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

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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.

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that IND is a real entity stems from animal models. Recently, two different Hox11L1 knockout mouse models have been generated.\textsuperscript{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\textsuperscript{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.\textsuperscript{34,35} The ENS contains more neurons than the spinal cord and is responsible for the coordination of normal bowel motility and secretory activities.\textsuperscript{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.\textsuperscript{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.\textsuperscript{38}

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.\textsuperscript{12,38} NC cells give rise to neuronal, endocrine and paraendocrine, craniofacial, conotruncal heart, and pigmented tissues. Neurocristopathies encompass tumors, malformations, and single or multiple abnormalities of tissues mentioned above in various combinations.\textsuperscript{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 cranio-caudal 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.\textsuperscript{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.\textsuperscript{39-42} Studies in the avian system provide strong evidence for the contribution of the sacral NC to the hindgut ENS.\textsuperscript{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.\textsuperscript{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.\textsuperscript{1} A familial incidence of 15% to 21% has been reported in total colonic aganglionosis and 50% in the rare total intestinal aganglionosis.\textsuperscript{1,46} Schiller et al\textsuperscript{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 and 50% in the rare total intestinal aganglionosis.\textsuperscript{1,46} Schiller et al\textsuperscript{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 and 50% in the rare total intestinal aganglionosis, and only 1 had rectosigmoid HSCR. Engum et al\textsuperscript{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\textsuperscript{49} 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...
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.\(^{34,36,49,50}\) The RET receptor is the signaling component of receptor complexes with four ligands, glial-derived neurotrophic factor (GDNF), neurturin (NTN), artemin (ATM), and persephin (PSP).\(^{34,36}\) 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 1. Recurrence Risk to Siblings

<table>
<thead>
<tr>
<th>Relative to Patient</th>
<th>Recurrence Risk (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brothers of patients with rectosigmoid HSCR</td>
<td>4</td>
</tr>
<tr>
<td>Sisters of patients with rectosigmoid HSCR</td>
<td>1</td>
</tr>
<tr>
<td>Brothers of female with long segment HSCR</td>
<td>24</td>
</tr>
<tr>
<td>Sons of females with long segment HSCR</td>
<td>29</td>
</tr>
</tbody>
</table>

### Table 2. Gene Mutations Associated with Hirschsprung’s Disease

<table>
<thead>
<tr>
<th>Gene</th>
<th>Locus</th>
<th>Function</th>
<th>Frequency in Humans</th>
<th>Animal Homologues</th>
</tr>
</thead>
<tbody>
<tr>
<td>RET</td>
<td>10q11.2</td>
<td>Tyrosin kinase receptor</td>
<td>70-80% long segment</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>50% familial</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>15-20% sporadic</td>
<td></td>
</tr>
<tr>
<td>GDNF</td>
<td>5p12-13.1</td>
<td>Glial cell derived neurotropic factor</td>
<td>&lt;10%</td>
<td></td>
</tr>
<tr>
<td>NTN</td>
<td>19q13.3</td>
<td>Neurturin, RET ligand</td>
<td>&lt;1%</td>
<td></td>
</tr>
<tr>
<td>GFRα1</td>
<td>10q26</td>
<td>GDNF family receptor alpha 1</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>EDN3</td>
<td>20q13</td>
<td>Endothelin-B</td>
<td>&lt;10%</td>
<td></td>
</tr>
<tr>
<td>EDNRB</td>
<td>13q22</td>
<td>Endothelin-B-receptor</td>
<td>&lt;10%</td>
<td></td>
</tr>
<tr>
<td>ECE-1</td>
<td>1p36.1</td>
<td>Endothelin converting enzyme</td>
<td>&lt;1%</td>
<td></td>
</tr>
<tr>
<td>SOX10</td>
<td>22q13.1</td>
<td>Sry/HMG box transcription factor</td>
<td>&lt;1%</td>
<td></td>
</tr>
<tr>
<td>Phox2b</td>
<td>4p12</td>
<td>Paired-like homeobox 2b</td>
<td>&lt;1%</td>
<td></td>
</tr>
<tr>
<td>Pax3</td>
<td>2q35</td>
<td>Paired box gene 3</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>SIP1</td>
<td>2q22</td>
<td>Siah-interacting protein</td>
<td>6 cases</td>
<td></td>
</tr>
</tbody>
</table>
mutations are involved in modulation of the HSCR phenotype via its interaction with other susceptibility loci such as RET. Shen and coworkers described hypoganglionosis in gdnf+/− mice. 

**ENDOTHELIN SIGNALING PATHWAY**

The endothelins (EDN1, EDN2, and EDN3) are intercellular local messengers that act via cell surface receptors, EDNRA and EDNRB. EDNRA shows high affinity binding for EDN1 and does not bind EDN3 at physiologic concentrations, whereas EDNRB accepts all these ligands with high affinity. 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. Two ECE genes have been described, ECE1 and ECE2.

EDN3 and EDNRB have a role in the migration and development of the ENS. 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. 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. EDNRB or EDN3 mutations have been demonstrated in less than 10% of cases of HSCR. 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. 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. A heterozygous ECE1 mutation has been identified in a patient with HSCR who also had craniofacial and cardiac defects.

**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. The involvement of SOX10 in the development of enteric neurons was demonstrated in the Dom (Dominant megacolon) mouse model of HSCR. 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.

**PHOX2B**

Phox2B gene is a homeodomain-containing transcription factor that is involved in neurogenesis and regulates RET expression in mice, in which disruption of the Phox2B gene results in a HSCR-like phenotype. 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
plexus. Recently, Garcia-Bercelo et al reported that Phox2B A-G_{1364} polymorphism may predispose to HSCR.

**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. Many investigators have raised doubts about the existence of IND as a distinct histopathologic entity. 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. Recently, strong evidence has emerged from animal models that suggests that INDB is a real entity (Table 3).

**Table 3. Gene Mutations Associated with Intestinal Neuronal Dysplasia**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Locus</th>
<th>Function</th>
<th>Frequency in Humans</th>
<th>Animal Homologues</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDNRB</td>
<td>13q22</td>
<td>Endothelin-B-receptor</td>
<td>–</td>
<td>ednrB +/– Piebal lethal</td>
</tr>
<tr>
<td>HOX11L1</td>
<td>2p12-p13</td>
<td>T-cell leukemia, homeobox 2</td>
<td>–</td>
<td>Enx +/–</td>
</tr>
</tbody>
</table>

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. In both cases, homozygous mutant mice were viable but developed mega colon 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 substitution. 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.

**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. When colonization of the gut by NC cells is incomplete, the distal part of the bowel is left aganglionic. Recently Von Boyen et al 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**


