The **THAI**
***Journal of* SURGERY**

Official Publication of the Royal College of Surgeons of Thailand

Vol. 21 October - December 2000 No. 4

Congenital Megacolon : New Insight into Molecular Level

Surasak Sangkhathat, MD

Pediatric Surgery Unit, Department of Surgery, Prince of Songkla University, Hadyai , Songkla, Thailand 90112

E-mail: sasurasa@ratree.psu.ac.th

Abstract **Hirschsprung's disease (HSCR) is a common pediatric surgical condition causing functional colonic obstruction. Although surgical treatment for the disease is well known, the underlying etiology and genetic basis have just gained more interest in recent years. This review focuses on the current concepts of molecular genetic and basic science as related to HSCR.**

RET proto-oncogene on chromosome 10q11.2 is the first susceptibility gene for HSCR. The gene and its ligand 'GDNF' play an important role in transduction of proliferation and differentiation signal into the neuronal precursors. Mutations of RET proto-oncogene are detected in 10 - 40 % of HSCR cases, the majority is long-segment cases. The second gene, ENDRB, maps to chromosome 13q22. ENDRB ligands are the endothelin molecules. Most of ENDRB mutations are found in short-segment HSCR.

Migratory arrest in HSCR could be related to the genetic derangement of the neural crest cells or the expression deficit of the genes. Moreover, alteration of neurotrophic factors as well as alterations of the intestinal microenvironment are also involved.

HSCR is classified as a simple form of neurocristopathy. Syndromic neurocristopathy, including MEN2A and Waardenberg syndromes, are closely linked to the disease, regarding embryology and genetic predisposition. Screening for oncogenic mutations in familial or long-segment HSCR is recommended.

Key word : **Hirschsprung's disease, Congenital megacolon**

Hirschsprung's disease (HSCR) is a congenital anomalies of the colon affecting about 1:5,000 livebirths.[1-4] The principle pathology is the absence of ganglion cells in the terminal segment of the large bowel together with increase in cholinergic innervation in the submucosa. The aganglionosis leads to sustained contraction of the diseased colon with secondary dilatation and hypertrophy of the more proximal segment. "Congenital megacolon" was named after this secondary change.

Although surgical treatment of HSCR has been established since 1950's,[5,6] the underlying genetic basis and embryopathogenesis of the disease are not clearly understood. Recent advances in molecular genetic research and histochemical studies reactivated the interest in this area among pediatric surgical society. HSCR has been classified as a form of neurocristopathies.[7,8] The susceptibility genes and ligands for HSCR, especially the long segment disease, have been discovered and some have certain clinical implication.[9] This review is focused on advanced concepts in basic science research of HSCR which may give us a better understanding of the etiology and pathophysiology of the disease.

HSCR as a genetic disease

Sexual preponderance and familial occurrence have been demonstrated in HSCR. In general, boys are affected 4 times more often than girls but the ratio is less for long-segment disease.[10,11] A familial incidence of 8 percent[11,12] has been reported and the evidence is more likely in patients with long-segment aganglionosis, compared to those who have the short-segment disease. The risk in siblings is about 4 percent.[13,14] The association between HSCR and various genetic syndromes has been observed. These linked syndromes include Down's syndrome, Waardenberg's syndrome, cartilage-hair hypoplasia, central hypoventilation syndrome (Ondine's curse) and multiple endocrine neoplasia (MEN 2A and MEN 2 B).[1,3,4]

The epidemiological evidences mentioned above suggest genetic contribution in the etiology of HSCR, especially in the long-segment form.[15] In 1990, Badner A and his colleagues analysed 487 probands from HSCR patients and their families in order to characterize modes of inheritance.[14] The families were classified into separate categories by the extent of aganglionosis. The inheritance pattern in cases with aganglionosis beyond the sigmoid colon is compatible with a dominant gene with incomplete penetrance. While for cases with aganglionosis extending no further than the sigmoid colon, the pattern is equally likely to be either multifactorial or due to a recessive gene with very low penetrance.

Interstitial deletion of the long arm of chromosome 10 was observed in an infant with total colonic aganglionosis.[16] Linkage analyses in families with autosomal dominant pattern mapped HSCR gene to 10q11.2,[17,18] the same region where MEN 2A gene and genes associated with other neurocristopathy had been earlier located.[19,20] The gene locus was then matched with the RET proto-oncogene by mutation studies.[21,22] This locus has been the first demonstrable gene related to congenital megacolon (HSCR 1). Mutations of HSCR 1 in human are heterozygous with incomplete penetrance.[23-25] Apart from RET-protooncogene, another major susceptibility locus was identified in HSCR cases with recessive inheritance. Puffenberger and his co workers performed their research in a group of inbred families and detected the locus on chromosome 13q22 (HSCR2).[26,27]

HSCR genes

To date, there are two systems of major susceptibility genes for HSCR, the RET system and endothelin receptor-B (ENDRB) system.[9,15,28]

RET proto-oncogene and its ligand genes

RET (REarranged during Transfection) proto-oncogene encodes a receptor protein "tyrosine kinase" which can be detected in the developmental period of neural crest-derived cells including the neurons of the enteric nervous system.[15,28,29] A receptor tyrosine kinase molecule consists of three domains; an intracellular tyrosine kinase domain, a transmenbrane domain and an extracellular domain which harbors a catherin-like, cysteine rich region.[30] This ligand-dependent receptor functions as a signal transduction pathway to phosphorylate a second messenger protein in the cytoplasm. The signaling process plays a critical role in the development of the enteric nervous system.

The incidence of the RET proto-oncogene mutation has been reported in the range of 10 - 40 per cent of all HSCR, depending on familial or sporadic occurrence of the disease and the length of aganglionosis.[23-25,31-33] In general, rates of mutation are higher in familial cases and cases with long-segment disease. Missence and nonsence mutations and a few base-pair deletions or insertions were detected through the length of 20 exons by different investigators.[8,30] Homozygous RET knockout mice exhibits total intestinal aganglionosis together with renal agenesis whereas the heterozygous mutants have normal phenotype.[34] The alleic status in RET mutant humans are almost always heterozygous. This discrepancy may be attributable to the difference in genetic backgrounds among the two species and to the different

threshold of enteric precursors to RET proto-oncogene derangement. Heterozygous mutation in human is embryonically lethal.[9, 28]

Ligand of RET receptor has recently been discovered as glial-cell-line-derived neurotrophic factor (GDNF).[35,36] GDNF is a distant member of the transforming growth factor-beta superfamily, a group of molecules which play an important roles in the regulation of cell survival, proliferation and differentiation.[37-40] GDNF gene was mapped to chromosome 5p12 - p13.1.[41] Moreover, a new glycosylphosphatidylinositol-linked protein GDNFR-alpha was found to be the RET's co-receptor for GDNF binding. GDNF-GDNFR-alpha-RET complex provides dimerization and phosphorylation of the tyrosine kinase domains of RET receptor, leading to intracellular signaling.[8,42] Mutation of GDNF was detected in 0.9 - 5.5 per cent of all HSCR cases.[8,43,44] Although some can be regarded as dominant mutations, other GDNF mutations may not be related to HSCR phenotype.[15,28]

ENDRB and its ligands

In the same year when RET proto-oncogene was recognized as HSCR1, Puffenberger E and his colleagues demonstrated that endothelin receptor B (ENDRB) was the affected gene in their previously detected locus at chromosome 13q22.[26,27] ENDRB is one of the receptors for endothelins (ET) peptides which comprise of ET1, ET2 and ET3. In human fetuses, ENDRB and ET3 are expressed in enteric neurons and intestinal mesenchymal cells, suggesting their roles in regulation of neurons-mesenchyme interaction during developmental period.[9] Missence and nonsence mutations of ENDRB gene were reported in both isolated and syndromic HSCR.[27,45,46] Penetrance in human mutation is 74 % for homozygotes and 21 % for heterozygotes.[27] The effect of ENDRB mutation in HSCR is, therefore, dosage dependent and the mutation is neither fully dominant nor fully recessive. Contrary to RET proto-oncogene mutation, in which the majority of cases has long segment HSCR, aganglionosis in most of the ENDRB mutants confines to the rectosigmoid colon.[46,47]

Homozygous mutations of the ET3 gene on chromosome 20 have been identified in consanguineous HSCR families with associated Shah-Waardenberg syndrome (pigmentation defects and deafness).[48,49] However, mutation of ET3 gene in non-syndromic HSCR is rare.[50]

Complete deletion of ENDRB gene can be found in the piebald lethal (s'/s') mutant mouse which expresses phenotype of megacolon and white spotting in coat color.[51] Natural mutation of ET3 is also found in lethal spotted mouse mutant (ls/ls), which habor a point mutation on ET3 gene and distal colonic aganglionosis.[52] The inheritance pattern of both genes in mice is autosomal recessive. These animal models confirm the roles of endothelin signaling system in the etiology of HSCR.

Other candidate genes for HSCR were identified in animal aganglionosis. Dominant megacolon genes of mice (Dom) is the only gene that shows semidominant inheritance pattern, whereas the other natural and artificial mutation in mice inherited as autosomal recessive.[53] Endothelin converting enzyme 1 (ECE1) gene is suspected to play an essential role for the endothelins signaling pathways.[54] Recently Sry related SOX10 was the third susceptibility gene detected in patients with HSCR associated with Shah-Waardenberg syndrome.[55]

Embryopathogenesis of HSCR

The enteric ganglion cells are derived from the vagal neural crest cells which migrate in head-to-toe direction from the neural tube to the esophagus in the fifth week of gestation, and to the entire length of gastrointestinal tract within the twelfth week.[56-59] Repeated cycles of cellular proliferation, differentiation and cell-to-cell and cell-to-matrix interaction are essential in the progression of the migratory process.[59]

The premature arrest of the craniocaudal migration of the neural crest-derived enteric neuron suggested by Okamoto and Ueda in 1967 is the most widely accepted hypothesis of the HSCR.[58] However, it is unclear whether the cessation of migration belongs to the incompetency of the neuroblasts in the migratory pathway or at the intestinal microenvironment where the cells should settle down.

Cellular genetic defects

Alteration of gene function is not only explainable by the mutated genetic structures, but could be the result of defects in the transcription or translation into the functional protein. Study of RET protein expression and tyrosine kinase activity in human fetal rectum by immunohistochemical techniques demonstrated that RET protein immunostaining was more intense in the enteric nervous system during the

early development and the tyrosine kinase activity of ganglion cells increases progressively with advancing gestational age.[29] The small ganglion cells in hypoganglionic transitional segment of HSCR also showed strongly staining of the antibodies against RET protein and low tyrosine kinase activity.[60,61] The evidences suggest that the pattern of "developing" arrest at the hypoganglionic segment could be secondary to functional disorder of the protein product of RET, the tyrosine kinase receptor, or the lack of an appropriate ligand. Kusafuga T. conducted an evaluation of RET mRNA expression in the colon of HSCR patients.[62] The signal intensity in aganglionic segment was low compared to the transitional segment and ganglionic segment, suggesting the contribution RET expression deficit at the diseased segment.

Neurotrophic factors deficiency

The ligand of RET receptor, GDNF, is previously known as a neurotrophic factor, which is necessary for the survival of various kinds of neurons including enteric ganglia.[37-40] GDNF mRNA expression in newborn rectum is prominent in the muscularis mucosae whereas the ganglion cells of the enteric nervous plexuses showed no expression by in situ hybridization.[63] Muscularis mucosa is then suspected to play an important role in the development of enteric nervous system. Immunofluorescence study showed reduction of GDNF immunoreactivity in aganglionic colon compared to normoganglionic segment.[64,65] GDNF expression deficit in the aganglionic segment may explain missed activation and phosphorylation of the RET receptor in the RET non-mutated patients.[8]

To test the neurotrophic roles of GDNF and ET3, cells from vagal neural crest were isolated and conditionally cultured.[66] Surprisingly, the stimulatory effect of GDNF on neuron numbers was strikingly diminished by the simultaneous presence of ET3. Hearn CJ and his co-authors discussed that ET3 modulated the action of GDNF by means of inhibiting neuronal differentiation to maintain the amount of precursor cells, and ensure sufficient cell population numbers to reach the entire length of gastrointestinal tract.

Certain neurotrophic factors have an important role in the development of central and peripheral nervous system.[67,68] Nerve growth factors (NGF), brain derived neurotrophic factors (BDNF) and the neurotrophins 3 interact with tyrosine kinase family of receptors in the enteric neurons to promote cellular development and survival.[69] Hoehner et al investigated the intensity of neurotrophin receptors in normal colon and segments from HSCR patients.[70] They reported that all three neurotrophin receptors are localized with cellular specificity to the enteric nervous system of normal and proximal ganglionic colon of HSCR. However, none was detected in the hypertrophic nerve fibers of aganglionic segments from HSCR. Kuroda T and his co-workers reported that the signal of NGF mRNA in the aganglionic segment of congenital megacolon mice was less intense than in the rectum of normal mice.[71]

Alteration in intestinal microenvironment

Abnormality in the development of enteric nervous system may be attributable to the road where they migrate and/or the house where they have to colonize and mature. The extracellular matrix proteins have been recognized as an important microenvironmental factor of neuronal processing pathway, a matrix for cellular adhesion and locomotion.[72] Moreover, the extracellular matrix molecules may serve as a latticework on which neurotrophic or signalling molecules might accumulate.[70] Fujimoto T and colleagues studied the extracellular matrix proteins as the migration pathway of neural crest cells in human embryo, using immunohistochemistry techniques.[73] The authors postulated that enteric neurogenesis is dependent on extracellular matrices. Fibronectin and hyaluronic acid provide a migration pathway for neural crest-derived cells in the developing gut whereas laminin and collagen type IV promote maturation of neurons. The abnormal in these extracellular matrix molecules, could inhibit the neuronal precursor cells from reaching their ultimate location and getting matured.

Distribution and intensity of different extracellular matric proteins in HSCR were studied by immunohistochemistry technique. The immunoreactivity of laminin and collagen type IV was less intense in aganglionic bowel compared to normal and ganglionic segment whereas those of fibronectin and tenascin were increased.[74]

HSCR as a neurocristopathies

Neural crest cells migrate and differentiate into diverse cell types, not only the ganglion cells of enteric nervous system, but also the neurons and glia of the

sensory, autonomic ganglia, neuroendocrine cells, adrenal medulla, pigmented cells, and facial cartilages.[75] The term "neurocristopathies" was first stated by Bolande P in 1974 to describe diseases or syndromes, of which the pathophysiological processes were related to maldevelopment of neural crest cells.[76] Diseases and syndromes arising from neurocristopathies are diverse in organ systems involvement. They include neurological, endocrinological, and cutaneous disorders. While HSCR is classified as the simple neurocristopathy, most of the syndromes, known to be epidemiologically associated with HSCR, are complex neurocristopathies.[7] The most studied HSCR-related neurocristopathies are MEN 2A and Waardenberg syndromes.

HSCR and MEN2A share the same susceptible gene.[77-79] Point mutations of RET proto-oncogene in MEN2A are observed in 97 percent of cases.[77-80] MEN2A-identical RET proto-oncogene mutations in sporadic and familial HSCR patients are 2.5- 5 per cent.[23,24,31,81] Although the RET mutations in HSCR span thorough the entire length of RET gene and cause functional deficit, mutations detected in HSCR-related MEN2A patients limit to the cysteine residue in the exon 10 of extra cellular domain (codons 609, 618 and 620), resulting in auto-phosphorylation of the RET receptor.[82,83] Penetrance of the index lesion, the medullary thyroid carcinoma, is essentially 100 per cent.[82] Besides MEN2A, other HSCR-associated neoplastic neurocristopathies which harbor mutated RET at exon 10 include MEN2B and familial medullary thyroid carcinoma.[79]

Mutation of ENDRB gene and ET3 gene affect in aganglionosis associated with pigmentary defect in mice.[51,52] In Puffenberger's study, nine of seventeen inbred population having point mutation of ENDRB gene exhibited phenotypes compatible to Shah-Waardenberg syndromes without aganglionosis.[27]

Clinical perspectives

Clinical utility of the basic information regarding genetic background of HSCR can be demonstrated in the aspects of prenatal diagnosis and oncologic prevention. The ability to determine the presence of relevant gene mutations in material acquired by amniocentesis may prove to be advantageous for the management of HSCR. Prenatal diagnosis in families with previously affected children may lead to earlier postnatal management, before the occurrence of complications from colonic obstruction.[9]

Certain points of mutation, especially at the exon 10 of RET proto-oncogene predispose the HSCR patients to MEN2A. Children with familial HSCR are then recommended for genetic screening of this cancer. Because medullary thyroid carcinoma (MTC) develops in essentially all patients with MEN2, and is the only component of the syndrome that is uniformly malignant, prophylactic thyroidectomy is indicated as early as the age of two or five years in a child with disease-specific mutation.[7,82,84] In sporadic cases, family history of MTC should be obtained.

Summary

Researches in molecular genetic and immunohistopathology have become a new frontier in the understanding of this old surgical disease. There are strong evidences of genetic contribution in the etiology of HSCR, although the single gene model is not applicable. More detailed research in the interactive roles of multiple genes and their products should clarify not only the cause of the disease, but our comprehension in the regulation of enteric nervous system development in molecular language.

References

1. Skinner MA. Hirschsprung's disease.Curr Prob Surg 1996; 33: 391-461.
2. Titelbaum DH, Coran AG, Weitzman JJ, et al. Hirschsprung's disease and related neuromuscular diseases of the colon, in O'Neill JA,Rowe MI, Grosfeld JL, et al (eds): Pediatric Surgery (5th ed), St Louis, MO, Mosby, 1998, pp 1381-424.
3. Holschneider A,Ure BM. Hirschsprung's disease. In Ashcraft KW. (ed): Pediatric Surgery (3rd ed), Philadelphia, W.B. Saunders, 2000, pp 543-472.
4. Ryan ET, Ecker JL, Christakis NA,et al. Hirschsprung's disease: associated anomalies and demographics. J Pediatr Surg 1992; 27: 76-81.
5. Swenson O. My early experience with Hirschsprung's disease. J Pediatr Surg 1989; 24: 839-45.
6. Ashcraft KW. Pull-through procedures for Hirschsprung's disease. Semin Pediatr Surg 1998; 7: 83-4.

7. Martuciello G. Hirschsprung's disease as a neurocristopathy. Pediar Surg Int 1997; 12: 2-10.
8. Martuciello G,Ceccherini I,Lerone M, et al. Pathogenesis of Hirschsprung's disease. J Pediatr Surg 2000; 35: 1017-25.
9. Robertson K, Mason I. Hirschsprung's disease : genetic mutations in mice and men. Gut 1997; 41: 436-41.
10. Kleinhaus S, Boley S, Sheran M, et al. A survey of the members of the American Association of Pediatrics. J Pediatr Surg 1979; 14: 588-97.
11. Russel MB, Russel Ca, Niebuhr E. Anepidemiologic study of Hirschsprung's disease and additional anomalies. Acta Pediatr 1994; 83: 68-71.
12. Ikeda K, Goto S. Diagnosis and treatment of Hirschsprung' s disease in Japan: An analysis of 1,628 patients. Ann Surg 1984; 199: 400-5.
13. Passarge E. The genetics of Hirschsprung's disease: evidence for heterogenous etiology in a study of sixty- three families. N Engl J Med 1697; 276: 138-43.
14. Badner JA, Sieber WK, Garver KL, et al. A genetic study of Hirschsprung's disease. Am J Hum Genet 1981; 18: 87-90.
15. Wartiovaara K, Salo M, Sariola H. Hirschsprung's disease genes and the development of the enteric nervous system. Ann Med 1998; 30: 66-74.
16. Martuciello G, Bicocchi MP, Dodero P, et al. Total colonic aganglionosis associated with interstitial deletion of the long arm of chromosome 10. Pediatr Surg Int1992; 7: 308-10.
17. Lyonnet S, Bolino A, Pelet A, et al. A gene for Hirschsprung' s disease maps to the proximal long arm of chromosome 10. Nat Genet1993; 4: 346-50.
18. Angrist M, Kauffman E, Slaugenhaupt S, et al. A gene for Hirschsprung's disease (megacolon) in the mericentro-metric region of human chromosome 10. Nat Genet 1993; 4: 351-6.
19. Donis-Keller H, Dou S, Chi D, et al. Mutation in the RET proto-oncogene are associated with MEN2A and FMTC. Hum Mol Genet 1993; 2: 851-6
20. Mulligan LM, Eng C, Healey CS, et al. Specific mutations of the RET proto-oncogene are related to disease phenotype in MEN2A and FMTC. Nat Genet 1994; 6: 70-4.
21. 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.
22. Edery P, Lyonnet S, Mulligan LM, et al. Mutations of the RET proto-oncogene in Hirschsprung's disease. Nature 1994; 367: 378-80.
23. Attie T, Pelet A, Ederly P, et al. Diversity of RET proto-oncogene mutations in familial and sporadic Hirschsprung's disease. Hum Mol Genet 1995; 4: 1381-6.
24. Angrist M, Bolk S, Thiel B, et al. Mutation analysis of the RET receptor tyrosine kinase in Hirschsprung's disease. Hum Mol Genet 1995; 4: 821-30.
25. Kusafuga T, Wang Y, Puri P. Mutation analysis of the RET, the endothelin-B receptor, and the endothelin-B genes in sporadic patients with Hirschsprung's disease. J Pediatr Surg 1997; 32: 501-4.
26. Puffenberger EG, Kauffman ER, Bolk S, et al. Identity by descent and association mapping of a recessive gene for Hirschsprung's disease on human chromosome 13q22. Hum Mol Genet1994; 3: 1217-25.
27. Puffenberger EG, Hosoda K, Washington SS, et al. A missence mutation of the endothelin-B receptor gene in multigenic Hirschsprung's disease. Cell 1994; 79: 1257-66.
28. Kusafuga T, Puri P. Genetic aspects of Hirschsprung's disease. Semin Pediatr Surg1998; 7: 148-55.
29. Tam PK, Gould SJ, Martuciello G, et al. RET protein in the human fetal rectum. J Pediatr Surg1996; 31: 568-71.
30. Kusafuga T, Puri P. The RET proto-oncogene:a challenge to our understanding of disease pathogenesis. Pediatr Surg Int 1997; 12: 11-8.
31. Seri M, Yin L, Barone V, et al. Frequency of RET mutations in long- and short- segment Hirschsprung's disease. Hum Mutat1997; 9: 243-9.
32. Svensson PJ, Molander ML, Eng C, et al. Low frequency of RET mutations in Hirschsprung's disease in Sweden. Clin Genet 1998; 54: 39-44.
33. Sancandi M, Ceccherini I, Costa, et al. Incidence of RET mutation in patients with Hirschsprung's disease. J Pediatr Surg 2000; 35: 139-43.
34. Schuchardt A, D'Agati V, Larsson-Blomberg L, et al. Defects in the kidney and enteric nervous system of mice lacking the tyrosine receptor ret. Nature 1994; 367: 380-3.
35. Durbec P, Marcos-Gutierrez C, Kilkenny C, et al. GDNF signalling through the RET receptor tyrosine kinase. Nature 1996; 381: 789-93.
36. Trupp M, Arenas E, Fainzilber M, et al. Functional receptor for GDNF encoded by the c-ret proto-oncogene. Nature 1996; 381: 785-9.
37. Lin LH, Doherty DH, Lile JD, et al. GDNF:A glial cell line derived neurotrophic factors. Science 1993; 260: 1130-2.
38. Henderson CE, Phillips HS, Pollock RA, et al. GDNF: A potent survival factor for motorneurons present in peripheral nerve and muscle. Science 1994; 266: 1062-4.
39. Yan Q, Matheson C, Lopez O. In vivo neurotrophic effects of GDNF on neonatal and adult facial motor neurons. Nature 1995; 373: 341-4.
40. Oppenheim RW, Houenou LJ, Johnson JE, et al. Developing motor neurons rescued from programmed and axotomy-induced cell death by GDNF. Nature1995; 373: 344-6.
41. Schindelhauer D, Schuffenhauer S, Gasser Y, et al. The gene coding for glial cell line derived neurotrophic factor (GDNF) maps to chromosome 5p12-p13.1. Genomics 1995; 28: 605-7.
42. Jing S, Wen D, Yu Y, et al. GDNF-induced activation of the ret protein tyrosine kinase is mediated by GDNFR-alpha, a novel receptor for GDNF. Nature 1996; 382: 80-3.
43. Angrist M, Bolk S, Halushka M, et al. Germline mutations in glial cell line-derived neurotrophic factor(GDNF) and RET in a Hirschsprung's disease patient. Nat Genet 1996; 14: 341-4.
44. Ivanchuck SM, Myers SM, Eng C, et al. De novo mutation of GDNF, ligand for the RET/GDNFR-a receptor complex in

Hirschsprung's disease. Hum Mol Genet 1996; 5: 2023-6.
45. Tanaka H, Moroi K, Iwai J, et al. Novel mutation of the endothelin S receptor gene in patients with Hirschsprung's disease and their characterization. J Biol Chem 1998; 273: 11378-83.
46. Oue T, Puri P. Altered endothelin3 and endothelin B receptor mRNA expression in Hirschsprung's disease. J Pediatr Surg 1999; 34: 1257-60.
47. Kusafuga T, Puri P. Mutation of the endothelin B receptor and endothelin 3 genes in Hirschsprung's disease. Pediatr Surg Int 1997; 12: 19-23.
48. Hoftra RMW, Osinga J, Tan-Sindhunata G, et al. A homozygous mutation in the endothelin 3 gene associated with a combined Waardenberg type 2 and Hirschsprung phenotype (Shah-Waardenberg syndrome). Nat Genet 1996; 12: 445-7.
49. Edery P, Attie T, Amiel J, et al. Mutation of the endothelin 3 gene in the Waardenberg-Hirschsprung's disease(Shah-Waardenberg syndrome). Nat Genet 1996; 12: 442-4.
50. Bidaud C, Solomon R, Van Camp G, et al. Endothelin 3 gene mutation in isolated and syndromic Hirschsprung's disease. Eur J Hum Genet 1997; 5: 247-51.
51. 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 1994; 79: 1267-76.
52. 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 1994; 79: 1277-85.
53. Lane P, Liu H. Association of megacolon with a new dominant spotting gene (Dom) in the mouse. J Hered 1984; 75: 435-9.
54. Xu D, Emoto N, Giaid A, et al. ECE-1, A membrane-bound metalloproteinase that catalyzes the proteolytic activation of big endothelin-1. Cell1994; 78: 473-85.
55. Pingault V, Bondurand N, Kuhlbrodt K, et al. SOX 10 mutations in patients with Waardenberg-Hirschsprung's disease. Nat Genet 1998; 18: 171-3.
56. Yntema CL, Hammond WS. The origin of intrinsic ganglia of trunk viscera from vagal neural crest in the chick embryo. J Comp Neurol 1954; 101: 515-54.
57. Allan IJ, Newgreen DF. The origin and differentiation of enteric neurons of the intestine of fowl embryo. Am J Anat 1980; 157: 137-54.
58. Okamato E, Ueda T. Embryogenesis of intramural ganglia of the gut and its relation to Hirschsprung's disease. J Pediatr Surg 1967; 2: 437-43.
59. Puri P, Ohshiro K,Wester T. Hirschsprung's disease :A search for etiology. Semin Pediatr Surg 1998; 7: 140-7.
60. Watanabe Y, Ito T, Harada T, et al. Expression of RET proto-oncogene products in the hypoganglionic segment of the small intestine of congenital aganglionosis rats. J Pediatr Surg 1995; 30: 641-5.
61. Martuciello G,Favre A,Takahashi M,et al. Immunohistochemical localization of RET protein in Hirschsprung's disease. J Pediatr Surg 1995; 30: 433-6.
62. Kusafuga T, Puri P. Alterted RET gene mRNA expression in Hirschsprung's disease. J Pediatr Surg 1997; 32: 600-4.
63. Wartiovaara K, Salo M, Sainio K, et al. Distribution of glial cell line-derived neurotrophic factor mRNA in human colon suggests roles for muscularis mucosae in innervation. J Pediatr Surg 1998; 33: 1501-6.
64. Ohshiro K, Puri P. Reduced glial cell line-derived neurotrophic factor level in aganglionic bowel in Hirschsprung's disease. J Pediatr Surg 1998; 33: 904-8.
65. Zhan J, XIU Y, Gu J, et al. Expression of RET proto-oncogene and GDNF deficit in Hirschsprung's disease. J Pediatr Surg 1999; 34: 1606-9.
66. Hearn CJ, Murphy M, Newgreen D. GDNF and ET-3 differentially modulate the numbers of avian enteric neural crest cells and enteric neurons in vitro. Dev Biol 1998; 197: 93-105.
67. Thoenen H. The changing scene of neurotrophic factor. Trends Neurosci 1991; 14: 165-70.
68. Thoenen H, Edgar D. Neurotrophic factors .Science 1985; 229: 238-42.
69. Eide FF, Lowenstein DH, Reichardt LF. Neurotrophins and their receptors-current concepts and implications for neurologic disease. Exp Neurol 1993; 121: 200-14.
70. Hoehner JC, Wester T, Pahlman S. Alteration in neurotrophin and neurotrophin-receptor localization in Hirschsprung's disease. J Pediatr Surg 1996; 31: 1524-9.
71. Kuroda T, Ueda M, Nakano M, et al. Altered production of nerve growth factor in aganglionic intestines. J Pediatr Surg 1994; 29: 288-92.
72. Brauer PR, Markwald RR. Attachment of neural crest cells to endogenous extracellular matrices. Anat Rec 1987; 219: 275-85.
73. Fujimoto T, Hata J, Yogoyama S, et al. A study of the extracellular matrix protein as the migration pathway of neural crest cells in the gut: An analysis in human embryos with special referrence to the pathogenesis of Hirschsprung's disease. J Pediatr Surg 1989; 24: 550-6.
74. Parikh DH, Tam PKH, VanVelzen D, et al. Abnormalities in the distribution of laminin and collagen typeIV in Hirschsprung's disease. Gastroenterology 1992; 102: 1236-41.
75. Bronner-Fraser M. Segregation of cell lineage in the neural crest. Curr Opin Genet Dev 1993; 3: 641-7.
76. Bolande RP. The neurocristopathies. A unifying concept of disease arising in neural crest maldevelopment. Hum Pathol 1974; 5: 409-29.
77. Donis-Keller H, Dou S, Chi D,et al. Mutations in the RET proto-oncogene are associated with MEN 2A and FMTC. Hum Mol Genet 1993; 2: 851-56.
78. Mulligan L, Kwok J, Healy C, et al. Germ-line mutations of the RET proto-oncogene in multiple endocrine neoplasia type 2A. Nature 1993; 363: 458-60.
79. Mulligan L,Eng C, Attie T, et al. Diverse phenotypes associated with exon 10 mutations of the RET proto-oncogene. Hum Mol Genet 1994; 3: 2163-7.
80. Mulligan L, Marsh D, Robinson B, et al. Genotype-phenotype correlation in multiple endocrine neoplasia type 2 : report of the international RET mutation consortium. J Intern Med

1995; 238: 343-6.
81. Sijmon RH, Hoftra RMW, Wijburg FA, et al. Oncological implications of RET gene mutations in Hirschsprung's disease. Gut 1998; 43: 542-7.
82. Decker RA, Peacock ML. Occurrence of MEN 2A in Familial Hirschsprung's disease: A new indication for genetic testing of the RET proto-oncogene. J Pediatr Surg 1998; 33: 207-14.
83. Inoue K, Shimotake T, Inoue K, et al. Mutational analysis of the RET proto-oncogene in a kindred with multiple endocrine neoplasia type 2A and Hirschsprung's disease. J Pediatr Surg 1999; 34: 1152-4.
84. Wells SA, Chi D, Toshima K, et al. Predictive DNA testing and prophylactic thyroidectomy in patients at risk for multiple endocrine neoplasia type 2A. Ann Surg 1994; 220: 237-50.