTERIC NERVOUS SYSTEM

The enteric nervous system (ENS) is the largest and the most complex division of the peripheral nervous system.[34,35] The ENS contains more neurons than the spinal cord and is responsible for the coordination of normal bowel motility and secretory activities.[36] As our understanding of the ENS improves, it becomes clear that it is no longer sufficient to simply determine whether enteric ganglion cells are present. We are learning the importance of determining whether the correct number and types of ganglion cells are present. This is further complicated by the fact that the morphology of the myenteric plexus varies with the age as well as location of the gastrointestinal tract.[37] For many years, the problem of neural crest (NC) development was addressed primarily at the cellular level, using avian embryos as an experimental system. The past few years have seen an explosion of information about genes that control the development of NC cell.[36]

The embryonic NC arises in the neural tube, originating with the central nervous system, but NC cells detach from this tissue via reduction of cell-cell and cell-matrix adhesion. The epithelio-mesenchymal transformation allows NC cells to migrate along pathways of defined routes to various tissues, where they stop moving and differentiate into various cell types. Pathway selection is most likely achieved by balanced combinations of molecules that promote and reduce adhesion.[12,38] NC cells give rise to neuronal, endocrine and paraendocrine, craniofacial, conotruncal heart, and pigmentary tissues. Neurocristopathies encompass tumors, malformations, and single or multiple abnormalities of tissues mentioned above in various combinations.[10]

In the human fetus, NC-derived cells first appear in the developing esophagus at the 5th week of gestation, and then migrate down to the anal canal in a cranio-caudal direction during the 5th to 12th week of gestation. The NC cells first form the myenteric plexus just outside the circular muscle layer. The mesenchymally derived longitudinal muscle layer then forms, sandwiching the myenteric plexus after it has been formed in the 12th week of gestation. In addition, after the craniocaudal migration has ended, the submucous plexus is formed by the neuroblasts, which migrate from the myenteric plexus across the circular muscle layer and into the submucosa; this progresses in a cranio-caudal direction during the 12th to 16th week of gestation.[12] The absence of ganglion cells in HSCR has been attributed to a failure of migration of NC cells. The earlier the arrest of migration, the longer the aganglionic segment is.

It is generally accepted that the enteric ganglion cells are derived primarily from the vagal NC cells.[39-42] Studies in the avian system provide strong evidence for the contribution of the sacral NC to the hindgut ENS.[43-45] Whether the sacral NC contributes to the ENS in the mammalian hindgut is less clear. Failure of the vagal-derived NC cells to colonize the hindgut results in failure of hindgut ENS development, suggesting that interaction between sacral and vagal enteric NC cells may be necessary for sacral NC cell contribution to the ENS.[36]

GENES INVOLVED IN ENS DEVELOPMENT AND HSCR

Genetic factors have been implicated in the aetiology of HSCR. HSCR is known to occur in families. The reported incidence of familial cases in rectosigmoid HSCR varied from 3.6% to 7.8% in different series.[1] A familial incidence of 15% to 21% has been reported in total colonic aganglionosis and 50% in the rare total intestinal aganglionosis.[4,46] Schiller et al[47] reported 22 infants belonging to four families from Gaza, who had either documented or clinically suspected HSCR. Of these infants, 13 underwent laparotomy and multiple intestinal biopsies, 10 had total intestinal aganglionosis, I had total colonic aganglionosis, I had near total colonic aganglionosis, and only 1 had rectosigmoid HSCR. Engum et al[48] reported 20 cases of HSCR in 12 kindreds. The level of aganglionosis was rectal or rectosigmoid in eight cases, left colon in two, transverse or right colon in two and total colonic ganglionosis with variable small bowel involvement in eight.

Recurrence risk to siblings is dependant upon the sex of the person affected and the extent of aganglionosis. Badner et al[9] calculated the risk of HSCR transmission to relatives and found that the recurrence risk to siblings increase as the aganglionosis becomes more extensive (Table 1). The brothers of patients with rectosigmoid HSCR have a higher risk (4%) than sisters (1%). Much higher risks are observed in cases of long segment

Table 1. Recurrence Risk to Siblings

Relative	Recurrence Risk (%)
Brothers of patients with rectosigmoid HSCR	4
Sisters of patients with rectosigmoid HSCR	1
Brothers of female with long segment HSCR	24
Sons of females with long segment HSCR	29

HSCR. The brothers and sons of affected females have a 24% and 29% risk of being affected, respectively.

Recently, several genes have been identified that control morphogenesis and differentiation of the ENS. These genes, when mutated or deleted, interfere with ENS development.[10,36,49,50] So far, nine genes are known to be involved in HSCR in humans (Table 2).

RET/GDNF/GFRα1 SIGNALING SYSTEM

This signaling pathway is of importance for subpopulations of both peripheral and central neurons, having been shown by in vitro and in vivo assays to promote survival of neurons, mitosis of neuronal progenitor cells, differentiation of neurons and neurite extension.[34,51,52] The RET receptor is the signaling component of receptor complexes with four ligands, glial-derived neurotropic factor (GDNF), neurturin (NTN), artemin (ATM), and persephin (PSP)[34,36] (Fig 1). The complete receptor complex includes the RET receptor tyrosine kinase and a glycosylphosphatidylinositol-anchored binding component (GFRα1, GFRα2, GFRα3, and GFRα4).[36,53,54] These act as specific binding components such that RET/GFRα1 binds GDNF, RET/GFRα2 binds NTN, RET/GFRα3 binds ATM, and RET/GFRα4 binds PSP. In vivo the absence of GDNF/GFRα1-mediated signaling leads to the failure of ENS development, whereas absence of NTN/GFRα2 mediated signaling leads to more subtle abnormalities in ENS development.[36]

The importance of RET in mammalian organogenesis has been further illustrated by the generation of RET knockout mice.[55] These mice exhibit total intestinal aganglionosis and renal agenesis. The RET proto-oncogene has been demonstrated to be a major gene causing HSCR.[56-60] Mutations of RET account for 50% of familial and 15% to 20% of sporadic cases of HSCR.[60,61] Mutation screening of this gene in familial and sporadic HSCR patients resulted in the detection of over 90 mutations, including missense, nonsense, and deletion/insertion mutations. These mutations are scattered throughout the gene, and have no particular hot spots. In addition, mutations occur at higher incidence in long segment HSCR, compared with short segment HSCR in both familial and sporadic patients.[10,62] Total aganglionosis occurring throughout the digestive tract observed in RET knockout mice appears to reflect a close association between RET mutations and long segment HSCR in humans.[56,63] Germline point mutations of RET are also responsible for the inheritance of MEN type 2 cancer syndromes, which are usually divided into three different clinical subtypes: MEN2A, MEN2B, and familial medullary thyroid carcinoma (FMTC).[10,61,64] MEN2A, MEN2B, and FMTC are autosomal dominant cancer syndromes. Both MEN2A and FMTC can be associated with HSCR in some families. Because up to 5% of patients with HSCR also have MEN2A or FMTC, it is argued whether all patients with HSCR regardless of a family history, should be screened for RET exon 10 and 11 mutations to rule out cancer predisposition.[10]

The development of the ENS is dependent upon the actions of GDNF, which stimulates the proliferation and survival of NC-derived precursor cells in the embryonic gut.[65-68] It has been reported that GDNF is the ligand of RET. Mice carrying homozygous null mutation in GDNF have been generated, and these mice demonstrate the lack of kidneys and ENS, confirming the crucial role of GDNF in the development of the ENS.[70,71] Although a causative role for GDNF mutations in some patients with HSCR has been suggested, the occurrence of such cases is uncommon, and it is more likely that the GDNF

Table 2. Gene Mutations Associated with Hirschsprung's Disease

Gene	Locus	Function	Frequency in Humans	Animal Homologues
RET	10q11.2	Tyrosin kinase receptor	70-80% long segment 50% familial 15-20% sporadic	ret $-/-$
GDNF	5p12-13.1	Glial cell derived neurotropic factor	$<10\%$	gdnf $-/-$
NTN	19q13.3	Neurturin, RET ligand	$<1\%$	ntn $-/-$
GFRα1	10q26	GDNF family receptor alpha 1	–	gfrα $-/-$
EDN3	20q13	Endothelin-B	$<10\%$	etr3 $-/-$, lethal spotted
EDNRB	13q22	Endothelin-B-receptor	$<10\%$	ednrB $-/-$ Piebal lethal
ECE-1	1p36.1	Endothelin converting enzyme	$<1\%$	ece 1 $-/-$
SOX10	22q13.1	Sry/HMG box transcription factor	$<1\%$	Sox 10^{DOM}, Dom
Phox2b	4p12	Paired-like homeobox 2b	$<1\%$	–
Pax3	2q35	Paired box gene 3	–	Pax3 $-/-$
SIP1	2q22	Siah-interacting protein	6 cases	–

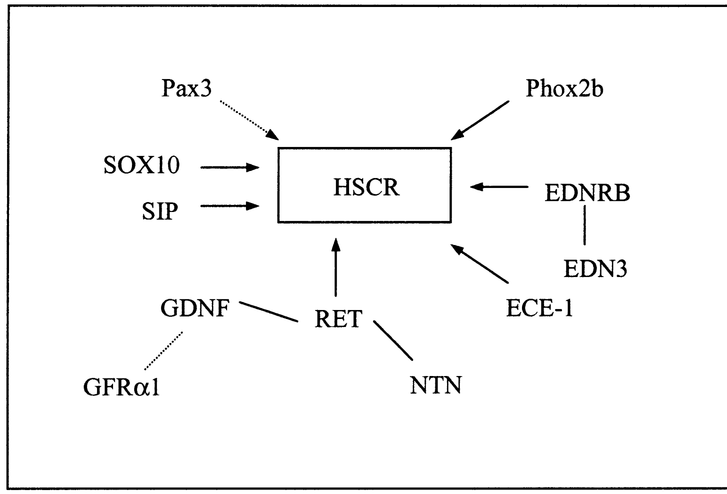

Fig 1. Genes involved in the pathogensis of Hirschsprung disease.

mutations are involved in modulation of the HSCR phenotype via its interaction with other susceptibility loci such as RET.[10,72] Shen and coworkers described hypoganglionosis in gdnf+/− mice.[73]

ENDOTHELIN SIGNALING PATHWAY

The endothelins (EDN1, EDN2, and EDN3) are intercellular local messengers that act via cell surface receptors, EDNRA and EDNRB.[34] EDNRA shows high affinity binding for EDN1 and does not bind EDN3 at physiologic concentrations, whereas EDNRB accepts all these ligands with high affinity.[36] EDN is initially produced as an inactive preproendothelin that undergoes two proteolytic steps to produce an active peptide. The first cleavage produces inactive big endothelins, and these are finally cleaved by a specific protease, endothelin-converting enzyme (ECE) to produce biologically active EDN.[10,34,36] Two ECE genes have been described, ECE1 and ECE2.[10]

EDN3 and EDNRB have a role in the migration and development of the ENS.[74-76] In mice in which the EDN3 or EDNRB gene was disrupted, intestinal aganglionosis was demonstrated experimentally. Furthermore, in natural mutants exhibiting aganglionic colon (piebald lethal and lethal spotted mice), a deletion of the entire EDNRB gene and a point mutation of the EDN3 gene have been confirmed respectively.[76,77] Although EDNRB binds all these endothelins, the similarity of phenotype of the EDNRB knockout mice suggests that EDNRB's major ligand is EDN3 in NC-derived cells.

EDNRB and EDN3 mutations have been described in isolated HSCR and Waardenburg-Shah syndrome.[78-84] EDNRB or EDN3 mutations have been demonstrated in less than 10% of cases of HSCR.[10,61,79] However, a 50% reduction in the EDN3 expression in the ganglionic and aganglionic segment of sporadic HSCR patients in the absence of mutations in the EDN3 gene has been reported.[85,86] This suggests that the downregulation of EDN3 expression may play a role in the pathogenesis of HSCR in the sporadic cases.

ECE1 knockout mice show craniofacial and cardiac abnormalities in addition to colonic aganglionosis.[87] A heterozygous ECE1 mutation has been identified in a patient with HSCR who also had craniofacial and cardiac defects.[88]

SOX10

A comparative study of human/mouse sequences led to the identification of a new member of the SRY/Sry-like, high mobility group box gene family, SOX10. It is expressed by ENS precursors before and throughout colonization of the gut mesenchyme.[36] The involvement of SOX10 in the development of enteric neurons was demonstrated in the Dom (Dominant megacolon) mouse model of HSCR.[89,90] SOX10, Dom/+ mice exhibit distal intestinal aganglionosis, they die shortly after birth and they are a naturally occurring model of HSCR. Mutations in SOX10 have been identified as a cause of the dominant megacolon mouse and Waardenberg-Shah syndrome in humans, both of which include defects in the ENS (distal intestinal aganglionosis) and pigmentation abnormalities.[91]

PHOX2B

Phox2B gene is a homeodomain-containing transcription factor that is involved in neurogenesis and regulates RET expression in mice,[92] in which disruption of the Phox2B gene results in a HSCR-like phenotype.[93] Enteric Phox 2B expression begins in vagal and truncal NC-derived cells as they invade the foregut mesenchyme and is contained in the adult submucosal and myenteric

Table 3. Gene Mutations Associated with Intestinal Neuronal Dysplasia

Gene	Locus	Function	Frequency in Humans	Animal Homologues
EDNRB	13q22	Endothelin-B-receptor	–	ednrB −/− Piebal lethal
HOX11L1	2p12-p13	T-cell leukemia, homeobox 2	–	Enx −/−

plexus.[36,93] Recently, Garcia-Bercelo et al reported that Phox2B A-G_{1364} polymorphism may predispose to HSCR.[94]

GENES ASSOCIATED WITH INTESTINAL NEURONAL DYSPLASIA

IND type B (INDB) is often encountered in children presenting with intractable constipation and grossly abnormal intestinal transit time and ENS abnormalities.[11-15] Many investigators have raised doubts about the existence of IND as a distinct histopathologic entity.[27-29] It has been suggested that the pathologic changes seen in IND may be part of normal development or may be secondary phenomenon induced by congenital obstruction and inflammatory disease.[28,30] Recently, strong evidence has emerged from animal models that suggests that INDB is a real entity (Table 3).

HOX11L1

Hox11L1 is a homebox gene involved in peripheral nervous system development and is reported to play a role in the proliferation or differentiation of NC cell lines. Two different Hox11L1 knockout mouse models have been generated.[31,32] In both cases, homozygous mutant mice were viable but developed megacolon at the age of 3 to 5 weeks. Histological and immunohistochemical analysis showed hyperplasia of myenteric ganglia, a phenotype similar to that observed in human INDB. However, the mutation screening of this gene in 48 patients with IND did not show any sequence variant, either causative missense mutation or neutral susbtitution.[95] Despite this, the future knowledge of the pathways in which Hox11L1 is involved, for example, the genes modulated by Hox11L1 protein would produce candidate genes for involvement in INDB, and if so, possible diagnostic markers.[34]

EDNRB GENE

Ontogenetic studies revealed that mutations in the EDNRB gene or its specific ligand endothelin-3 (EDN3) lead to defects in the development of NC cells.[76,77] When colonization of the gut by NC cells is incomplete, the distal part of the bowel is left aganglionic. Recently Von Boyen et al[33] reported abnormalities of the ENS in heterozygous EDNRB-deficient rats resembling IND in humans. They showed that a heterozygous 301 bp deletion of the EDNRB gene led to abnormalities of the ENS. Malformations of the ENS observed in +/sl rats included hyperganglionosis, giant ganglia, and hypertrophied nerve fibers in the submucous plexus resembling the histopathological features of INDB in humans. These findings support the concept that IND may be linked to a genetic defect. Systematic genetic screening or a heterozygous mutation in the EDNRB gene in a group of IND patients in whom the diagnosis is based on clearly defined histopathological criteria may provide new insights into the etiology of this condition.

REFERENCES

1. Puri P: Hirschspurung's disease, in Puri P (ed): Newborn Surgery London, UK, Arnold, 2003, pp 513-533

2. Ikeda K, Goto S: Diagnosis and treatment of Hirschsprung's disease in Japan: An analysis of 1628 patients. Ann Surg 199:400-405, 1984

3. Ziegler MM, Ross AJ III, Bishop HC: Total intestinal aganglionosis: A new technique for prolonged survival. J Pediatr Surg 22:82-83, 1987

4. Nemeth L, Yoneda A, Kader M, et al: Three-dimensional morphology of gut innervation in total intestinal aganglionosis using whole-mount preparation. J Pediatr Surg 36:291-295, 2001

5. Passarge E: The genetics of Hirschsprung's disease. N Engl J Med 276:138-143, 1967

6. Spouge D, Baird PA: Hirschsprung's disease in large birth cohort. Teratology 32:171-177, 1985

7. Orr JD, Scobie WG: Presentation and incidence of Hirschsprung's disease. Br Med J 287:1671, 1983

8. Goldberg E: An epidemiological study of Hirschsprung's disease. Int J Epidemiol 13:479-485, 1985

9. Badner JA, Seiber WK, Garver KL, et al: A genetic study of Hirschsprung's disease. Am J Hum Genet 46:568-580, 1990

10. Amiel J, Lyonnet S: Hirschsprung's, associated syndromes and genetics: A review. J Med Genet 38:729-739, 2001

11. Puri P: Variant Hirschsprung's disease. J Pediatr Surg 32:149-157, 1997

12. Rolle U, Nemeth L, Puri P: Nitrergic innervation of the normal gut and in motility disorders of childhood. J Pediatr Surg 37:551-567, 2002

13. Gillick J, Tazawa H, Puri P: Intestinal neuronal dysplasia: Results of treatment in 33 patients. J Pediatr Surg 36:777-779, 2001

14. Montedonico S, Acevedo S, Fadda B: Clinical aspects of intestinal neuronal dysplasia. J Pediatr Surg 37:1772-1774, 2002

15. Meier-Ruge W: Uber ein Ekrankungsbild des colon mit Hirschsprung-Symptomatik. Vehr Dtsch Ges Pathol 55:506-510, 1971

16. Fadda B, Maier WA, Meier-Ruge W, et al: Neuronale intestinal dysplasie: Eine Kritische 10-Jahres-Analyse klinischer und bioptischer diagnostik. Z Kinderchir 38:305-312, 1983

17. Kobayashi H, Hirakawa H, Surana R, et al: Intestinal neuronal dysplasia is a possible cause of persistent bowel symptoms after pull-

through operation for Hirschsprung's disease. J Pediatr Surg 30:253-259, 1995

18. Holschneider A, Puri P: Intestinal neuronal dysplasia, in Holschneider A, Puri P (eds): Hirschsprung's disease and allied disorders Singapore, Harwood Academic Publishers, 2000, pp 147-154

19. Kobayashi H, Hirakawa H, Puri P: What are the diagnostic criteria for intestinal neuronal dysplasia? Pediatr Surg Int 10:459-464, 1995

20. Borchard F, Meier-Ruge W, Wiebecke B, et al: Innervationsstrungen des Dickdarms-Klassifikation und Diagnostik. Pathlogie 12: 171-174, 1991

21. Koletzko S, Ballauff A, Hadziselimovic F, et al: Is histological diagnosis of neuronal intestinal dysplasia related to clinical and manometric findings in constipated children?: Results of a pilot study. J Pediatr Gastroenterol Nutr 17:59-65, 1993

22. Smith VV: Isolated intestinal neuronal dysplasia: A descriptive pattern or a distinct clinicopathological entity? in Hadziselimomic F, Herzog B (eds): Inflammatory Bowel Disease and Morbus Hirschsprung Dordrecht, The Netherlands, Kluwer Academic, 1992, pp 203-214

23. Martuciello G, Caffarena PE, Lerone M, et al: Neuronal intestinal dysplasia: Clinical experience in Italian patients. Eur J Pediatr Surg 4:287-292, 1994

24. Meier-Ruge W: Epidemiology of congenital innervation defects of the distal colon. Virchows Arch A Pathol Anat 420:171-177, 1992

25. Puri P, Wester T: Intestinal neuronal dysplasia. Semin Pediatr Surg 7:181-186, 1998

26. Scharli AF: Neuronal intestinal dysplasia. Pediatr Surg Int 7:2-7, 1992

27. Csury L, Pena A: Intestinal neuronal dysplasia: Myth or reality? Literature review. Pediatr Surg Int 10:441-446, 1995

28. Lake BD: Intestinal neuronal dysplasia: Why does it only occur in parts of Europe? Virchows Arch 426:537-539, 1995

29. Milla PJ, Smith VV: Intestinal neuronal dysplasia. J Pediatr Gastroenterol Nutr 17:356-357, 1993

30. Sacher P, Briner J, Hanimann B: Is neuronal intestinal dysplasia (NID) a primary disease or a secondary phenomenon? Eur J Pediatr Surg 3:228-230, 1993

31. Hatano M, Aoki T, Dezawa M, et al: A novel pathogenesis of megacolon in NCX/HOX 11L1 deficient mice. J Clin Invest 100:795-801, 1997

32. Shirisawa S, Yunker AMR, Roth KA, et al: ENX (HOX 11L1) deficient mice develop myenteric neuronal hyperplasia and megacolon. Nat Med 3:646-650, 1997

33. Von Boyen GBT, Krammer HJ, Suss A, et al: Abnormalities of the enteric nervous system in heterozygous endothelin B receptor deficient (spotting lethal) rats resembling intestinal neuronal dysplasia. Gut 51:414-419, 2002

34. Newgreen D, Young HM: Enteric nervous system: Development and developmental disturbances part 1. Pediatr Develop Pathol 5:224-247, 2002

35. Newgreen D, Young HM: Enteric nervous system: Development and developmental disturbances part 2. Pediatr Develop Pathol 5:329-349, 2002

36. Gariepy CE: Intestinal motility disorders and development of the enteric nervous system. Pediatr Res 49:605-613, 2000

37. Wester T, O'Briain DS, Puri P: Notable postnatal alterations in the myenteric plexus of normal human bowel. Gut 44:666-674, 1999

38. Puri P, Ohsiro K, Wester T: Hirschspurung's disease: A search for etiology. Semin Pediatr Surg 7:140-147, 1998

39. Gershon MD, Chalazonitis A, Rothman TP: From neural crest to bowel: Development of the enteric nervous system. J Neurobiol 24: 199-214, 1993

40. Goyal RK, Hirano I: The enteric nervous system. N Engl J Med 334:1106-1115, 1996

41. Gershon MD: The enteric nervous system: A second brain. Hosp Pact (Off Ed) 31-2,34,35-38,41-42, 1999

42. Yntema CL, Hammond WS: The origin of intrinsic ganglia of trunk viscera from vagal neural crest in the chick embryo. J Comp Neurol 101:515-534, 1954

43. Le Douarin NM, Teillet MA: The migration of neural crest cells to the wall of the digestive tract in avian embryo. J Embryol Exp Morphol 30:31-48, 1973

44. Pomeranz HD, Gershon MD: Colonization of the avian hindgut by cells derived from the sacral neural crest. Dev Biol 137:378-394, 1990

45. Burns AJ, Douarin NM: The sacral neural crest contributes neurons and glia to the postumbilical gut: Spatiotemporal analysis of the development of the enteric nervous system. Development 125: 4335-4337, 1998

46. Caniano DA, Ormsbee HS III, Polito W: Total intestinal aganglionosis. J Pediatr Surg 20:456-460, 1985

47. Schiller M, Levy P, Shawa RA, et al: Familial Hirschsprung's disease?: A report of 22 affected siblings in four families. J Pediatr Surg 25:322-325, 1990

48. Engum SA, Petrites M, Rescorla FJ, et al: Familial Hirschsprung's disease: 20 cases in 12 kindreds. J Pediatr Surg 28:1286-1289, 1993

49. Parisi MA, Kapur RP: Genetics of Hirschspurung's disease. Curr Opin Pediatr 12:610-617, 2000

50. Passarge E: Dissecting Hirschspurung's disease. Nat Genet 31: 11-12, 2002

51. Montgomery RK, Mulberg AE, Grant RJ: Development of the human gastrointestinal tract: Twenty years of progress. Gastroenterology 116:702-731, 1999

52. Taraviras S, Pachnis V: Development of the mammalian enteric nervous system. Curr Opin Gen Dev 9:321-327, 1999

53. Jing S, Wen D, Yu Y, et al: GDNF-induced activation of the ret protein tyrosine kinase is mediated by GDNFR-α, a novel receptor for GDNF. Cell 85:1113-1124, 1996

54. Jing S, Yu Y, Fang M, et al: GFRα-2 and GFRα-3 are two new receptors for ligands of the GDNF family. J Biol Chem 272:33111-33117, 1997

55. Schuchardt A, D'Agati V, Larsson-Blomberg L, et al: Defects in the kidney and enteric nervous system of mice lacking the tyrosine kinase receptor Ret. Nature 367:380-383, 1994

56. Luo Y, Cecchernin I, Pasini B, et al: Close linkage with the RET protooncogene and boundaries of deletion mutations in autosomal dominant Hirschsprung's disease. Hum Mol Genet 21:803-1808, 1993

57. 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 367:378-380, 1994

58. Edery P, Lyonnet S, Mulligan LM, et al: Mutations of the RET proto-oncogene in Hirschsprung's disease. Nature 367:378-380, 1994

59. Kusafuka T, Puri P: Altered RET gene mRNA expression in Hirschsprung's disease. J Pediatr Surg 32:600-604, 1997

60. Kusafuka T, Puri P: The RET proto-oncogene: A challenge to understanding of disease pathogenesis. Pediatr Surg Int 12:11-18, 1997

61. Martucciello G, Checcherini I, Lerone M, et al: Pathogenesis of Hirschsprung's disease. J Pediatr Surg 35:1017-1025, 2000

62. Seri M, Yin L, Barone V, et al: Frequency of RET mutations in long- and short-segment Hirschsprung's disease. Hum Mutat 9:243-249, 1997

63. Kusafuka T, Puri P: Genetic aspects of Hirschsprung's disease. Semin Pediatr Surg 7:148-155, 1998

64. Hofstra RM, Landsvater RM, Ceccherini I, et al: A mutation in the RET proto-oncogene associated with multiple endocrine neoplasia

type 2B and sporadic medullary thyroid carcinoma. Nature 367:375-376, 1994

65. Hellmich HL, Kos L, Cho ES, et al: Embryonic expression of glial-line derived neurotrophic factor (GDNF) suggests multiple developmental roles in neuronal differentiation and epithelial-mesenchymal interactions. Mech Dev 54:95-105, 1996

66. Worley DS, Pisano JM, Choi ED, et al: Developmental regulation of GDNF response and receptor expression in the enteric nervous system. Development 127:4383-4393, 2000

67. Fock PJ, Schiltz CA, Jones SE, et al: Enteric neuroblasts require the phosphatidylinositol 3-kinase pathway for GDNF-stimulated proliferation. J Neurobiol 47:306-317, 2001

68. Young HM, Hearn CJ, Farlie PG, et al: GDNF is a chemoattractant for enteric cells. Dev Biol 229:503-516, 2001

69. Durbec P, Marcos-Gutierrez CV, Kilkenny C, et al: GDNF signaling through the ret receptor tyrosine kinase. Nature 381:789-793, 1996

70. Sanches M, Silos-Santiago I, Frisen J, et al: Renal agenesis and the absence of enteric neurons in mice lacking GDNF. Nature 382:70-73, 1996

71. Pichel JG, Shen L, Sheng HZ, et al: Defects in enteric innervation and kidney development in mice lacking GDNF. Nature 382:73-76, 1996

72. Angrist M, Bolk S, Halushka M, et al: Germline mutations in glial cell line-derived neurotrophic factor (GDNF) and RET in Hirschsprung's disease patients. Nat Genet 14:341-344, 1996

73. Shen L, Pichel JG, Mayeli T, et al: GDNF haploinsufficiency causes Hirschsprung's-like intestinal obstruction and early onset lethality in mice. Am J Hum Genet 70:435-447, 2002

74. Baynash AG, Hosoda K, Giaid A, et al: Interaction of endothelin-3 with endothelin-B receptor is essential for development of epidermal melanocytes and enteric neurons. Cell 79:1277-1285, 1994

75. Leibl MA, Ota T, Woodward MN, et al: Expression of endothelin-3 by mesenchymal cells of embryonic mouse caecum. Gut 44: 246-252, 1999

76. Hosoda K, Hammer RE, Richardson JA, et al: Targeted and natural (piebald-lethal) mutations of endothelin-B receptor gene produce megacolon associated with spotted coat color in mice. Cell 79: 1267-1276, 1994

77. Rice J, Doggett B, Sweetser DA, et al: Transgenic rescue of aganglionosis and piebaldism in lethal spotted mice. Dev Dyn 217:120-132, 2000

78. Kusafuka T, Wang Y, Puri P: Novel mutation of the endothelin-B receptor gene in isolated patients with Hirschsprung's disease. Hum Mol Genet 5:347-349, 1996

79. Kusafuka T, Puri P: Mutations of the endothelin-B receptor and endothelin-3 genes in Hirschsprung's disease. Pediatr Surg Int 12:19-23, 1997

80. Kusafuka T, Wang Y, Puri P: Mutation analysis of the RET, the endothelin-B receptor, and the endothelin-3 genes in sporadic cases of Hirschsprung's disease. J Pediatr Surg 32:501-504, 1997

81. Amiel J, Attie T, Jan D, et al: Heterozygous endothelin receptor B (EDNRB) mutations in isolated Hirschsprung's disease. Hum Mol Genet 5:355-357, 1996

82. Bidaut C, Pelet A, Van Camp G, et al: Endothelin-3 gene in isolated and syndromic Hirschsprung's disease. Eur J Hum Genet 5:247-251, 1997

83. Hofstra RM, Osinga J, Tan-Sindhunata G, et al: A homogenous mutation in the endothelin-3 gene associated with a Combine Waardenburg type 2 and Hirschsprung's phenotype (Shah-Waardenburg syndrome). Nat Genet 12:445-447, 1996

84. Edery P, Attie T, Amiel J, et al: Mutations of the endothelin-3 gene in Waardenburg-Hirschsprung's disease (Shah-Waardenburg syndrome). Nat Genet 12:442-444, 1996

85. Oue T, Puri P: Altered endothelin-3 and endothelin-B receptor mRNA expression in Hirschsprung's disease. J Pediatr Surg 34:1257-1260, 1999

86. Kenny SE, Hofstra RM, Buys CH, et al: Reduced endothelin-3 expression in sporadic Hirschsprung's disease. Br J Surg 87:580-585, 2000

87. 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. Development 125:825-836, 1998

88. Hofstra RM, Valdenaire O, Asch E, et al: A loss of function mutation in the endothelin converting enzyme-1 (ECE-1) associated with Hirschsprung's disease, cardiac defects and autonomic dysfunction. Am J Hum Genet 64:304-308, 1999

89. Hersarth B, Pinganlt V, Bondurand H, et al: Mutation of the Sry-related SOX 10 gene in dominant megacolon, a mouse model for human Hirschsprung's disease. Proc Natl Acad Sci USA 95:5161-5168, 1998

90. Soultared-Smith EM, Kos L, Pavan WJ: Sox 10 mutation disrupts neural crest development in Dom Hirschsprung's mouse model. Nat Genet 18:60-64, 1998

91. Kuhlbrodt K, Schmidt C, Sock E, et al: Functional analysis of Sox10 mutations found in human Waardenburg-Hirschsprung patients. J Biol Chem 273:23033-23038, 1998

92. Paltyn A, Morin X, Cremer H, et al: Expression and interactions of the two closely related homeobox genes Phox2a and Phox 2b during neurogenesis. Development 124:4065-4075, 1997

93. Paltyn A, Morin X, Cremer H, et al: The homebox gene Phox2b is essential for the development of autonomic neural crest derivates. Nature 399:366-377, 1999

94. Garcia-Barcelo M, Sham MH, Lui VCH, et al: Association study of Phox2b as a candidate gene for Hirschsprung's disease. Gut 52:563-567, 2003

95. Costa M, Fava M, Seri M, et al: Evaluation of the HOX11L1 gene as a candidate for congenital disorders of intestinal innervation. J Med Genet 37:E9 (Letter), 2000