Animal Models of Aganglionosis

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4.1 Introduction

Humans are not the only mammals to suffer from aganglionosis. Aganglionosis has also been described in mice, rats, horses, cats and dogs. Rodent animal models have contributed significantly to our understanding of Hirschsprung's disease (HSCR). Over the last decade, the understanding of the genetics and cell biology of the development of the enteric nervous system (ENS) has made great progress. Rodent animal models have shown many points of correlation with humans in regard to ENS development, both normal and abnormal. Nevertheless, the link between the genotype and the phenotype is often indirect, and so many questions have yet to be answered. This chapter deals with the characteristics of aganglionosis in rodents with emphasis on how knowledge of the animal models has contributed to our understanding of the genetics and pathogenesis of HSCR and allied disorders.

4.2 History

4.2.1 Rodents

The first description of aganglionosis in mice was by Derrick and St George-Grambauer in 1957 [1]. They found approximately 3.2 per 1000 of their colony developed aganglionosis. The average length of aganglionosis was 15–20 mm with colonic distension extending into the first few millimeters of aganglionic colon. There was no association with a white or patched color coat. The next description was by Bielschowsky and Schofield in 1962 [2]. In this colony 10% of the offspring were affected and there was an association with a white colored coat. These mice also had a high incidence of mammary cancer and pituitary adenomas. Outbreeding experiments suggested an autosomal recessive trait with modification of the trait by other genetic factors.

In 1966, Lane described two strains of mice which developed aganglionosis as an autosomal recessive condition [3]. The lethal spotting (ls) mice have approximately 2 mm of aganglionosis with a patched coat, and later studies linked the defect to chromosome 2. Piebald lethal (s^1) mice had approximately 10 mm of aganglionosis and linkage studies suggested that the defect was on chromosome 14. In 1979, Ikadai et al. [4] described aganglionosis in spotting lethal (sl) rats. The animals had two lengths of aganglionosis: total colonic aganglionosis (TCA) and mid colon. These animals again showed autosomal recessive inheritance and had a white colored coat. A fourth rodent model is the Dominant megacolon (Dom) mouse

in which the aganglionic colon has a long hypoganglionic transition zone (Table 4.1) [5].

4.2.2 Other Mammals

There have been isolated reports of aganglionosis in a range of animals including cats [6, 7], horses [8–11] and pigs [12, 13].

4.3 Histologic Anatomy

Are rodents good histologic models of HSCR? The first histologic studies were performed by Lane [3]. These were restricted to showing there was aganglionosis in the terminal bowel, and in documenting the length of aganglionosis.

Bolande and Towler [14] and Bolande [15] investigated the lethal spotting mouse using histochemical and ultrastructural studies. Histology showed hypoganglionosis in the distal bowel but there was no dense ingrowth of nerve fibers. Boley [16] suggested that these findings of no hypertrophied nerve trunks indicated that these mice were not a good model of human disease. In the distal narrowed segment there was a reduction in adrenergic and cholinergic fibers. In the dilated part of the bowel there was an increase in adrenergic fibers. The ultrastructural studies showed that just above the transition zone, there were secondary degenerative changes in the ganglion cells, which increased with age, resulting in what appeared to be secondary cell death and abiotrophy. Webster [17, 18] performed detailed studies in lethal spotting and piebald lethal mice using cholinesterase stains and fluorescence to delineate the adrenergic nerves. In postnatal mice of both strains he demonstrated normal innervation in the proximal bowel followed by a transition zone and then an aganglionic zone with increased nerve trunks and a decrease in the innervation of the circular muscle fibers. In the most distal aganglionic colon, just above the anal sphincters, there appeared to be a variable, but denser, innervation to the circular muscle involving cholinergic nerves. Bu'Lock et al. [19] found a selective depletion of substance P in the transitional zone in piebald lethal mice. However, the change was from 10% in normal mice to 5% in mutant mice, and the study failed to confirm a previous report from the same laboratory of a decrease in substance P in the mutant ileum, indicating that the variability between animals and sensitivity of the techniques can make conclusions difficult.

In the spotting lethal (sl) rat model Ikadai et al. [4, 20] and Horie et al. [21] studied the length of aganglionosis and found that there were two subgroups, one in which there was TCA and a second, less numerous group, in which ganglion cells extended to the proximal half of the colon. The visible cone was often distal to the commencement of aganglionosis. In a histologic study of sl rats using whole-mounts and AchE, tyrosine hydroxylase and substance P, Nagahama et al. [22] showed aganglionosis of the colon along with increased nerve trunks. These changes are similar to those seen in humans with TCA. However, even in the proximal ganglionated duodenum there were changes in the two dimensional structure of the enteric plexus, with the lattice pattern being irregular. This raises the question as to whether the proximal gut is entirely normal, and if there is histologic abnormality in some of the subtle architecture, does this mean diminished function? The bowel also has many functions, so it may be possible for example that water absorption is affected while propulsive activity is normal.

An ultrastructural study in the sl rat model confirmed that almost no nerve terminals were present in the circular muscle layer of any regions of the constricted intestine, but some terminals were observed in the longitudinal muscle layer of that segment. The authors concluded that the denervated circular muscle layer is related to the production of a constricted segment, irrespective of the presence or absence of nerve terminals in the longitudinal muscle layer [23].

Table 4.1 Naturally occurring rodent animal models of HSCR

Name	Length of aganglionosis	Pigment	Inheritance	Locus	Genetic defect	Percent in human HSCR
Lethal spotting mice (ls)	2mm	Patched	Recessive	Chr.2	Point mutation of EDN3	5
Piebald lethal mice (sl)	10mm	White	Recessive	Chr.14	Absent EDNRB	<10
Spotting lethal rat (sl)	TCA	White	Recessive	Chr.6	301 bp deletion in EDNRB	<10
Dominant megacolon (Dom)	Variable	White	Dominant	Chr.15	Point mutation of SOX10	<1

Studies in our laboratory using AchE whole-mounts have demonstrated that the three rodent animal models have a histologic picture identical to that of humans. There was distal aganglionosis with increased nerve trunks, followed by a transition zone which was often asymmetric and variable in length, and more proximally the plexus was near normal in two-dimensional architecture. In spotting lethal rats, particularly those with TCA, the proximal small bowel had an abnormal architecture, but inconsistently so. We could not demonstrate as clearly as Nagahama et al. [22] that the duodenal architecture was abnormal. In our experiments the normal two-dimensional architecture of the enteric plexus was itself variable with some areas looking open and other areas with a tight regular lattice-like structure.

In the lethal spotting mice we were able to identify increased nerve trunks, in contrast to Bolande [15] who could not detect them. The animals we investigated had a longer length of aganglionosis (about 4 mm) and it may be that the genetic background of the animals used by Bolande was such that they had a very short length of aganglionosis, and were more a hypoganglionic model.

The published literature suggests that the length of aganglionosis in each animal model is relatively consistent within each strain [3]. However, in reality there is a considerable variability and while in the majority of cases the length of aganglionosis in piebald lethal is greater than in lethal spotting mice, there is overlap. We have noticed that if heterozygote animals with the least pigment are chosen as mating pairs then the length of aganglionosis tends to be longer. Alternatively, if the mutant animals are back-crossed with C57 or Castaneus stock, the length of aganglionosis reduces. Some F2 offspring from a lethal spotting mouse crossed with Castaneus apparently had anatomically normal distal colon although the color of the coat was that of a mutant animal. The most dramatic alteration in length of aganglionosis occurs when spotting lethal rats are crossed with DA rats: the predominant ileal aganglionosis changes to distal colonic aganglionosis. The change is a dramatic step-like decrease in the length of aganglionosis, suggesting that an extra quantum of enteric neuronal precursors has been created or there are regional differentiations in the bowel such that a region is either filled or remains aganglionic. It is of interest that in humans there also appear to be two common points of cessation of enteric innervation, either the sigmoid colon (in 80% of patients) or about 10 cm proximal to the ileocecal valve (in 10% of patients).

The sl rat has also been instructive in further elucidating the distal aganglionic bowel, namely that the gut is not completely aganglionic. There are groups of ganglion cells (seen best with NPDH-diaphorase staining) associated with the hypertrophied nerve fibers. We postulate that these can only have arisen from the sacral neural crest (there is a gap of many centimeters before ganglion cells are seen in the small bowel). Furthermore, we postulate that the presence of occasional clusters of ganglion cells in the "aganglionic" distal bowel in HSCR patients, if sampled on rectal biopsy, may cause a temporary diagnostic error. This may be the basis of the rarely described patients with "acquired aganglionosis", where the patient clinically has HSCR but the first biopsy suggests the presence of ganglion cells.

Morphologic studies in mice have shown that the density and distribution of the interstitial cells of Cajal (ICC) in the aganglionic region of the colons are similar to those of ICC at the same level of the colon in age-matched wild-type controls [24, 25]. Therefore, it appears that the enteric neurons are not necessary for the development of ICC. Data from humans are inconsistent which could be attributed to the regional differences in the density of ICC in the colon [26–28]. More recently Taniguchi et al. [29] have shown that the aganglionic intestine of ls/ls mice induces secondary disturbances during the normal development of ICC, in the form of fewer cytoplasmic processes and lack of attachment to the intermuscular nerves.

4.4 Physiology

The principle work, which has been on the piebald lethal mouse, is that of Wood et al. [30–32]. In a series of experiments the colon was inspected under video cameras and at the level of aganglionosis there was a functional obstruction. Pellets would move down the bowel and as soon as they reached the aganglionic zone they would stop and at that stage reverse peristalsis would commence [31].

Electrophysiologic studies in piebald lethal mice have shown that there are abnormal discharges of myogenic action potentials in the aganglionic bowel associated with tonic constriction and a reduction in the luminal diameter [30]. Furthermore, the proliferated extrinsic cholinergic nerve fibers appear to be not related to the narrowing of the aganglionic colon [33].

Electrophysiologic experiments in our laboratory have demonstrated that the aganglionic bowel in mouse models has no inhibitory neuromuscular junction potentials (IJP) and only occasional excitatory junction potentials which on repeated stimulation fatigue quickly. The aganglionic circular muscle, lacking inhibition, writhe in an uncontrolled manner and tend to contract [34]. The visual and tension studies confirm the impression that the aganglionic smooth muscle lacks the stabilizing influence of the nonadrenergic noncholinergic (NANC) inhibitory nerves. The smooth muscle appears hyperexcitable and in constant motion. This would confirm the hypothesis proposed by Alvarez [35] that the simplest explanation for the finding in HSCR is that nerves to smooth muscle normally function to keep the muscle from contracting into a knot. This is also the conclusion of Richardson

[36] who performed a pharmacologic study on the lethal spotting mouse.

The simple lack of nerve fibers is sufficient explanation for the functional obstruction seen in the rodent models and in patients with HSCR. There is no need to invoke selective and subtle disorders of various components of the autonomic nervous system to account for the clinical variability.

Thorough studies on the electrophysiology of the mutant rat colon have shown an absence of IJPs except at the sphincter where there is an evoked inhibitory response (in addition to an excitatory response) [37–40]. Another study in piebald lethal mice has shown an increase in basal contractile activity and a reduction in responsiveness to vasoactive intestinal peptide [41]. This supports a generalized reduction in the function of the inhibitory innervation of the aganglionic colon.

In 1990, Bult et al. [42] provided evidence that nitric oxide (NO) is released on stimulation of the inhibitory NANC nerves of canine ileocolonic junction. Since then, substantial evidence has accumulated indicating that NO is the primary nitrergic inhibitory neurotransmitter in the gut of various species [43–46]. More recently de Lorijn et al. [47] have shown that the inhibitory innervation of the murine internal anal sphincter and the rectoanal inhibitory reflex are mediated by NO, and the rectoanal inhibitory reflex requires an intact network of ICC in the internal anal sphincter. Thus both loss of nitrergic innervation and deficiency of ICC lead to impaired anal relaxation and may play an important role in rectal evacuation disorders.

4.5 Embryologic Studies on Rodent Models of Aganglionosis

Webster [17, 18] reported studies on both the ls and s^l mice in which he used a nonspecific esterase stain to follow migratory patterns of enteric neuronal precursors in embryos of mutant mice. In both cases there was slowing of migration such that the migrating vagal neural crest cells (VNCC) did not keep pace with the rapidly elongating gut, although the cells still showed signs of distal migration for several days after the usual time of cessation of migration of VNCC in the normal embryos. Webster interpreted these results as suggesting a defect in the vagal neural crest. Using the nonspecific esterase strain in the mutant and control animals there was no evidence of a sacral neural crest input. However, Rothman and Gershon found different results studying the same ls mouse strain [48, 49]. They found that the gut microenvironment in the embryo is unreceptive and cannot support enteric neuronal precursors. The principal experiments in reaching this conclusion were cocultures where isolated segments of aganglionic colon from mutant mice were placed next to sources of neural crest cells (either vagal crest or proximal gut). Aganglionic gut from the mutants was never normally innervated whereas distal gut from the normal embryo was receptive to ingrowth of enteric neuronal precursors. Rather, enteric neuronal precursors tended to avoid aganglionic gut tissue [50]. Other studies looking at the extracellular matrix have shown increases in laminin, collagen type IV and chondroitin sulfate in the distal aganglionic gut [51, 52]. These authors concluded that it is an excess of these extracellular matrix molecules, in particular laminin, which results in a hostile local microenvironment and this causes aganglionosis. They proposed that there is no defect in the émigrés from the neural crest.

Commencing in 1984 our laboratory studied the ls, s^l mouse and sl rat animal models, using histology, tissue culture and the kidney capsule techniques. We used three techniques in an attempt to overcome possible artifacts with any one technique, and three animal models to detect any differences between each of the animal strains. The results in all animals, and with each of the three techniques, agreed with those of Webster. In particular, there was slowing of migration in what was the one predominant source of enteric neurons, namely vagal neural crest cells. The slowing in the migration of these cells occurred well before the eventual aganglionic zone was reached and migration spluttered on for several days after it would normally have ceased. In the ls and s^l mice the slowing commenced in the terminal small bowel whereas in the sl rats the slowing was apparent even in the proximal small bowel. The appearances were most consistent with the interpretation of a lesser population of enteric precursors in the mutants and insufficient numbers to fully colonize the embryonic gut [53].

We made one new finding which shed some light on the contentious debate as to the existence and extent of a contribution of the second sacral neural crest to the vagal neural crest cells: there were small numbers of enteric precursors in the most distal hindgut. These cells were usually in groups of two to four (maximum ten) in contrast to the vagal émigrés which proliferated into the thousands. There was no obvious difference in the numbers of these sacral neural crest cells in the mutants compared to normal embryos [34]. This result was confirmed using the three different experimental techniques. Therefore, as in most good debates, both sides are correct—there is a sacral neural crest contribution to the ENS, but this contribution is functionally insignificant. Nevertheless, failure of the vagal derived neural crest cells to colonize the hindgut is the prime cause of failure of hindgut enteric nervous development. Furthermore, the interaction between sacral and vagal enteric neural crest cells may be necessary for sacral neural crest cell contribution to the ENS [54, 55].

However, the use of immunohistochemistry, special stains and techniques such as the kidney capsule were limited in achieving a full understanding of how aganglionosis arises in the animal models. Ultimately, all the techniques are indirect, and the debate between the

various theories (see below) could not be settled. Therefore, the new techniques of molecular genetics, that is linkage studies and gene knockouts, were utilized. Initially, the first strategy was linkage studies, as there was no knowledge of which genes were likely to be involved and so knocked out. Theoretically the linkage strategy was simple but during the laborious application of these techniques the answers came from knocking out genes known to have a completely unrelated physiologic function with the unexpected finding that aganglionosis resulted.

4.6 Molecular Genetics

We will focus here on the animal work that lead to a better understanding of the molecular genetics of HSCR and allied disorders (for further details see Chapter 5).

4.6.1 Backcross and Linkage

As the rodent model animals are inbred, if polymorphisms are introduced by outbreeding (backcrossing) then a linkage strategy should allow the chromosomal area responsible for the mutation to be progressively narrowed and finally the gene isolated [56].

In several laboratories (including ours) a lethal spotting backcross strategy was used to try and localize the gene. It was already known that the chromosomal location was on mouse chromosome 2, and backcross studies narrowed the area to between GNAS and endothelin 3 (*edn3*) (Ramu E et al., unpublished work; [57]). However before further work could be done to walk into the mutation area, the answer came from knockout experiments (see below). A similar backcross strategy was used in the s^{l} mouse. Work was more successful in these experiments, in that the regions of interest were localized and cloned, but it remained difficult to identify the gene involved [58]. Similarly in the sl rat, a project was initiated in our laboratory to localize the gene responsible for aganglionosis in the rat, using the mutant animals crossed with the DA rat. The DA rat was chosen because of its heavy pigmentation which allowed the wild types and heterozygotes to be clearly distinguished from mutants in the F2 offspring litter. However, while this work was progressing the answer came from knockouts of genes found initially in humans and adult animals whose full function was being explored by the use of experiments in which the gene was removed and then what happened in the offspring was observed.

4.6.2 Knockout Models

With advances in knockout and transgenic technology, many molecules and several signaling pathways have been identified as important in the control of mammalian ENS development (Table 4.2).

4.6.2.1 *Ret/Gdnf/Gfrα1* and *Ret/Ntn/Gfrα2* Knockout Mouse

The *Ret/Gdnf/Gfrα1* signaling pathway is of importance in ENS development, having been shown to promote survival of neurons, mitosis of neuronal progenitor cells, differentiation of neurons and neurite extension [59, 60].

The first targeted gene deletion knockout model was of the *ret* gene which unexpectedly produced a phenotype similar to aganglionosis. The *ret* gene had been initially isolated in a tumor cell line [61]. Subsequent examination of these animals showed a total absence of ganglion cells throughout the gut and associated renal anomalies [62]. *Ret* is normally expressed in the embryonic gut [63]. *Ret*–/– mice exhibit a failure of neural crest colonization of the gut distal to the gastric cardia. The esophagus and gastric cardia also exhibit a reduced population of neurons and glia [64, 65].

This animal work was critically important in identifying *RET* as a firm human candidate gene in the area previously identified as deleted on chromosome 10 [66–69]. Without this vital research, *RET* would have remained only one of a dozen or so candidate genes in this deleted area of the human chromosome. It was a combination of both the human and animal works that allowed the early identification of *RET* as the first gene responsible for HSCR [70, 71].

Likewise, *gdnf*–/– and *gfrα1*–/– mice have almost identical phenotypes to *ret*–/– mice [72, 73]. To date, a few *GDNF* mutations and no *GFRα1* mutations have been identified in humans with HSCR [74–76].

Gfrα2 and *ntn* are, like *gfrα1* and *gdnf*, capable of forming a *ret*-activation complex and are thought to be required for the maintenance of a subset of enteric ganglia [77]. *Ntn*–/– and *gfrα2*–/– mice exhibit a decrease in the density of cholinergic neurons in the ENS but no renal abnormalities, and the mice survive and breed [77, 78]. It appears that *NTN* mutation alone does not result in HSCR, but could contribute to the severity of HSCR due to other mutations [79]. To our knowledge, the *GFRα2* mutation has not been identified yet in humans with HSCR.

4.6.2.2 *Ednrb/Edn3/Ece1* Knockout Mouse

Endothelins had been discovered while searching for contractile substances in the pig aorta [80]. Scientists interested in the biologic function of endothelin genes also produced a series of targeted gene deletion knockouts in an attempt to see how animals without the gene would function. The first of these was an endothelin-1 (*edn1*) deletion, due to disruption of the endothelin-convert-

Table 4.2 Knockout mouse models of Hirschsprung's disease and allied disorders [54, 59, 119]

Mice	Gene	Function	Phenotypea	Human locus	Percent in HSCR
Ret−/−	RET	Tyrosine kinase receptor	Total intestinal aganglionosis	10q11.2	70–80% long segment, 50% familial, 15–20% sporadic
Gdnf−/−	GDNF	Glial cell-derived neurotrophic factor	Total intestinal aganglionosis	5p12–13.1	<10%
G Gfrα1−/−	GFRα1	GDNF family receptor alpha 1	Total intestinal aganglionosis	10q26	–
Ntn−/−	NTN	Neurturin, RET ligand	Hypoganglionosis	19q13.3	<1%
Gfrα2−/−	GFRα2	GDNF family receptor alpha 2	Hypoganglionosis	–	–
Etr3−/−, ls	EDN3	Endothelin-3	Distal hindgut aganglionosis	20q13	<10%
EdnrB−/−,Sl	EDNRB	Endothelin-B-receptor	Distal hindgut aganglionosis	13q22	<10%
Ece 1−/−	ECE-1	Endothelin-converting enzyme	Distal hindgut aganglionosis	1p36.1	<1%
Sox 10DOM, DOM	SOX 10	Sry/HMG box transcription factor	Complete gut aganglionosis	22q13.1	<1%
Phox2b−/−	Phox2b	Paired-like homeobox 2b	Complete gut aganglionosis	4p12	<1%
Pax3−/−	PAX3	Paired box gene 3	Total intestinal aganglionosis	2q37	–
Ncx−/−	HOX11L1	Homeobox 2	INDB-like condition	2p13.1	–
Ihh−/−	IHH	Indian hedgehog	Skip intestinal aganglionosis	2q33–q35	–
Shh−/−	SHH	Sonic hedgehog	Ectopic mucosal neurons	7q36	–

[a]Of homozygous mice

ing enzyme-1 gene (*ece1*), which produced craniofacial and cardiac defects in addition to colonic aganglionosis, suggesting that the endothelins were important in neural crest development [81, 82]. In follow-on experiments, when the endothelin-3 gene (*edn3*) was made functionally null, a phenotype resulted which looked identical to the lethal spotting mouse. Similarly when the endothelin B receptor (*ednrb*) was made nonfunctional, the offspring resembled piebald lethal animals. Examination of the gut confirmed aganglionosis. Researchers then examined the lethal spotting mice and piebald lethal mice for defects in *edn3* and *ednrb*, respectively. They confirmed that *edn3* and *ednrb* have a role in the migration and development of the ENS, and defects of the endothelin signaling pathway are responsible for the phenotypes of these animal models. Namely, in the lethal spotting mouse there was a point mutation in the proendothelial-3 gene which prevented cleavage and resulted in no *edn3*; and in the piebald lethal mouse there was a complete deletion of *ednrb* (Table 4.1) [83–86].

The shorter length of aganglionosis in the lethal spotting mouse is thought to be due to the other endothelins (*edn1* and *edn2*) partially reacting with the *ednrb* and producing a milder form of aganglionosis. This would explain why in the piebald lethal animals the length of aganglionosis is on average about 10 mm, whereas in the lethal spotting mouse the length of aganglionosis is about 2 mm.

In our laboratory we have crossed both lethal spotting and piebald lethal animals and the resultant length of

aganglionosis appears to be similar or only slightly longer than that of the piebald lethal mouse. Therefore there appears to be no additive factor between the two genes and the defect produced by the piebald lethal lesion which is due to the absence of *ednrb* is the limiting factor in the length of aganglionosis. However, when the animals are backcrossed with either C57 or Castaneus animals, the innervation of the most distal colon appears to be near normal. Therefore, there are modifier genes affecting the length of aganglionosis in these mouse models.

Knowledge that *ret* and *ednrb* defects are involved in mice suggested that defects in these genes were candidates for the spotting lethal rat. First a defect in *ret* was excluded, along with *edn3*. Working initially on a cDNA from the rat *ednrb*, we noted a 250–300 bp deletion. Further experiments using both the cDNA and genomic DNA localized the defective area to the end of the first translating exon and the next 17 bp sequence of the first intron of the *ednrb*. This 301 bp deletion results in alternative splicing which results in either a stop codon or an in-frame 270 bp deletion and a protein product with an inability to insert into the cell membrane [87, 88].

A recent study has shown that abnormalities of the ENS in heterozygous *ednrb*-deficient spotting lethal rats resemble those in intestinal neuronal dysplasia B (INDB) [89].

These mouse animal experiments again led the way to the discovery of defects in the same genes in humans. Subsequently, defects in *EDNRB* [90–94] and *EDN3* [95, 96] have been found in humans.

4.6.2.3 *Sox10* Knockout Mouse

Sox10 was identified while doing a comparative study of human/mouse sequences [97]. It is a member of the sry-related family of transcription factors [98]. The naturally occurring Dom mouse model of HSCR was used to identify the role of *sox10* in ENS development [99]. It appears that early death of neural crest cells is responsible for the complete aganglionosis of the gut in *sox10Dom*/*sox10Dom* mice [100]. *Sox10Dom*/+ mice exhibit distal hindgut aganglionosis and pigmentation abnormalities [101]. A similar gene mutation has been identified in patients with Waardenburg-Shah syndrome [102].

4.6.2.4 *Phox2B* Knockout Mouse

Phox2b is a homeodomain-containing transcription factor that regulates *ret* expression and thus it is essential for ENS development [103, 104]. *Phox2b*–/– mice exhibit neural crest colonization of foregut only. Subsequently, the foregut neural crest cells undergo apoptosis. At birth, animals exhibit complete aganglionosis of the gut [104]. *PHOX2B* is proposed to be a candidate gene in patients with Haddad syndrome [104, 105].

4.6.2.5 *Pax3* Knockout Mouse

Pax3 is a member of the paired-box-containing family of transcription factors [106]. It appears that *pax3* is required for the formation of enteric ganglia and functions with *sox10* to modulate *ret* expression, and thus there are no enteric neurons caudal to the stomach in *pax3*–/– mice [107]. Patients with Waardenburg syndrome without HSCR usually have mutations in *PAX3* [108].

4.6.2.6 *Hox11L1* Knockout Mouse

Hox11L1 is a homeobox transcription factor which may play a role in neural crest cell proliferation or differentiation [109]. *Hox11L1*–/– mice develop an INDB-like condition, followed by death of some of the enteric neurons [109, 110]. The *Hox11L1*–/– mouse has been proposed as a model for INDB. Further knowledge of the regulatory genes and the transcriptional targets of *Hox11L1* may produce candidate genes for involvement in INDB, and thus a better understanding of this controversial disease entity [59, 111].

4.6.2.7 *Ihh*–/– and *Shh*–/– Knockout Mouse

Indian hedgehog (*Ihh*) and Sonic hedgehog (*Shh*) genes may influence survival and/or differentiation of neural crest cells [112]. *Ihh*–/– and *Shh*–/– mice die during early embryonic stages. Late fetal *Ihh*+/– mice exhibit a dilated region of the colon, with missing enteric neurons in some parts of the small intestine and the dilated region of the colon [113]. *Shh*+/– mice do not lack an ENS in any part of the gut, but nerve cell bodies are present within the mucosa [113]. Both *IHH* and *SHH* are possible candidate genes for ENS defects in humans [59].

4.7 Contribution of Animal Models to Theories as to the Cause of Aganglionosis

There are two broad theories as to the cause of aganglionosis (with many internal minor refinements of the theories being possible), and workers investigating the animal models have found evidence for both theories.

4.7.1 Defect of Central Vagal Neural Crest Cell Production and Migration

Webster's and our interpretation of the slowing of migration found in mutant embryos is that this suggests an early defect in the vagal neural crest in the production of sufficient neural crest cells to adequately populate the gut.

This hypothesis would fit with the ablation experiments of Yutema and Hammond (see Chapter 2).

As a refinement to this theory we hypothesize that the uneven distribution we see in humans and mutant animal experiments suggests evidence of clones or discrete quanta of precursors or mother cells, perhaps as low as four to six quanta from which arise all progeny that normally populate the gut. If one quantum is missing, distal colonic aganglionosis results; if two quanta are missing, ileal aganglionosis results; if three are missing, mid-small-gut aganglionosis results; if four quanta are missing, total intestinal aganglionosis results [34]. Certainly in the mutant rats we do not see an even or random distribution of the site of the commencement of aganglionosis; rather there are three most frequent "nodal regions" where aganglionosis commences. Our hypothesis is that the quanta are generated at a vagal neural crest level over several somites and the defect in the mutants occurs initially at a premigratory stage, with the eventual extent of bowel aganglionosis being merely a later playing out of this early vagal neural crest defect. It is as if there is a "checkerboard" of potential spaces to be filled and a number of precursor families to fill the spaces. Because the spaces are always filled from a proximal direction, no matter which family is missing, the result will be distal aganglionosis. The only variable is that if there are more families missing then the length of aganglionosis will be longer (Cass, First World Workshop in Hirschsprung's Disease, Sestri Levante, 1993, unpublished data).

4.7.2 Defect in the Local Gut Microenvironment

Gershon and coworkers extensive experimental work supports defects in the mutants being in the gut microenvironment itself and being specifically related to excess laminin causing migrating enteric neuronal precursors to mature early and hence not to continue to divide or migrate [114]. Nishijima et al. [115] found migration down the mouse embryonic gut was not an even process but rather proceeded in bursts followed by a pause. In the lethal spotting mouse mutants, migration proceeded normally but then suddenly stopped at the last of these boundaries, resulting in aganglionosis. The authors interpreted this result as indicating that the gut had subtly different microenvironments, and an intrinsic defect in the last 2 mm of the mouse colon resulted in aganglionosis. Similarly Kapur et al. [116], using transgenic mice with a cell label DβH-inlacZ and chimeric animals, showed that the enteric neurons from ls/ls could populate the distal gut. The explanation was that the enteric neuronal precursor from the normal embryo contributed a factor that overcomes the microenvironmental defect [116–118]. In our experiments, we could not demonstrate defects in the extracellular matrix in early embryos [34, 53]. Rather the increase in the extracellular matrix components appeared to be a secondary event [34, 53].

4.8 Summary

In summary, the animal models of aganglionosis have been pivotal in the discovery of the genes of HSCR. In future, animal models will continue to contribute to the understanding of how the genes interact and are modified by yet other genes. In addition, animal models of aganglionosis will continue to contribute to the anatomic, physiologic and pharmacologic understanding of aganglionosis.

References

1. Derrick EH, St George-Grambauer BM (1957) Megacolon in mice. J Path Bacteriol 73:569–571
2. Bielschowsky M, Schofield GC (1962) Studies on megacolon in piebald mice. Aust J Exp Biol Med Sci 40:395–404
3. Lane PW (1966) Association of megacolon with two recessive spotting genes in the mouse. J Hered 57:181–183
4. Ikadai H, Agematsu Y, Imamichi T (1979) Observation of congenital aganglionosis rat (Hirschsprung's disease rate) and its genetic analysis (in Japanese). Congen Anom 19:31–36
5. Lane PW, Liu HM (1984) Association of megacolon with a new dominant spotting gene (Dom) in the mouse. J Hered 75:335–339
6. Dietzmann VU (1968) uber das Vorkommen des kongenitalen Megakolons (Hirschsprungsches Megakolon) bei der Katz. Mh Veterinermed 23:349–352
7. Yoder R (1968) Colectomy in cats. Vet Med Small Anim Clin 63:1049
8. Hultgren BD (1982) Ileocolonic aganglionosis in white progeny of overa spotted horses. J Am Vet Med Assoc 180:289–292
9. McCabe L, Griffin LD, Kinzer A, Chandler M, Beckwith JAB, McCabe ERB (1990) Overo lethal white foal syndrome: equine model of aganglionic megacolon (Hirschsprung disease). Am J Med Genet 36:336–340
10. Kyke TM, Laing EA, Hutchins DR (1990) Megacolon in two related Clydesdale foals. Aust Vet J 67:463–464
11. Yang GC, Croaker GD, Zhang AL, Manglick P, Cartmill T, Cass DT (1998) A dinucleotide mutation in the endothelin-β receptor gene is associated with lethal white foal syndrome (LSWF): a horse variant of Hirschsprung's disease (HSCR). Hum Mol Genet 7:1047–1052
12. Kernkampe HCH, Kanning HH (1995) Primary megacolon (Hirschsprung's disease) in swine. North Am Vet 36:642–643
13. Osborne JC, Davis JW, Farley H (1968) Hirschsprung's disease: a review and report of the entity in a Virginia swine herd. Vet Med Small Anim Clin 63:451–453
14. Bolande RP, Towler WF (1992) Ultrastructural and histochemical studies of murine megacolon. Am J Pathol 69:139–162

15. Bolande RP (1975) Animal model: aganglionic megacolon in piebald and spotted mutant mouse strains. Am J Pathol 79:189–192
16. Boley SJ (1975) The pathogenesis of Hirschsprung's disease – a continuing research. J Pediatr Surg 10:861–863
17. Webster WS (1973) Embryogenesis of enteric ganglia in normal mice and in mice that develop congenital aganglionic megacolon. J Embryol Exp Morphol 30:573–585
18. Webster W (1974) Aganglionic megacolon in Piebald-lethal mice. Arch Pathol 97:111–117
19. Bu'Lock A, Vaillant C, Dockray GJ (1984) Selective depletion of Substance P-immunoreactive neurons in the transition zone of the colon in Piebald lethal mice. Neurochem Int 6:55–61
20. Ikadai H, Suzufi K, Fujita H, Imamichi T (1981) Animal models of human disease. Hirschsprung's disease. Comp Pathol Bull 13:3–4
21. Horie H, Ikadai H, Iwasaki I, Ide G, Takahashi H (1980) Pathological studies on newly established congenital aganglionosis rat in Japan. J Jpn Soc Pediatr Surg 16:549–560
22. Nagahama M, Ozaki T, Hama K (1985) A study of the myenteric plexus of the congenital aganglionosis rat (spotting lethal). Anat Embryol 171:285–296
23. Nagahama M, Semba R, Tsuzuki M, Ozaki T (2001) Distribution of peripheral nerve terminals in the small and large intestine of congenital aganglionosis rats (Hirschsprung's disease rats). Pathol Int 51:145–157
24. Ward SM, Ordog T, Bayguinov JR, Horowitz B, Epperson A, Shen L, Westphal H, Sanders KM (1999) Development of interstitial cells of Cajal and pacemaking in mice lacking enteric nerves. Gastroenterology 117:584–594
25. Ward SM, Gershon MD, Keef K, Bayguinov YR, Nelson C, Sanders KM (2002) Interstitial cells of Cajal and electrical activity in ganglionic and aganglionic colons of mice. Am J Physiol Gastrointest Liver Physiol 283:G445–456
26. Horisawa M, Watanabe Y, Torihashi S (1998) Distribution of c-kit immunopositive cells in normal colon and in Hirschsprung's disease. J Pediatr Surg 33:1209–1214
27. Vanderwinden JM, Rumessen JJ, Liu H, Descamps D, De Laet MH, Vanderhaeghen JJ (1996) Interstitial cells of Cajal in human colon and in Hirschsprung's disease. Gastroenterology 111:901–910
28. Yamataka A, Kato Y, Tibboel D, Murata Y, Sueyoshi N, Nishiye H, Miyano T (1995) A lack of intestinal pacemaker (c-kit) in aganglionic bowel of patients with Hirschsprung's disease. J Pediatr Surg 30:441–444
29. Taniguchi K, Matsuura K, Matsuoka T, Nakatani H, Nakano T, Furuya Y, Sugimoto T, Kobayashi M, Araki K (2005) A morphological study of the pacemaker cells of the aganglionic intestine in Hirschsprung's disease utilizing ls/ls model mice. Med Mol Morphol 38:123–129
30. Wood JD (1973) Electrical activity of the intestine of mice with hereditary megacolon and absence of enteric ganglion cells. Am J Dig Dis 18:477–488
31. Brann L, Wood JD (1976) Motility of the large intestine of piebald lethal mice. Am J Dig Dis 21:633–640
32. Brann L, Furtado D, Migliazzo CV, Baxendale J, Wood JD (1977) Secondary effects of aganglionosis in the piebald-lethal mouse model of Hirschsprung's disease. Lab Anim Sci 27:946–954
33. Nakai Y, Okasora T, Okamoto E (1994) Studies on cholinergic nerve function of the aganglionic colon in murine model. J Smooth Muscle Res 30:73–84
34. Cass DT (1993) The treatment and cause of aganglionosis. Vol 2: Studies in rodents. PhD Thesis, Department of Paediatric Surgery, Sydney University, Sydney, Australia
35. Alvarez WC (1949) A simple explanation for cariospasm and Hirschsprung's disease. Gastroenterology 13:422–429
36. Richardson J (1975) Pharmacologic studies of Hirschsprung's disease on a murine model. J Pediatr Surg 10:875
37. Chakder S, McHugh KM, Rattan S (1997) Inhibitory neurotransmission in lethal spotted mutant mice: a model for Hirschsprung's disease. Gastroenterology 112:1575–1585
38. Kubota M, Ito Y, Taguchi T, Ikeda K, Ikadai H (1989) Regional differences in the pattern of neurogenic responses in the aganglionic colon from congenitally aganglionic rats. J Pediatr Surg 24:911–919
39. Okasora T, Okamoto E, Toyosaka A, Nose K, Nakai Y, Tomimoto Y (1990) Study on function of aganglionic colon musculature of Hirschsprung's disease murine model. Nippon Heikatsukin Gakkai Zasshi 26:131–136
40. Wood JD, Brann LR, Vermillion DL (1986) Electrical and contractile behavior of large intestinal musculature of piebald mouse model for Hirschsprung's disease. Dig Dis Sci 31:638–650
41. Caniano DA, Grace GT, Sun CC, Ormsbee HS 3rd, Hardy FE, Hill JL (1986) Functional response to vasoactive intestinal peptide in piebald lethal mice. J Pediatr Surg 21:1128–1132
42. Bult H, Boeckxstaens GE, Pelckmans PA, Jordaens FH, Van Maercke YM, Herman AG (1990) Nitric oxide as an inhibitory non-adrenergic no-cholinergic neurotransmitter. Nature 345:346–347
43. Boeckxstaens GE, Pelckmans PA, Bult H, et al (1990) Non-adrenergic non-cholinergic relaxation mediated by nitric oxide in the canine ileocolonic junction. Eur J Pharmacol 190:239–246
44. Rolle Udo, Nemeth L, Puri P (2002) Nitrergic innervation of the normal gut and in motility disorders of childhood. J Pediatr Surg 37:551–567
45. Sanders KM, Ward SM (1992) Nitric oxide as a mediator of nonadrenergic noncholinergic neurotransmission. Am J Physiol 262:G379–G392
46. Stark ME, Bauer AJ, Starr MG, et al (1993) Nitric oxide mediated inhibitory input in human and canine jejunum. Gastroenterology 103:398–409
47. de Lorijn F, de Jonge WJ, Wedel T, Vanderwinden JM, Benninga MA, Boeckxstaens GE (2005) Interstitial cells of Cajal are involved in the afferent limb of the rectoanal inhibitory reflex. Gut 54:1107–1113
48. Rothman TP, Gershon MD (1982) Phenotypic expression in the developing murine enteric nervous system. J Neurosci 2:381–393
49. Rothman TP, Gershon MD (1984) Regionally defective colonization of the terminal bowel by the precursors of enteric neurons in lethal spotted mutant mice. Neuroscience 12:1293–1311

50. Jacob-Cohen RJ, Payette RF, Gershon MD, Rothman TP (1987) Inability of neural crest cells to colonise the presumptive aganglionic bowel of ls/ls mutant mice: requirements for a permissive microenvironment. J Comp Neurol 255:425–438
51. Payette RF, Tennyson VM, Pham TD, Mawe GM, Pomeranz HD, Rothman TP (1987) Origin and morphology of nerve fibers in the aganglionic colon of the lethal spotted (ls/ls) mutant mouse. J Comp Neurol 257:237–252
52. Payette RF, Tennyson VM, Pham TD, Mawe GM, Pomeranz HD, Rothman TP, Gershon MD (1988) Accumulation of components of basal laminae: association with the failure of neural crest cells to colonize the presumptive aganglionic bowel of ls/ls mutant mice. Dev Biol 125:341–360
53. Cass DT, Zhang AL, Morthorpe J (1992) Aganglionosis in rodents. J Pediatr Surg 27:351–356
54. Gariepy CE (2001) Intestinal motility disorders and development of the enteric nervous system. Pediatr Res 49:605–613
55. Newgreen D, Young HM (2002) Enteric nervous system: development and developmental disturbances – part 2. Pediatr Dev Pathol 5:329–349
56. Rothwell NV (1993) Understanding genetics – a molecular approach. Wiley-Liss, New York
57. Pavan WJ, Mac S, Cheng M, Tilghman SM (1995) Quantitative trait loci that modifies the severity of spotting in piebald mice. Genome Res 5:29–41
58. Metallinos DL, Oppenheimer AJ, Rinchik EM, Russell LB, Dietrich W, Tilghman SM (1994) Fine structure mapping and deletion analysis of the murine piebald locus. Genetics 136:217–223
59. Newgreen D, Young HM (2002) Enteric nervous system: development and developmental disturbances – part 1. Pediatr Dev Pathol 5:224–247
60. Taraviras S, Pachnis V (1999) Development of the mammalian enteric nervous system. Curr Opin Genet Dev 9:321–327
61. Takahashi M, Buma Y, Iwamoto T, Inaguma Y, Ikeda H, Hiai H (1988) Cloning and expression of the ret proto-oncogene encoding a tyrosine kinase with two potential transmembrane domains. Oncogene 3:571–578
62. Schuchardt A, D'Agayi V, Larsson-Blomberg L, Costanini F, Pachnis V (1994) Defects in the kidney and enteric nervous system of mice lacking the tyrosine kinase receptor Ret. Nature 367:380–383
63. Pachnis V, Mankoo B, Costantini F (1993) Expression of the c-ret proto-oncogene during mouse embryogenesis. Development 119:1005–1017
64. Durbec PL, Larsson-Blomberg LB, Schuchardt A, Costantini F, Pachnis V (1996) Common origin and developmental dependence on c-ret of subsets of enteric and sympathetic neuroblasts. Development 122:349–358
65. Taraviras S, Marcos-Gutierrez CV, Durbec P, Jani H, Grigoriou M, Sukumaran M, Wang LC, Hynes M, Raisman G, Pachnis V (1999) Signalling by the RET receptor tyrosine kinase and its role in the development of the mammalian enteric nervous system. Development 126:2785–2797
66. Martucciello G, Biocchi M, Dodero P, Lernone M, Cirillo MS, Puliti A, et al (1992) Total colonic aganglionosis associated with interstitial deletion of the long arm of chromosome 10. J Pediatr Surg 7:308–310
67. Lo L, Anderson DJ (1995) Postmigratory neural crest cells expressing c-RET display restricted developmental and proliferative capacities. Neuron 15:527–539
68. Angrist M, Kauffman EG, Slaugenhaupt SA, Matise TC, Puffenberger EG, Washington SS, et al (1993) A gene for Hirschsprung's disease (megacolon) in the pericentromeric region of chromosome 10. Nat Genet 4:351–356
69. Lyonnet S, Bolino A, Pelet A, Abel L, Nihoul-Fekete C, Briard M, et al (1993) A gene for Hirschsprung disease maps to the proximal long arm of chromosome 10. Nat Genet 4:346–350
70. Edery P, Lyonnet S, Mulligan L, Pelet A, Dow E, Holder, S, et al (1994) Mutations of the RET proto-oncogene in Hirschsprung disease. Nature 367:378–380
71. Romeo G, Rochetto P, Luo Y, Barone V, Seri M, Ceccherini I, et al (1994) Point mutations affecting the tyrosine kinase domain of the RET proto-oncogene in Hirschsprung disease. Nature 367:377–378
72. Enomoto H, Araki T, Jackman A, Heuckeroth RO, Snider WD, Johnson EM Jr, et al (1998) GFR alpha1-deficient mice have deficits in the enteric nervous system and kidneys. Neuron 21:317–324
73. Moore MW, Klein RD, Farinas I, Sauer H, Armanini M, Philips H, et al (1996) Renal and neuronal abnormalities in mice lacking GDNF. Nature 382:76–79
74. Angrist M, Bolk S, Halushka M, Lapchak PA, Chakravarti A (1996) Germline mutations in glial cell line-derived neurotrophic factor (GDNF) and RET in a Hirschsprung disease patient. Nat Genet 14:341–344
75. Ivanchuk SM, Myers SM, Eng C, Mulligan LM (1996) De novo mutation of GDNF, ligand for the RET/GDNFR-alpha receptor complex, in Hirschsprung disease. Hum Mol Genet 5:2023–2026
76. Martucciello G, Thompson H, Mazzola C, Morando A, Bertagnon M, Negri F, et al (1998) GDNF deficit in Hirschsprung's disease. J Pediatr Surg 33:99–102
77. Rossi J, Luukko K, Poteryaev D, Laurikainen A, Sun YF, Laakso T, et al (1999) Retarded growth and deficits in the enteric and parasympathetic nervous system in mice lacking GFR alpha2, a functional neurturin receptor. Neuron 22:243–252
78. Heuckeroth RO, Enomoto H, Grider JR, Golden JP, Hanke JA, Jackman A, et al (1999) Gene targeting reveals a critical role for neurturin in the development and maintenance of enteric, sensory, and parasympathetic neurons. Neuron 22:253–263
79. Doray B, Salomon R, Amiel J, Pelet A, Touraine R, Billaud M, et al (1998) Mutation of the RET ligand, neurturin, supports multigenic inheritance in Hirschsprung disease. Hum Mol Genet 7:1449–1452
80. Sakurai T, Yanagisawa M, Masaki T (1992) Molecular characterization of endothelin receptors. Trends Pharmacol Sci 13:103–108
81. Kurihara Y, Kuihara H, Suzuki H, Kodama T, Maemura K, Nagai R, et al (1994) Elevated blood pressure and craniofacial abnormalities in mice deficient in endothelin-1. Nature 368:703–710
82. Yanagisawa H, Yanagisawa M, Kapur RP, Richardson JA, Williams SC, Clouthier DE, et al (1998) Dual genetic pathways of endothelin-mediated intercellular signaling revealed by targeted disruption of endothelin converting enzyme-1 gene. Development 125:825–836

83. Baynash AG, Hosoda K, Giaid A, Richardson J, Emoto N, Hammer R, et al (1994) Interaction of endothelin-3 with endothelin-B receptor is essential for development of epidermal melanocytes and enteric neurons. Cell 79:1277–1285
84. Hosoda K, Hammer R, Richardson J, Baynash A, Cheung J, Giaid A, et al (1994) Targeted and natural (Piebald-Lethal) mutations of endothelin-B receptor gene produces megacolon associated with spotted coat color in mice. Cell 79:1267–1276
85. Leibl MA, Ota T, Woodward MN, et al (1999) Expression of endothelin-3 by mesenchymal cells of embryonic mouse caecum. Gut 44:246–252
86. Rice J, Doggett B, Sweetser DA, et al (2000) Transgenic rescue of aganglionosis and piebaldism in lethal spotted mice. Dev Dyn 217:120–132
87. Ceccherini I, Zhang A, Matera I, Yang G, Devoto M, Romeo G, et al (1995) Interstitial deletion of the endothelin-B receptor gene in the spotting lethal (sl) rat. Hum Mol Genet 4:2089–2096
88. Gariepy CE, Cass DT, Yanagisawa M (1996) Null mutation of endothelin-B receptor in spotting lethal rats causes aganglionic megacolon and white coat color. Proc Natl Acad Sci U S A 93:867–872
89. Von Boyen GBT, Krammer HJ, Suss A, et al (2002) Abnormalities of the enteric nervous system in heterozygous endothelin B receptor deficient (spotting lethal) rats resembling intestinal neuronal dysplasia. Gut 51:414–419
90. Puffenberger EG, Hosoda K, Washington SS, Nako K, deWit D, Yanigisawa M, et al (1994) A missense mutation of endothelin-B receptor gene in multigenic Hirschsprung disease. Cell 79:1257–1266
91. Auricchio A, Cassari G, Staiano A, Ballabio A (1996) Endothelin-B receptor mutations in patients with isolated Hirschsprung disease from a non-inbred population. Hum Mol Genet 5:351–354
92. Kasafuka T, Wang Y, Puri P (1996) Novel mutations of the endothelin-B receptor gene in isolated patients with Hirschsprung disease. Hum Mol Genet 5:347–349
93. Amiel J, Attie T, Jan D, Pelet A, Edery P, Bidaud C, et al (1996) Heterozygous endothelin receptor B (EDNRB) mutations in isolated Hirschsprung disease. Hum Mol Genet 5:355–357
94. Attie T, Till M, Pelet A, Amiel J, Edery P, Boutrand L, et al (1995) Mutation of the endothelin-receptor B gene in Waardenburg-Hirschsprung disease. Hum Mol Genet 4:2407–2409
95. Edery P, Attie T, Amiel J, Pelet A, Eng C, Hofstra RMW, et al (1996) Mutation of the endothelin-3 gene in the Waardenburg-Hirschsprung disease (Shah-Waardenburg syndrome). Nat Genet 12:442–444
96. Hofstra RMW, Osinga J, Tan-Sindhunata G, Wu Y, Kamsteeg E-J, Stulp RP, et al (1996) A homozygous mutation in the endothelin-3 gene associated with a combined Waardenburg type 2 and Hirschsprung phenotype (Shah-Waardenburg syndrome). Nat Genet 12:445–447
97. Push C, Hustert E, Pfeifer D, Sudbeck P, Kist R, Roe B, et al (1998) The SOX10/Sox10 gene from human and mouse: sequence, expression, and transactivation by the encoded HMG domain transcription factor. Hum Genet 103:115–123
98. Kulbrodt K, Herbarth B, Sock E, Hermans-Borgmeyer I, Wegner M (1998) Sox10, a novel transcriptional modulator in glial cells. J Neurosci 18:237–250
99. Southard-Smith EM, Kos L, Pavan WJ (1998) Sox 10 mutation disrupts neural crest development in Dom Hirschsprung mouse model. Nat Genet 18:60–64
100. Kapur RP (1999) Early death of neural crest cells is responsible for total enteric aganglionosis in Sox10(Dom)/Sox10(Dom) mouse embryos. Pediatr Dev Pathol 2:559–569
101. Herbarth B, Pingault V, Bondurand N, Kuhlbrodt K, Hermans-Borgmeyer I, Puliti A, et al (1998) Mutation of the Sry-related Sox10 gene in Dominant megacolon, a mouse model for human Hirschsprung disease. Proc Natl Acad Sci U S A 95:5161–5165
102. Kuhlbordt K, Schmidt C, Sock E, Pingault V, Bondurand N, Goossens M, et al (1998) Functional analysis of Sox10 mutations found in human Waardenburg-Hirschsprung patients. J Biol Chem 273:23033–23038
103. Pattyn A, Morin X, Cremer H, Goridis C, Brunet JF (1997) Expression and interactions of the two closely related homeobox genes Phox2a and Phox2b during neurogenesis. Development 124:4065–4075
104. Pattyn A, Morin X, Cremer H, Goridis C, Brunet JF (1999) The homeobox gene Phox2b is essential for the development of autonomic neural crest derivatives. Nature 399:366–370
105. Garcia-Barcelo M, Sham MH, Lui VCH, et al (2003) Association study of Phox2b as a candidate gene for Hirschsprung's disease. Gut 52:563–567
106. Goulding MD, Chalepakis G, Deutsch U, Erselius JR, Gruss P (1991) Pax-3, a novel murine DNA binding protein expressed during early neurogenesis. EMBO J 10:1135–1147
107. Lang D, Chen F, Milewski R, Li J, Lu MM, Epstein JA, et al (2000) Pax3 is required for enteric ganglia formation and functions with Sox10 to modulate expression of c-ret. J Clin Invest 106:963–971
108. Tassabehji M, Read AP, Newton VE, et al (1992) Waardenburg's syndrome patients have mutations in the human homologue of the Pax-3 paired box gene. Nature 355:635–636
109. Shirasawa S, Yunker AM, Roth KA, Brown GA, Horning S, Korsmeyer SJ (1997) Enx (Hox11L1)-deficient mice develop myenteric neuronal hyperplasia and megacolon. Nat Med 3:646–650
110. Hatano M, Aoki T, Dezawa M, Yusa S, Iitsuka Y, Koseki H, et al (1997) A novel pathogenesis of megacolon in Ncx/Hox11L1 deficient mice. J Clin Invest 100:795–801
111. Costa M, Fava M, Seri M, et al (2000) Evaluation of the HOX11L1 gene as a candidate for congenital disorders of intestinal innervation (letter). J Med Genet 37:E9
112. Yang JT, Liu CZ, Villavicencio EH, Yoon JW, Walterhouse D, Iannaccone PM (1997) Expression of human GLI in mice results in failure to thrive, early death, and patchy Hirschsprung-like gastrointestinal dilatation. Mol Med 3:826–835
113. Ramalho-Santos M, Melton DA, McMahon AP (2000) Hedgehog signals regulate multiple aspects of gastrointestinal development. Development 127:2763–2772
114. Gershon MD (1995) Neural crest development. Do developing enteric neurons need endothelins? Curr Biol 5:601–604

115. Nishijima E, Meijers JHC, Tibboel D, Luider TM, Peters-van der Sanden MMJ, van der Kamp AWM, et al (1990) Formation and malformation of the enteric nervous system in mice: an organ culture study. J Pediatr Surg 25:627–631
116. Kapur RP, Yost C, Palmiter RD (1993) Aggregation chimeras demonstrate that the primary defect responsible for aganglionic megacolon in lethal spotted mice is not neuroblast autonomous. Development 117:993–999
117. Coventry S, Yost C, Palmiter RD, Kapur RP (1994) Migration of ganglion cell precursors in the ileoceca of normal and lethal spotted embryos, a murine model for Hirschsprung disease. Lab Invest 71:82–93
118. Kapur RP, Yost C, Palmiter RD (1992) A transgenic model for studying development of the enteric nervous system in normal and aganglionic mice. Development 116:167–175
119. Puri P, Shinkai T (2004) Pathogenesis of Hirschsprung's disease and its variants: recent progress. Semin Pediatr Surg 13:18–24