Developmental Biology 417 (2016) 139–157

Contents lists available at ScienceDirect

Developmental Biology

journal homepage: www.elsevier.com/locate/developmentalbiology

Mouse models of Hirschsprung disease and other developmental disorders of the enteric nervous system: Old and new players

Nadege Bondurand [a,b,*], E. Michelle Southard-Smith [c,**]

[a] INSERM, U955, Equipe 6, F-94000 Creteil, France
[b] Universite Paris-Est, UPEC, F-94000 Creteil, France
[c] Vanderbilt University Medical Center, Department of Medicine, 2215 Garland Ave, Nashville, TN 37232, USA

ARTICLE INFO

Article history:
Received 8 March 2016
Received in revised form
27 June 2016
Accepted 27 June 2016
Available online 28 June 2016

Keywords:
Hirschsprung
Enteric nervous system
Neural crest
Mouse models

ABSTRACT

Hirschsprung disease (HSCR, intestinal aganglionosis) is a multigenic disorder with variable penetrance and severity that has a general population incidence of 1/5000 live births. Studies using animal models have contributed to our understanding of the developmental origins of HSCR and the genetic complexity of this disease. This review summarizes recent progress in understanding control of enteric nervous system (ENS) development through analyses in mouse models. An overview of signaling pathways that have long been known to control the migration, proliferation and differentiation of enteric neural progenitors into and along the developing gut is provided as a framework for the latest information on factors that influence enteric ganglia formation and maintenance. Newly identified genes and additional factors beyond discrete genes that contribute to ENS pathology including regulatory sequences, miRNAs and environmental factors are also introduced. Finally, because HSCR has become a paradigm for complex oligogenic diseases with non-Mendelian inheritance, the importance of gene interactions, modifier genes, and initial studies on genetic background effects are outlined.

1. Introduction

Identifying the factors that lead to enteric ganglia deficiencies, such as Hirschsprung disease (HSCR), has been a long-term goal of many investigators who study innervation of the intestine. Efforts have been particularly focused on genetic factors and the molecular effects of discrete coding and non-coding mutations as well as post-translational mechanisms that impact the enteric neural crest cells (ENCCs) that populate the fetal intestine to form the mature enteric nervous system (ENS). Current knowledge has greatly benefited from exchanges between human geneticists and researchers using animal models. Because gene targeting techniques and inbred strains have long been available in laboratory mice, advances in identifying genes that contribute to HSCR susceptibility and subsequent studies of cellular mechanism have been possible through analysis of ENS development. In recent years, the impact of environmental factors on ENS development has been identified and has been advanced by studies in mouse models (Fu et al., 2010; Heuckeroth and Schafer, 2016; Schill et al., 2016).

The ENS is an extensive network of neurons and glial cells within the wall of the bowel that controls gut motility, regulates transport of ions across the epithelium, and modulates blood flow (Furness, 2012). In the small and large intestine, neurons and glial cells are mostly found in two main plexuses: the myenteric plexus located between the circular and longitudinal muscle layers, and the submucosal plexus found within the connective tissue of the submucosa. The essential role of enteric neurons in peristalsis control is exemplified by bowel obstruction that occurs in aganglionic regions of patients presenting with HSCR (Chakravarti et al., 2004). This multigenic disorder exhibits variable penetrance and severity with a general population incidence of 1/5000 live births and a prominent gender bias of 4:1 in males compared to females (Badner et al., 1990; Spouge and Baird, 1985). Studies using animal models have contributed to our understanding of HSCR genetic complexity through genome targeting efforts in mice that have identified many causative genes for aganglionosis. Continued studies in mice with complementary work in chick and zebrafish, have identified many other molecules that are crucial for ENS development and have aided in understanding cellular processes that occur in normal and abnormal ENS development (see examples included in recent reviews (Goldstein et al., 2013; Harrison and Shepherd, 2013; Lake and Heuckeroth, 2013; Obermayr et al., 2013; Zimmer and Puri, 2015). As a result, we now know that that neural crest cells, mainly from vagal levels of the neural tube,

** Corresponding author.
* Correspondence to: INSERM, U955, Equipe 6, F-94000 Creteil, France.
E-mail addresses: nadege.bondurand@inserm.fr (N. Bondurand), Michelle.southard-smith@vanderbilt.edu (E.M. Southard-Smith).

http://dx.doi.org/10.1016/j.ydbio.2016.06.042
0012-1606/

enter the foregut and migrate to colonize the whole length of the intestine (Burns and Douarin, 1998; Le Douarin and Teillet, 1974; Young et al., 1998). The principal pathway from vagal levels occurs in a rostro-caudal wave down the length of the developing intestine. Upon reaching the hindgut, ENCCs can proceed either through a trans-mesenteric pathway (Nishiyama et al., 2012) or migrate through the cecum to populate the distal colon (Druckenbrod and Epstein, 2005). Because the gut lengthens substantially while it is being colonized, vagal ENCCs migrate further than any other neural crest cell population. Vagal progenitors are complemented by truncal and sacral ENCC populations that make smaller contributions to total cell numbers (Burns and Douarin, 1998; Kapur, 2000; Wang et al., 2011). In humans, it takes three weeks for these cell populations to colonize the whole length of the bowel, while in mice it takes five days, which is ¼ of the gestation period (for recent review see McKeown et al., 2013; Obermayr et al., 2013). During the whole process, coordinated proliferation, migration, and differentiation is required, as perturbations to ENCCs number, migratory behavior or rate of differentiation can result in aganglionosis of the distal bowel. Although apoptosis is not prominent while ENCCs are colonizing the gut (Gianino et al., 2003), early cell death before vagal crest cells enter the gut has been reported in the chick (Wallace et al., 2009) and is also known to occur among mutants that later exhibit intestinal aganglionosis (Durbec et al., 1996; Kapur, 1999; Stanchina et al., 2006).

This review summarizes recent progress in understanding control of ENS development through analysis of mouse models. An overview of signaling pathways that have long been known to control the migration, proliferation and differentiation of ENCCs into and along the developing gut is provided as a framework for the latest information on factors that influence enteric ganglia formation and maintenance. Newly discovered genes that cause HSCR or other abnormalities of enteric ganglia density such as hypoganglionosis or hyperganglionosis are described. Beyond discrete gene identification, the role of regulatory sequences, miRNAs and environmental factors in the etiology of ENS disorders are also introduced. Finally because HSCR has become a paradigm for complex oligogenic diseases with non-Mendelian inheritance, the importance of gene interactions, modifier genes, and initial studies on genetic background effects are included.

2. Molecular mediators that control ENS development and maturation: Key historical genes and new players

Over the years, multiple naturally occurring (“spontaneous”) or gene-targeted mutations that alter molecules involved in the colonization of the gut by ENCCs have been described. These include factors secreted by the gut mesenchyme that act on receptors expressed by ENCCs, transcription factors, guidance factors and morphogens, as well as proteins that transmit signals from the cell surface to the cytoskeleton and the nucleus, including adhesion molecules. Mutations in genes encoding many of these components have been associated with HSCR in human patients, and the majority of these factors are known to affect multiple cellular processes during development.

Between 2012 and 2013, several reviews described in detail many of the identified molecules that are known to play key roles during ENS development (Bergeron et al., 2013; Bondurand and Sham, 2013; Butler Tjaden and Trainor, 2013; Goldstein et al., 2013; Harrison and Shepherd, 2013; Lake and Heuckeroth, 2013; McKeown et al., 2013; Musser and Southard-Smith, 2013; Obermayr et al., 2013; Young, 2012). For details concerning what we refer to here as “key historical genes”, we encourage readers to go back to these elegant reviews. In order to highlight new data published over the last five years, we provide here an overview of individual genes that cause aganglionosis and their roles in ENS development when known (Table 1). We complement this with a summary of known genes that impact the ENS although aganglionosis is not evident (Table 2) and a summary of environmental factors that influence ENS development (Table 3). Finally we include a summary of known genetic interactions that affect ENS development (Table 4).

The first mouse model of HSCR was generated by targeting the *Ret* gene (Schuchardt et al., 1994). This tyrosine kinase receptor interacts with four distinct ligands [glial cell line-derived neurotrophic factor (Gdnf), neurturin (Nrtn), artemin (Artn) and persephin (Pspn)]. Each of these activates Ret by binding to the glycosylphosphatidylinositol-linked Gdnf family of co-receptors (Gfra1 to 4). In mice, total *Ret* deficiency causes complete intestinal aganglionosis. $Ret^{-/-}$ mice additionally present with kidney agenesis and die at birth. *Gdnf* and *Gfra1* deletions cause nearly identical phenotypes, indicating that they are the critical Ret activators during fetal development (Table 1; (Cacalano et al., 1998; Durbec et al., 1996; Enomoto et al., 1998; Moore et al., 1996; Pichel et al., 1996a; Sanchez et al., 1996)). *Gdnf* haploinsufficiency also leads to severe hypoganglionosis (Table 1; (Gianino et al., 2003; Shen et al., 2002)). In contrast, mutants affecting other Ret ligands or co-receptors present with subtler defects, including reduced nerve fiber density, abnormalities in neurotransmitter release, or hypoganglionosis (Table 2, and see for example (Obermayr et al., 2013; Young, 2012; Zimmer and Puri, 2015)). A large variety of *Ret* mutant mice have been generated over time. These new alleles include mono-isoformic variants as well as serine and tyrosine phosphorylation mutation sites (Table 1). Each of the latter, as well as inactivation of Ret inhibitors, greatly helped decipher downstream signaling pathways involved in ENS ontogenesis (Tables 1 and 2 and references therein). In total, data from these models show that Ret signaling is essential for ENS precursor proliferation, migration, differentiation, survival, and neurite growth. Gdnf/Ret signaling can also influence formation of specific subtypes of neurons, with reduction of neuronal nitric oxide synthase (nNOS) in some mutants (Roberts et al., 2008; Uesaka and Enomoto, 2010). Interestingly, conditional inactivation of *Ret* or *Gfrα1* after gut colonization by ENCCs causes loss of neurons in the colon, suggesting that this signaling pathway is also essential for survival of colonic ENCCs (Uesaka et al., 2007, 2008).

Other cell populations in addition to vagal and sacral neural crest-derived progenitors contribute to formation of enteric ganglia and are Ret signaling dependent. Using genetic fate mapping in mice, Enomoto's group indeed definitively demonstrated that a subset of Schwann cell precursors (SCPs), that invade the gut along extrinsic nerves, adopt a neuronal fate in the postnatal period and contribute to the ENS (Uesaka et al., 2015, 2016). Genetic ablation of Ret in SCPs caused colonic aganglionosis, indicating that SCP-derived neurogenesis is essential for ENS integrity, providing novel insight into the development and disorders of neural crest-derived tissues.

Endothelin-3 (Edn3, a member of the 21 amino acid family of peptides, processed by the Ece1 enzyme) and its seven transmembrane G-coupled receptor (Ednrb) are members of a second pathway shown to play crucial roles in ENS development (Table 1, and references therein). Point mutations in *Edn3* and/or deletion of *Ednrb* are causative for spontaneous mouse mutants called *Lethal spotting*, *Piebald lethal* and *Piebald* respectively (Baynash et al., 1994; Hosoda et al., 1994). The first two mutants, in a manner similar to the genomic knock-out of these genes, present with delay in ENCCs migration within the small intestine, and distal hindgut aganglionosis (Barlow et al., 2003; Baynash et al., 1994; Hosoda et al., 1994). All mutants in the *endo*thelin pathway additionally present with pigmentation defects due to abnormal

Table 1
Single gene mutations in mice that lead to intestinal aganglionosis and their effects on ENS development.

Gene	Allele type	Developmental effects on ENS	Effect on mature ENS	Mouse model references	Initial gene report in HSCR patients*
Col6a4 Collagen 6α4	*Tg (Sox3-GFP,Tyr)^{HolNpln}* *(aka "Holstein")* Untargeted transgene insertion upstream of Col6a4 leads to up-regulated Col6a4 expression	Delayed colonization of fetal intestine by ENCCs. Reduced glial/progenitor cell ratios.	Homozygotes exhibit aganglionosis in distal colon accompanied by hypoganglionosis in mid colon.	(Soret et al., 2015)	Mutation not reported in GI patients
Ece1 Endothelin converting enzyme 1, Processes endothelins to active peptides	*Ece1 $^{tm1Reh\ /\ tm1/Reh}$* (aka *Ece1$^{-/-}$*) Targeted gene knockout	Defective migration of ENCCs into the developing hindgut.	Aganglionosis in distal colon	(Yanagisawa et al., 1998)	(Hofstra et al., 1999)
Edn3 Endothelin 3, peptide, Ednrb ligand,	*Edn3 $^{ls\ /\ ls}$* (aka *"Lethal Spotting"*) spontaneous point mutation	Defective migration of ENCCs into the developing hindgut.	Aganglionosis in distal colon; decreased neuronal numbers	(Baynash et al., 1994; Coventry et al., 1994; Rothman and Gershon, 1984)	(Hofstra et al., 1996)
	Edn3 $^{tm1Ywa/\ tm1Ywa}$ (aka *Edn3 $^{-/-}$*) Targeted gene knockout	uncharacterized	Aganglionosis in distal colon	(Baynash et al., 1994)	
Ednrb Endothelin receptor type B, G-coupled Protein Receptor	*Ednrb$^{s/s}$* (aka *"piebald"*) spontaneous insertion in intron causes reduced gene expression.	uncharacterized	Hypomorphic allele due to decreased gene expression. Very rare colonic aganglionosis	(Hosoda et al., 1994; McCallion et al., 2003; Yamada et al., 2006)	(Puffenberger et al., 1994)
	Ednrb$^{s\text{-}l/s\text{-}l}$ (aka *"piebald lethal"*) Spontaneous gene deletion	ENCCs exhibit delayed entry into the developing hindgut and do not fully populate the distal colon despite the fact that they migrate longer during development.	Aganglionosis of distal colon; Decreased intensity of AChE fibers in proximal intestine; Alterations in neuron types in ganglionated regions of colon in homozygotes; Decreased density of ganglia in heterozygotes.	(Cantrell et al., 2004; Fujimoto, 1988; Hosoda et al., 1994; Webster, 1973)	
	Ednrb$^{tm1Ywa\ /\ tm1Ywa}$ (aka *Ednrb $^{-/-}$*) Targeted gene knockout	ENCCs exhibit delayed entry into the developing hindgut.	Aganglionosis in distal colon; hypoganglionosis in regions of small intestine	(Cantrell et al., 2004; Hosoda et al., 1994)	
FoxD3 Forkhead Box D3,Transcription factor	*FoxD3 $^{tm3Lby/tm3Lby}$* (aka *Foxd3 $^{-/-}$*) Floxed allele in combination with either Wnt1cre or Ednrb-iCre	Maintenance of progenitors; control of progenitor proliferation, neural patterning, and glial differentiation	Lack of neurons in the entire gastrointestinal track	(Mundell et al., 2012; Teng et al., 2008)	Mutation not reported in GI patients
Gdnf Glial cell derived neurotrophic factor, Ligand for Ret	*Gdnf $^{tm1Lmgd\ /\ tm1Lmgd}$* (aka *Gdnf $^{-/-}$*) Targeted gene knockout	Gdnf promotes survival, proliferation, differentiation and migration.	Total intestinal aganglionosis in homozygotes; hypoganglionosis throughout intestine of heterozygotes	(Gianino et al., 2003; Pichel et al., 1996a, b; Sanchez et al., 1996; Shen et al., 2002; Wang et al., 2010)	(Angrist et al., 1996; Ivanchuk et al., 1996)
Gfra1 Gdnf family receptor alpha 1, co-receptor for Ret	*Gfra1 $^{tm1Jmi\ /\ tm1Jmi}$* (aka *Gfra1 $^{-/-}$*) Targeted gene knockout	Mediates signaling of Gdnf through Ret.	Total intestinal aganglionosis in homozygotes. Heterozygotes have decreased neuron size but normal numbers.	(Cacalano et al., 1998; Enomoto et al., 1998; Gianino et al., 2003; Hansen and Li, 2012; Wang et al., 2010)	(Eketjall and Ibanez, 2002)
Ihh Indian hedge hog	*Ihh$^{tm1Amc/tm1Amc}$* (aka *Ihh $^{-/-}$*) Targeted gene knockout	Promotes survival of a subpopulation of ENCCs.	Perinatal lethality in homozygotes. Colonic aganglionosis	(Ramalho-Santos et al., 2000)	Not reported in GI patients
Itgb1 Integrinβ1 subunit	*Itgb1 $^{tm3Ref/tm1Ref}$*, *Tg HtPA-Cre* *(aka Itgb1 $^{BGeo\ /\ flox}$; Tg.HtPA-Cre)* Conditional allele used in combination with Tg HtPA-Cre removed exons 2-7 in neural crest	ENCCs do not migrate beyond mid hindgut. ENCCs exhibit altered migration and increased aggregation.	Defect in colonization of cecum and proximal hindgut; Altered ganglion geometry.	(Breau et al., 2009; Breau et al., 2006)	Not reported in GI patients

Table 1 (*continued*)

Gene	Allele type	Developmental effects on ENS	Effect on mature ENS	Mouse model references	Initial gene report in HSCR patients*
Pax3$^{Sp/Sp}$ Paired Box 3, Transcription factor	*Pax3$^{Sp/Sp}$* Spontaneous Null allele	ENCCs fail to enter fetal intestine.	Total intestinal aganglionosis in homozygotes;	(Lang et al., 2000; Lang and Epstein, 2003)	Mutation not reported in GI patients
Pds5b PDS5 cohesin associated factor B, Cohesion regulatory protein involved in sister chromatid cohesion	*Pds5b $^{tm1Jmi/tm1Jmi}$* (aka *Pds5b $^{-/-}$*) Targeted gene knockout	Delayed entry of ENCCs into distal hindgut by 12dpc; Altered neuronal density in regions of small intestina that are colonized	Perinatal lethality with colonic aganglionosis.	(Zhang et al., 2007)	Mutations of PDS5B in Cornelia de Lange patients do not exhibit aganglionosis.
Phox2b Paired-like homeobox 2, Transcription factor	*Phox2b ^{tm1Jbr} / ^{tm1Jbr}* *(aka Phox2b$^{-/-}$)* Targeted gene knockout	Promotes survival of autonomic progenitors.	Total intestinal aganglionosis in homozygotes;	(Pattyn et al., 1999)	(Amiel et al., 2003)
	Phox2b$^{tm2Heno/+}$ and Phox2b $^{tm1Heno/+}$ *(aka Phox2b del5/+ and Phox2b del8/+)* 931 del5 and 693–700 del8 mutations lacking nucleotides 931–935 and 693–700 in the ORF	Impaired neuronal differentiation and decrease proliferation of the enteric ganglion progenitors	Perinatal lethality of heterozygous mutants;.Hypoganglionosis in Phox2bdel5/+ and colonic aganglionosis in *Phox2b$^{del8/+}$ and general hypoganlionosis in other gut segments*	(Nagashimada et al., 2012)	
Ret Rearranged during transfection; Receptor Tyrosine kinase,	*Ret $^{tm1Cos/tm1Cos}$* *(aka Ret $^{-/-}$)* Targeted gene knockout	Altered migration, proliferation, survival and neuronal differentiation of ENCCs.	Total intestinal aganglionosis in homozygotes; In heterozygotes normal neuronal numbers, decreased neuron size and altered cholinergic fibers are reported.	(de Graaff et al., 2001; Gianino et al., 2003; Schuchardt et al., 1994)	(Attie et al., 1995; Attie et al., 1994; Edery et al., 1994; Romeo et al., 1994)
	Ret – hypomorphic isoforms Humanized monomeric isoform, multiple alleles that produce only one isoform from the gene or that alter phosphorylation of the receptor including: *Ret$^{mi51/mi51}$*, *Ret$^{9/-}$*; *Ret^{S697A}*;	Decreased ENCCs and failure to migrate into fetal hindgut; compromised neuronal survival. Effects depend on isoform that is mutated	Colonic aganglionosis	(de Graaff et al., 2001; Schuchardt et al., 1994; Uesaka et al., 2008)	
	Ret $^{tm3(RET)Jmi/+}$ *(aka Ret $^{DN/+}$)* Targeted mutation of cytoplasmic domain	uncharacterized	Total intestinal aganglionosis in homozygotes; Heterozygotes severe hypoganglionosis and reduced nerve fiber density in ganglionated intestine	(Jain et al., 2004)	
	Ret $^{tm1Cti/+}$ *(aka Ret$^{C620R/+}$)* Targeted amino acid substitution	uncharacterized	Total intestinal aganglionosis in homozygotes; Heterozytoes exhibit decreased neuron numbers and fiber density	(Carniti et al., 2006)	
	Ret $^{tm3Cos/tm3Cos}$ *(aka Ret$^{S697A/S697A}$)* Targeted nucleotide substitution	Delayed migration of ENCCs into colon.	Homozygous mutants exhibit aganglionosis in mid and distal colon.	(Asai et al., 2006)	
Sox10 Sry box 10, Transcription Factor	*Sox10$^{Dom/+}$* (aka *"Dominant Megacolon")* Spontaneous mutation, dominant negative	Decreased ENCCs, Delayed migration in heterozygotes, vagal cell death in homozygotes.	Variable aganglionosis in colon of heterozygotes and imbalance of neuron subtypes in proximal ganglionated intestine. Homozygotes have complete intestinal aganglionosis	(Kapur, 1999; Musser et al., 2015; Southard-Smith et al., 1998; Walters et al., 2010)	(Pingault et al., 1998) Seen in patients with Waardenburg-Shah (WS4) and PCWH Syndromes
	Sox10 $^{tm1Weg/+}$ *(aka Sox10$^{Lacz/+}$)* Targeted gene knockout, haploinsufficient	Altered specification of neural crest lineages; Decreased numbers of ENCCs; Loss of mutipotency in ENCCs; Delayed migration in heterozygotes,	Aganglionosis in distal colon of heterozygotes.	(Britsch et al., 2001; Paratore et al., 2002; Paratore et al., 2001)	
TashT	*Tg(SRY-YFP,Tyr)^{TashTNpln}* (aka *"TashT"*) Untargeted transgene insertion in gene desert near Fam162b leads to overexpression	Slower migration of vagal ENCCs into the fetal intestine. Normal directionality, proliferation, and differentiation of vagal ENCCs was observed.	Aganglionosis in distal colon of subsets of homozygous transgenic mice with higher predominance in male TashT mutants.	(Bergeron et al., 2015)	Mutation not reported in GI patients.

Zeb2	*Zeb2* $^{tm1.1Yhi/tm1.1Yhi}$, *Tg.Wnt1-Cre*	Vagal neural crest cells do not develop or delaminate from the neural tube.	Zeb2 homozygotes are lethal at E9.5;	(Van de Putte et al., 2007; Van de Putte et al., 2003; Zimmer and Puri, 2015) (Wakamatsu et al., 2001)
Zinc finger E-box binding homeodomain 2; Transcription factor,	(aka *Zeb2*$^{flox(ex7)}$,*Wnt1Cre*) Targeted gene knockout; or neural crest ablation of floxed allele in combination with Wnt1-Cre		Zeb2$^{fl/(ex7)fl(ex7)}$ colonic and partial small intestinal aganglionosis	Van de Putte, 2003; Van de Putte, 2007

Phenotypes of spontaneous or engineered alleles in mice that lead to alterations ENS structure and the roles of each molecule in ENS development if known are listed. ** Mutant strains are identified by Mouse Genome Nomenclature (http://informatics.jax.org) that designates the specific allele studied. Fields marked as "unknown" indicate data not reported, or mouse model not tested. *Only the first report of disease gene mutation identified in patients is listed due to space constraints.

development of neural crest-derived melanocytes. Consistent with the phenotypes observed in mouse models, mutations in either gene have been found causative for isolated HSCR or Waardenburg-Hirschsprung disease (HSCR combined with pigmentation defects and deafness) in humans (Table 1, and for reviews see (Amiel et al., 2008; Pingault et al., 2010). During ENS development, the primary function of Ednrb and its ligand is to prevent neuronal differentiation of ENCC progenitors and to keep them in a proliferative state, thereby maintaining a pool of uncommitted cells with potential to colonize the gut (Bondurand et al., 2006; Hearn et al., 1998; Nagy and Goldstein, 2006; Wu et al., 1999). Endothelins also appear to promote ENCCs migration directly and perturbation of Ednrb principally affects individual ENCC speed rather than directionality (Druckenbrod and Epstein, 2009; Young et al., 2014), however the underlying mechanism is still not clear.

Over the years, a cohort of transcription factors, including *Sox10, Phox2b, Zeb2*, as well as *Foxd3, Hand2, Ascl1* (formerly known as *Mash1*), *Pax3, Tlx2 (formerly Hox11L1), Hoxb5, Hlx1, Dlx2* and interacting cofactors such as *HipK2* have been implicated in ENS development. Although each of the corresponding mouse models present with severe ENS defects (see Tables 1 and 2 and references therein), only mutations within the first 3 genes lead to aganglionosis and are observed in syndromic forms of HSCR in humans, namely Waardenburg-Hirschsprung disease, Haddad Syndrome (Central Congenital Hypoventilation Syndrome associated with HSCR) and Mowat-Wilson Syndrome (Intellectual disabilities associated with specific craniofacial abnormalities and HSCR in about 40% of cases), respectively. Homozygous loss of *Phox2b* in mice leads to a complete absence of ENS similar to the phenotype of the *Ret* mutants (Pattyn et al., 1999). More recently, generation of mutants bearing variations identical to the ones identified in CCHS patients (931 del5 and 693–700 del8), revealed heterozygous animals failing to breath and presenting with colonic aganglionosis or hypoganglionosis (Nagashimada et al., 2012). These mutants demonstrate that Phox2b is essential for normal ENS development but also showed *Phox2b* variants with altered transcriptional activation protein domains can function as dominant negatives leading to severe deficits in ENS development with resulting aganglionosis. The development of the ENS is even more sensitive to levels and function of *Sox10 and Zeb2* (Table 1 and references therein). Haploinsufficiency of *Sox10* leads to distal colonic aganglionosis, while homozygous mutants die between E13.5 and birth, with total absence of ENCCs even within the esophagus (Herbarth et al., 1998; Kapur, 1999; Southard-Smith et al., 1998). *Sox10* is a member of the Sry related family of transcription factors that is expressed early in neural crest development, including in the vagal and sacral regions, and later in ENCCs when they reach the gut and migrate along it. Similar to Edn3 signaling, Sox10 promotes the maintenance of a pool of progenitor cells for ENS colonization (Bondurand et al., 2006; Kim et al., 2003). Suppression of *Sox10* expression is required for neuronal differentiation to occur and complete absence of ENCCs within the digestive tract of homozygous mutants is due to early cell death of vagal crest cells before they reach the gut (Kapur, 1999; Southard-Smith et al., 1998), illustrating the critical requirement of this transcription factor for ENCC survival and differentiation. More recently, Sox10 was also shown to control cell migration (Corpening et al., 2011) by modulating cell adherence properties (Watanabe et al., 2013). *In vitro* studies indicate that *Sox10* in concert with *Pax3* regulates expression of *Ret*, and additional molecular studies in vitro suggest that Sox10 is capable of binding regulatory regions for *Ednrb* and *Sox10* itself (Lang et al., 2000; Wahlbuhl et al., 2012; Zhu et al., 2004). While transcription factors that participate with *Sox10* auto-regulation have been identified (Wahlbuhl et al., 2012), cofactors that participate with Sox10 in regulation of *Ednrb* are not yet known. Analysis of *Sox10*^{Dom} mouse

Table 2
Gene alterations in mice that impact the ENS without overt intestinal aganglionosis.

Gene	Allele type[a]	Role in ENS development	Effect on mature ENS	References
Araf Araf proto-oncogene serine threonine kinase	*Araf*$^{tm1Mmc\ /\ tm1Mmc/}$ *(aka Araf*$^{-/-}$*)* Targeted gene knockout	Not described	Enteric ganglia present but functionally defective with abnormal architecture. Homozygous mutants exhibit colonic distension	(Pritchard et al., 1996)
Ascl1 achaete-scute family bHLH transcription factor	*Ascl1*$^{tm1And\ /\ tm1And}$ *(aka Mash1*$^{-/-}$*)* Targeted gene knockout	Promotes survival and development of neuronal subtypes	Decreased neuronal numbers; wide spacing and erratic arrangement of enteric ganglia	(Blaugrund et al., 1996)
Bmp4 Bone morphogenetic protein 4	*Bmp4* *Tg.(Eno2-Bmp4)3Jake* transgene over-expression in neurons and entero-endocrine cells using Neuron Specific Enolase promoter	Not described	Regional specific increases in 5HT+, dopaminergic, and TrkC+ neurons accompanied by increased glial numbers	(Chalazonitis et al., 2011a, 2008)
Celsr3 Cadherin, EGF LAG seven-pass G-type receptor 3	*Celsr3*$^{tm1Agof/tm1Agof}$ *(aka Celsr3*$^{-/-}$*)* Targeted gene knockout	Disrupted neurite network with fewer and shorter longitudinal processes and more circumferential and orally projecting processes on developing enteric neurons. Normal differentiation and proliferation of neurons.	Perinatal lethal. Defects in longitudinal tract formation and neurite organization at P0.	(Sasselli et al., 2013)
	Celsr3$^{tm2Agof/tm2Agof}$*, Tg. Wnt1-Cre)* (aka *Celsr3*$^{flox/flox}$*, Wnt1Cre*) Floxed allele used in combination with Wnt1-Cre	Not described	Required for organization of enteric plexus. Interganglionic strands are reduced in thickness and have irregular trajectories. Aberrant Colonic Migrating Motor Complexes and deficient ability to propel luminal contents in the distal bowel.	(Sasselli et al., 2013)
Cdh2 N-Cadherin cell adhesion molecule	*Cdh2*$^{tm1Glr\ /\ tm1Glr}$*; Tg. Ht-PA Cre* *(aka Ncad*$^{flox\ /\ flox}$*)* Conditional floxed allele used in combination with Ht-PA Cre	Promotes migration	Delayed but complete gut colonization	(Broders-Bondon et al., 2012)
Dicer1 Ribonuclease type III processes miRNAs	*Dicer1*$^{tm1Bdh\ /\ tm1Bdh}$*; Tg. Wnt1-Cre* *(aka Dicer*$^{flox\ /\ flox}$*; Tg. Wnt1-Cre)* Conditional inactivation of Dicer with Wnt1-Cre	Controls differentiation and cell survival. ENCCs migrate normally, but marked reductions in cell numbers is noticed by E17 with increased apotosis.	Homozygous lethal at birth.	(Zehir et al., 2010)
Dlx2 distal-less homeobox 2, Homeobox transcription factor	*Dlx2* *(aka Dlx2*$^{-/-}$ *)* Targeted gene knockout	Not described	Enteric neurons are present but newborn pups die perinatally with massive distension of the intestinal lumen	(Qiu et al., 1995)
ErbB2 Receptor of neuregulin1	*ErbB2*$^{tm1KLee/\ tm1Klee}$*; Tg. Nestin-Cre* *(aka ErbB2*$^{-/-}$ *)* conditional floxed allele used in combination with Nestin-Cre	Promotes postnatal survival of neurons and glial cells	Postnatal loss of neurons and glial cells in the colon upon deletion of ErbB2 in epithelial cells	(Crone et al., 2003)
ErbB3 erb-b2 receptor tyrosine kinase 3;	*ErbB3*$^{tm2Cbm\ /\ tm2Cbm}$ *(aka ErbB3*$^{-/-}$ *)*	Aberrant enteric glial development	Absent enteric glia	(Chalazonitis et al., 2011b; Riethmacher et al., 1997)
Fgf2 Fibroblast growth factor 2	*Fgf2*$^{tm1Zllr\ /\ tm1Zllr}$ *(aka Fgf2*$^{-/-}$ *)* Targeted gene knockout	Unknown	Hyperplastic enteric ganglia with open architecture of connectives; altered mucosal barrier function and Cl- secretion.	(Hagl et al., 2008, 2013)

Fzd3 frizzled class receptor 3, Wnt receptor	*Fzd3*$^{tm1Nat/tm1Nat}$ *(aka Fzd3*$^{-/-}$ *)*	Required for guidance and growth of enteric neuronal projections. Normal distribution of TuJ1^{+} neurons compared to controls, however the neurite network is disrupted.	Defects in longitudinal tract formation and neurite organization at P0	(Sasselli et al., 2013)
Gas1 Growth arrest specific1; blocks entry to S phase and plays a role in growth suppression	*Gas1*$^{tm2Fan/\ tm2Fan}$ *(aka Gas1*$^{LacZ\ /LacZ}$ *)* Targeted gene knock-in	Repels enteric axons	Homozygous lethal at birth; Increased numbers of enteric neurons, mislocalized within gut mesenchyme;	(Biau et al., 2013; Jin et al., 2015)
Gdnf glial cell line derived neurotrophic factor	*Gdnf* *Tg.(Myog-Gdnf)1Lich* Gdnf transgene over expression in muscle	Not described	Increased numbers submucosal neurons; changes in fiber density; accelerated intestinal transit	(Wang et al., 2010)
Gfra2 Gdnf family receptor alpha 2	*Gfra2*$^{tm1Msa/\ tm1MSa}$ *(aka Gfra2*$^{-/-}$ *)* Targeted gene knockout	Promotes neuron survival and outgrowth of neurites	Reduction of fibers and abnormal motility, defective pancreas innervation	(Rossi et al., 2003, 1999)
Hand2 Heart and neural crest derivatives expressed transcript 2; Transcription factor	*Hand2*$^{tm1Majh/tm1Majh}$ *or* *Hand2*$^{tm1Cse/tm1Cse}$ *(aka Hand2*$^{-/-}$*; Tg. Wnt1-Cre)* conditional floxed allele crossed to Wnt1-Cre	Promotes terminal differentiation of VIP and nNOS neuronal subtypes	Disorganized ENS plexus, decreased neuronal numbers, reduced GI transit	(D'Autreaux et al., 2011; Hendershot et al., 2007; Lei and Howard, 2011; Morikawa et al., 2007)
Hipk2 Homeodomain interacting protein kinase 2	*Hipk2*$^{tm1Ejh/tm1Ejh}$ *(aka Hipk2*$^{-/-}$ *)*	Not described	Postnatal increase in glia and progressive loss of neurons with concurrent arrest of synaptic maturation in enteric neurons	(Chalazonitis et al., 2011b)
Hlx1 H2.0-like homeobox, Transcription factor,	*Hlx1*$^{tm1Rph/\ tm1Rph}$ *(aka Hlx1*$^{-/-}$ *)* Targeted gene knockout	Reduction of ENCCs in fetal foregut, severe reduction of ENCCs in mid- and hindgut.	Homozygotes embryonic lethal with severe hypoganglionosis	(Bates et al., 2006)
Hoxb5 Homeobox B5 – engrailed fusion, transcription factor	*Tg.(CAG-en/Hoxb5-EGFP)#Vchl* dominant negative allele; inducible by Cre	Dominant negative allele antagonizes normal Hoxb5; Reduction of Ret expression, reduced migration of ENCCs	Reduction of neurons or aganglionosis affecting colon and ileum	(Lui et al., 2008)
Kif26a Kinesin family member 26 a; motor protein	*Kif26a*$^{tm1.1Noh/\ tm1.1Noh}$ *(aka Kif26a*$^{-/-}$ *)* Targeted gene knockout	Negative regulator of RET signaling	Enteric neuronal hyperplasia with pseudoobstruction	(Zhou et al., 2009)
L1cam L1 cell adhesion molecule	*L1cam*$^{tm1Mtei\ /\ tm1Mtei}$ *(aka L1cam*$^{-/-}$ *)* Targeted gene knockout	Enables ENCC migration	Delayed but complete gut colonization.	(Anderson et al., 2006)
Noggin noggin protein	*Tg.(Eno2-Nog-EGFP)Alch* *(aka NSE-Noggin)* Noggin transgene over expression in neurons and enteroendocrine cells using Neuron Specific Enolase promoter	Promotes development of TrkC+ neurons	Increased neuronal numbers in myenteric and submucosal plexi; reduction of TrkC+ neurons, altered proportions of 5HT+, GABA+ and CGRP+ neurons; altered intestinal transit and stool composition	(Chalazonitis et al., 2004, 2011a, 2008)
Nrtn Neurturin	*Nrtn*$^{tm1JMi\ /\ tm1Jmi}$ *(aka Nrtn*$^{-/-}$ *)* Targeted gene knockout	Ret Ligand; Promotes neurite outgrowth	Homozygotes have reduced neuron soma size, neuron fiber density, abnormal GI motility	(Heuckeroth et al., 1999; Yan et al., 2004)
Ntf3 Neurotrophin-3, ligand for p75 low affinity neurotrophin receptor	*Ntf3*$^{tm1Par\ /\ tm1Par}$ *(aka Ntf3*$^{-/-}$ *)* Targeted gene knockout	Expressed by mesenchymal cells; promotes survival and differentiation of developing neurons	Region specific decrease in enteric neuron number	(Chalazonitis et al., 2001)

Table 2 (*continued*)

Gene	Allele type[a]	Role in ENS development	Effect on mature ENS	References
Ntrk3 Receptor of neurotrophin-3	*Ntrk3*^{tm1Par} / ^{tm1Par} *(aka TrkC*$^{-/-}$*)* Targeted gene knockout	unknown	Reduction of myenteric and submucosal neuron subtypes	(Chalazonitis et al., 2001)
Phactr4 Phosphatase and actin regulator 4	*Phactr4*$^{humdy/humdy}$ ENU mutation missense change in C-terminus; hypomorphic allele	Promote migration; regulates directional migration of ENCCs through PP1, Itgb1signaling; reduced number of NADPH-diaphorase stained neurons in the E18 colon	Homozygous lethal at birth	(Zhang et al., 2012; Zhang and Niswander, 2012)
Pofut1 Protein O-fucosyltransferase 1	*Pofut1*^{tm1Ysa} / ^{tm1Ysa}*;Tg. Wnt1-Cre* *(aka Pofut1*$^{flox/flox}$ *)* Conditional Floxed allele crossed to Wnt1-Cre	Promotes proliferation and glial development	Premature neurogenesis and reduction of glial cells; hypoganglionosis	(Okamura and Saga, 2008)
Ptch1 Patched 1	*Ptch1*^{tm1Bjw} / ^{tm1Bjw} *(aka Ptch1*$^{-/-}$ *)* targeted gene knockout	Reduced proliferation of ENCCs; Reduced neurogenesis; Premature gliogenesis	Embryonic lethal at 12.5dpc	(Ngan et al., 2011)
Pten Phosphatase and tensin homology	*Pten*^{tm1Hwu} / ^{tm1Hwu} *(aka Pten*^{flox} / ^{flox}*; Tg. Tyr-Cre)* conditional inactivation using Tyr-Cre	Inhibits migration and proliferation	Hypertrophy and hyperplasia of the ENS; fatal intestinal pseudo-obstruction	(Puig et al., 2009)
Rb1 Retinoblastoma protein1	*Rb1*^{tm2Brn} / ^{tm2Brn}, Tg(Tyr-Cre) (aka *Rb1*$^{flox/flox}$, Tg(Tyr-Cre)) Conditional inactivation using Tyr-Cre	Not described	Abnormal intestinal motility; increased enteric glia; nNOS neurons exhibit large nuclei as a result of DNA replication without cell division	(Fu et al., 2013)
Shh Sonic Hedghog	*Shh*^{tm1Amc} / ^{tm1Amc} *(aka Shh*$^{-/-}$ *)* targeted gene knockout	Expressed by epithelial cells; promotes proliferation and concentric patterning	Increased number of neurons in mucosa of knockout mutant mice	(Ramalho-Santos et al., 2000)
Slc6a2 solute carrier family 6 (neurotransmitter transporter, noradrenalin), member 2	*Slc6a2*^{tm1Mca} /^{tm1Mca} *(aka Net -/-)* Targeted gene knockout	Unknown	Decreased numbers of myenteric neurons; Reduced numbers of neuronal subsets including 5HT+ and calretinin+	(Li et al., 2010)
Spry2 Sprouty homolog 2	*Spry2*$^{tm1Ayos/tm1Ayos}$ *(aka Sprouty2*$^{-/-}$ *)* Targeted gene knockout	Negative regulator of Gdnf signaling; Alters neonatal development or survival of enteric neurons	Enteric neuron hyperplasia, oesophagal achalasia	(Taketomi et al., 2005)
Sufu Suppressor of Fused negative regulator of Gli transcription factors	*Sufu*^{tm1Hui} / ^{tm1Hui}*; Tg. Wnt1-Cre* *(aka Sufu*$^{flox/flox}$*;Wnt1-Cre)* Conditional invalidation using Wnt1-Cre	Neurons and glia highly disorganized in all Sufu mutants at E13.5; neuron to glia ratios reduced. Severe axonal fasciculation defects; delay in gut colonization observed in 14% of mutants	Unknown- Sufu mutants died around E14 but show hypoganglionosis	(Liu et al., 2015)
Tcof1 Treacher Collins Franceschetti syndrome1, homolog; nucleolar factor	*Tcof1*$^{tm1Mjd/+}$ *(aka Tcof*$^{-/+}$*)* Targeted gene knockout	Delayed and prolonged migration of ENCCs	Neonatal lethality of heterozygotes prevents functional analysis of any motility deficits.	(Barlow et al., 2012)
Tfam Transcription factor A, mitochondrial	*Tfam*$^{tm1Lrsn/tm1Lrsn}$*;Tg. CNP-Cre* *(aka Tafm*$^{flox/flox}$ *)* Floxed allele crossed to CNP-Cre	Not described	Postnatal death of specific subsets of enteric neurons	(Viader et al., 2011)

Tlr2 Toll-like receptor 2	*Tlr2*$^{tm1Kir\ /\ tm1Kir}$ *(aka Tlr2*$^{-/-}$ *)* Targeted gene knockout	Not described	Aberrant connectivity of enteric ganglia; Altered intestinal contractility; Abnormal mucosal secretion; Altered inflammatory responses in colon	(Brun et al., 2013)
Tlx2 T cell leukemia, homeobox 2, Hox Transcription factor	*Tlx2*$^{tm1Sjk\ /\ tm1Sjk}$ *(aka Hox11L1*$^{-/-}$ *)* Targeted gene knockout	Not described	Hyperinnervation of the proximal colon accompanied by megacolon at 3–5 weeks of age	(Hatano et al., 1997; Shirasawa et al., 1997)
Tph2 Tryptophan Hydroxylase 2, neuronal expressed form of enzyme critical for serotonin synthesis	*Tph2*$^{tm1Lex/tm1Lex}$ *(aka Tph2*$^{-/-}$ *)* Targeted gene knockout	Promotes differentiation	Homozygotes show decreased neuronal density in the ileum, reduced proportions of dopaminergic neurons with resulting slowed GI transit and colonic emptying.	(Li et al., 2011)
Zic2 Zinc finger protein of the cerebellum 2, transcription factor	*Zic2*$^{m1Nisw/m1Nisw}$ ENU-induced missense mutation	Potential negative regulator of nerve fiber growth.	Homozygous lethal. Increased density of enteric ganglia and neurite extension	(Zhang and Niswander, 2013)

[a] Mutant strains are identified by Mouse Genome Nomenclature (http://informatics.jax.org) that designates the specific allele studied. Fields marked as "unknown" indicate data not reported, or mouse model not tested.

Table 3
Environmental factors that impact the ENS in mice.

Environmental factor	Gene	Allele type**	Role in ENS development	Effect on mature ENS	References
Vitamin A	*Aldh1a2* Aldehyde dehydrogenase family 1, enzyme essential for production of retinoic acid	*Aldh1a2*$^{tm1lpc\ /tm1lpc}$ *(aka Raldh2*$^{-/-}$*)* Targeted gene knockout	Severe reduction of post-otic vagal neural crest and absence of Ret+ ENCCs in foregut	Homozygous lethal	(Niederreither et al., 2003)
MPA mycophenolic acid	*Impdh2* inosine 5' monophosphate dehydrogenase	*Impdh2*$^{tm2Bmi\ /\ tm2Bmi}$; *Tg. Wnt1-Cre* *(aka Impdh2*$^{flox/flox}$, *Wnt1-Cre)*	Reduces the number of migrating ENCC with lamellipodia, reduces DNA synthesis and induces ENCC apoptosis.	Mycophenolate treatment induces bowel aganglionosis and increases the penetrance and severity of HSCR of *Ret*$^{9/-}$ and *Sox10*$^{LacZ/+}$ mutants Impdh2 mutants present with multiple NCC defects, including highly penetrant intestinal aganglionosis,	(Lake et al., 2016, 2013)
Vitamin A	*Rbp4* Retinal binding protein 4 plasma; binds Vitamin A in serum	*Rbp4*$^{tm1Gott\ /\ tm1Gott}$ Targeted gene knockout *(aka Rbp4 -/-)*	In absence of dietary Vitamin A, mutants lack ENCC migration into hindgut; Vitamin A affects lamellipodia formation and ENCC migration in response to GDNF	Colorectal aganglionosis; distal from stomach if deprived of dietary retinoid from E7.5	(Fu et al., 2010)
Ibuprofen	*Gene unknown*		Reduced migration in Ret$^{+/-}$ heterozygotes with decreased lamellipodia and levels of RAC1/CDC42.		(Schill et al., 2016)

Table 4
Summary of genetic interactions that impact the ENS based on double mutant mouse studies.

Interacting genes	Alleles used		Effect on mature ENS and developmental origin	Molecular basis	References
Ret x Ednrb	$Ret^{+/-}$	$Ednrb^{s}$, $Ednrb^{sl}$	Highly penetrant aganglionosis present in double mutants.	unknown	(Carrasquillo et al., 2002; McCallion et al., 2003)
Ret x Edn3	Ret^{51}	$Edn3^{ls}$	Removal of *Edn3* activity in $Ret^{51/51}$ animals results in increased intestinal aganglionosis length compared to *Edn3*-deficient or $Ret^{51/51}$ embryos. Presence of a single Ret^{51} allele partially rescues the aganglionosis of $Edn3^{ls/ls}$ animals. Synergistic effect and antagonistic roles of these pathways on proliferation and migration of ENS progenitors, respectively.	Intercrossed signaling pathways- PKA involvement	(Barlow et al., 2003)
L1cam x Ednrb	$L1cam^{-/y}$	$Ednrb^{s}$, $Ednrb^{sl}$,	Loss of *L1cam* exacerbates the hypoganglionosis in the caudal colon of $Ednrb^{s/s}$ mice. Haploinsufficiency of *L1cam* increases the severity of the aganglionosis in $Ednrb^{sl/sl}$.	unknown	(Wallace et al., 2011)
L1cam x Edn3	$L1cam^{-/y}$	$Edn3^{+/-}$	Loss or haploinsufficiency of *L1cam* increases the severity of the aganglionosis in *Edn3* mutant mice. Double mutants also present with hypoganglionosis.	unknown	(Wallace et al., 2011)
Sox10 x Ednrb	$Sox10^{LacZ}$ $Sox10^{Dom}$	$Ednrb^{s}$, $Ednrb^{sl}$,	Double heterozygotes present with increased intestinal aganglionosis length compared to *Sox10* heterozygotes. Defects partly due to increased cell death of vagal crest cells prior to entry into foregut and increased neuronal differentiation.	SOX10 activates *Ednrb* enhancer in vivo	(Cantrell et al., 2004; Stanchina et al., 2006; Zhu et al., 2004)
Sox10 x Edn3	$Sox10^{LacZ}$ $Sox10^{Dom}$	$Edn3^{ls}$	Double heterozygotes and double mutants present with increased intestinal aganglionosis length compared to single mutants.	SOX10 activates *Ednrb* enhancer	(Stanchina et al., 2006; Zhu et al., 2004)
Sox10 x Sox8	$Sox10^{LacZ}$	$Sox8^{LacZ}$	Loss or haploinsufficiency of *Sox8* increases the severity of the aganglionosis in *Sox10* heterozygotes. Defects are partly due to increased cell death of vagal crest cells before gut entry and reduction of glial cells in double heterozygotes.	unknown	(Maka et al., 2005)
Sox10 x Zeb2	$Sox10^{LacZ}$	$Zfhx1b^{\Delta ex7}$	*Zeb2* heterozygosity increases the severity of the aganglionosis in *Sox10* heterozygotes from E11.5 onwards. Double heterozygotes also present with hypoganglionosis. Defects are partly due to decreased progenitor proliferation and increased neuronal differentiation.	unknown	(Stanchina et al., 2010)
Sox10 x L1cam	$Sox10^{LacZ}$	$L1cam^{-/y}$	Loss of *L1cam* increases the penetrance of the aganglionic phenotype observed in $Sox10^{LacZ/+}$ embryos from E11.5 onwards. Double mutants present with additional hypoganglionosis. Defects partly due to increased cell death of vagal crest cells prior to entry into foregut.	SOX10 regulates *L1cam* expression	(Wallace et al., 2011)
Sox10 x Itgb1	$Sox10^{LacZ}$ $Sox10^{Dom}$	*Ht-PA-Cre;* $Itgb1^{f/neo}$	Double mutants present with increased intestinal aganglionosis length and more severe neuronal network disorganization from E11.5 onwards, due to altered cell migration capacities	Unknown	(Watanabe et al., 2013)
Sox10 x Ets1	$Sox10^{LacZ}$	*Variable spotting*	Hypoganglionosis in $Sox10^{LacZ/+}$; $Ets1^{-/-}$	Est1 activates Sox10 enhancer element MCS4/U3 in vitro	(Saldana-Caboverde et al., 2015)
Sox10 x Sufu	$Sox10^{NGFP}$	*Wnt1-Cre;* $Sufu^{flox\ /\ flox}$	Reduction of *Sox10* attenuates glial differentiation defects of *Sufu* mutants.	Gli1 and Gli2 activate SOX10 MCS4/U3 and MCS7/U1 enhancers + SOX10 represses Sufu	(Liu et al., 2015)
Tcof x Pax3	$Tcof1^{+/-}$	$Pax3^{Sp/+}$	Colonic aganglionosis in double heterozygotes. Phenotype partly due to cumulative apoptosis and decreased proliferative capacity.	unknown	(Barlow et al., 2013)
Cdx x Pax3	Gt(ROSA)26Sor$^{tm1(en/Cdx1\text{-}EGFP)Npln}$; Pax3Pro-Cre (aka, R26R^{EnRCdx1}, P3Pro-Cre)	$Pax3^{Sp/+}$	Hypoganglionosis is present in double heterozygotes.	Transactivation of a neural crest enhancer of Pax3 by Cdx	(Sanchez-Ferras et al., 2016; Sanchez-Ferras et al., 2012)

Dominant negative Engrailed knockdown of Cdx1 in neurectoderm					
Itgb1 x Cdh2	*Ncad*$^{fl/fl}$	*Ht-PA::cre; Itgb1*$^{fl/neo}$	Double mutants present with increased intestinal aganglionosis length, but rescued *Itgb1* aggregation anomalies and neuronal network disorganization. Phenotype partly due to altered speed of locomotion and directionality as well as changes in the balance of adhesion.	unknown	(Broders-Bondon et al., 2012)

Crosses performed and effects on mature ENS as well as developmental and molecular origin of defects observed are listed. Fields marked as "unknown" indicate data not reported.

mutants revealed that alterations in this transcription factor not only lead to distal aganglionosis, but can also disrupt normal proportions of neuron subtypes in ganglionated small intestine that lead to deficits in intestinal transit (Musser et al., 2015). Similarly, *Zeb2* plays essential roles in enteric neurogenesis by promoting ENCC proliferation and early migration within the fetal bowel (Stanchina et al., 2010; Van de Putte et al., 2003) via interactions with Sox10.

In addition to transcription factor networks, cell surface molecules, including the cell adhesion molecule L1CAM, β1Integrins, and N-cadherin, also play crucial roles during ENS cell migration and adhesion processes (Tables 1 and 2 and references therein). The gut microenvironment expresses several extracellular matrix components (ECM) molecules including fibronectin, laminins, collagens, tenascin-C and proteoglycans of various families (Akbareian et al., 2013; Breau et al., 2009). The major ECM receptors are integrins, and in 2006 deletion of *Itgb1*, encoding the Integrinβ1 subunit, in ENCCs was performed (Breau et al., 2006). Abnormal cellular adhesion, delayed migration and distal aganglionosis was documented in the resulting mutants. The migratory defect seems to occur specifically in the cecum/proximal hindgut and is thought to be due to a requirement for Integrinβ1-mediated interactions between ENCC and extracellular matrix components tenascin-C and fibronectin (Breau et al., 2009). More recently, studies that analyzed N-cadherin and Integrinβ1 interactions highlighted the complex regulation that exists between cell–cell and cell–matrix adhesion molecules and revealed how the correct balance between these two types of adhesion processes is crucial for ENS ontogenesis (Broders-Bondon et al., 2012).

In 2015, a novel role for collagen, the most abundant extracellular matrix protein, has been identified in ENS development. Soret and colleagues identified a new mechanism that causes HSCR-like disease in mice and involves deposition of excess collagen VI in the intestine by migrating ENS precursors as they colonize the fetal bowel (Soret et al., 2015). The description of a new mouse model, named *Holstein,* which was generated through an insertional mutagenesis screen that led to altered gene expression of the collagen-6α4 (*Col6a4)* gene is described further in the section entitled “Beyond genes: role of regulatory sequences, miRNAs and environmental factors”.

Besides these well-known molecules, additional factors have been implicated in the development of the ENS from animal studies including Neurotrophin-3, Sonic hedgehog (Shh), Indian Hedgehog (Ihh), Bone morphogenic proteins (Bmp2 and 4), Notch, Small GTPases, Neuregulin (Nrg1), Serotonin, planar cell polarity genes *Celsr3* and *Fzd3,* nucleolar protein Treacle (Tcof1) and Sprouty 2 among others (see Tables 1 and 2 for lists of genes and functional effects on the ENS). Some of these mice recapitulate simple aganglionosis that is the hallmark of HSCR (Table 1). However, a number of them are models of more complex gastrointestinal (GI) disease, affecting only specific neuronal subtypes or glial cells in late embryogenesis or adulthood and/or leading to hypo- or hyperganglionosis (Table 2 and references therein). For example, inactivation of negative regulators of the Ret signaling pathway (such as Sprouty 2 or Kinesin superfamily protein 26A; Kif26A) lead to hyperganglionosis and motility disorders due to over activated Ret signaling, such as esophageal achalasia (Taketomi et al., 2005; Zhou et al., 2009). Use of conditional gene inactivation to specifically ablate some of these signaling pathways in ENCCs or in the developing fetal bowel when ENCCs are emigrating has provided greater support for their involvement in ENS development. This is the case for intracellular components of signaling pathways with wide spatio-temporal functionality, such as Retinoblastoma protein (Rb1), Rho GTPases, the miRNA processing enzyme Dicer, the phosphatase and actin regulator Pten, the Tryptophan hydroxylase 2 enzyme Tph2 (Fu et al., 2013; Fuchs

et al., 2009; Huang et al., 2010; Li et al., 2011; Puig et al., 2009) and more recently Suppressor of Fused (*Sufu*) (Liu et al., 2015). The recent identification of missense mutations affecting the GLI1, 2 and 3 transcriptions factors in patients presenting with HSCR and the analysis of the *Sufu* mutants indeed confirmed the essential role of the whole hedgehog signaling pathway (Liu et al., 2015). Hedghog proteins have important roles as morphogens. Both ligands are expressed in the gut, but have very different effects. Targeted *Shh* mutation results in ectopic enteric neurons (Shh has been shown to promote proliferation, inhibits neuronal differentiation and prevents premature centripetal invasion of gut by ENCC), whereas loss of *Ihh* causes aganglionosis in parts of the gut (see Table 2 and references therein). Ablation of *Gas1* that mediates Shh signaling leads to ENS deficits comparable to targeted deletion of Shh (Biau et al., 2013). Ectopic expression of the transcriptional effector *Gli1* produces an effect similar to loss of *Ihh*, with hypoganglionosis (Yang et al., 1997). This data well correlates with the five gain of function missense mutations newly identified in GLI1, 2 and 3 in HSCR patients (Liu et al., 2015). Although prevalence of these mutations is surprisingly high, these data prompted Liu et al. to further analyze the role of this signaling pathway during ENS development, and focused on Sufu, which negatively regulates the activities of GLI proteins. Previous studies demonstrated that hedgehog signaling is required for the normal migration of enteric neural progenitors and glial cell generation through Sox10 regulation (Liu et al., 2015; Ngan et al., 2011). In *Sufu* mutants, the networks of enteric ganglia were disorganized and neuron:glial cell ratios were reduced, a defect reminiscent of that recently described in *Sox10* and *Raldh1,−2, and −3* mutants (Musser et al., 2015; Wright-Jin et al., 2013). Because mice lacking *Sufu* in neural crest cells die before the gut is completely colonized by ENCCs, the authors were however not able to determine whether loss of *Sufu* results in an absence of neurons in the distal bowel and thus a HSCR-like phenotype. Altogether, these studies emphasize the importance of conditional models in ENCC developmental studies. Use of Cre driver lines such as Wnt1-Cre, Ht-PA-Cre, Nestin-Cre or Tyr-Cre that differ in their temporal and cell type specific expression of Cre can produce gene deletions that affect distinct phases of neural crest and ENCC development. These conditional approaches are complementary and essential for understanding the complexity of genes that contribute to HSCR; however caution is needed in comparing phenotypes across different crosses if the Cre line used differs between studies.

Recent studies of the ENS in mouse models have also identified roles for specific molecules in enteric neuron neurotransmitter specification, axon growth and navigation, target selection, synapse formation and development of mature electrical properties in late development and in adult (see Tables 2 and 3 and references therein). The diversity of molecules and mechanisms that contribute to the entire process of ENS ontogeny is not surprising as ENS maturation continues after the gut has been initially colonized by ENCCs, with additional neuron types exiting the cell cycle even after birth (Pham et al., 1991). While an increasing number of genes that contribute to the etiology of HSCR have been identified, surprisingly these genes account for only a small proportion of known cases. Generation of new models, identification of gene interaction studies and environmental factors that affect ENS development is therefore of high importance. Recently, the pace of identifying new mutations that lead to aganglionosis in mice has declined. This may be due in part to the oligogenic inheritance of the disease that greatly complicates generation of relevant genetic models and the time required for thorough functional validation. Fortunately, candidate gene approaches have been replaced by more general unbiased strategies. Indeed, a number of the recently published models were generated via insertional mutation or N-Ethyl-N-Nitrosourea (ENU) screens, looking for new neural crest regulators. This is the case of of the *TashT* and *Holstein* mouse models that will be described in the next section (Bergeron et al., 2015), and of *Zic2*^{m1Nisw} presenting with enteric hyperplasia and dysplasia (Zhang and Niswander, 2013). Analysis of mutant phenotypes emerging from these screens will hopefully lead to the identification of novel HSCR genes in the coming years.

3. Beyond genes: Roles for regulatory sequences, miRNAs and environmental factors

Beyond HSCR gene identification, human genetic studies have highlighted the major importance of variations within regulatory sequences of known genes as causative factors in aganglionosis. The increasing number of non-coding mutations within or near the *RET* locus are a reminder that although these types of changes may have low penetrance, they are capable of acting synergistically with other mutations to affect a disease phenotype (see for examples (Brooks et al., 2005; Emison et al., 2005; Griseri et al., 2007; Sribudiani et al., 2011). Deletion or point mutations identified within regulatory sequences of other genes including *SOX10* and *DSCAM* are consistent with significant effects of regulatory variants on HSCR susceptibility (Jannot et al., 2013; Lecerf et al., 2014). Non-coding regulatory alterations around known HSCR genes and genes that may affect the ENS therefore need to be carefully considered, as some of the missing heritability in children with HSCR might result from noncoding variants that alter gene expression. In mice, at least three models bearing altered regulatory sequences have been described that present with neural crest defects, including aganglionosis phenotype, and are consistent with an effect of regulatory sequences perturbing essential gene function in the ENS. All three models were generated via insertional mutation screens looking for new neural crest regulators. In each case traditional “knockout” approaches would never have led to the discovery of these new HSCR susceptibility loci because the insertions alter noncoding regions of the genome that were poorly characterized at the time of publication and the affected genes are overexpressed in two of these models.

The first model, *Sox10*^{Hry}, was published in 2006, well before the full description of *Sox10* enhancers (Antonellis et al., 2006). This mutant presents with distal intestinal aganglionosis and severe hypopigmentation due to a 16 kb deletion upstream of the *Sox10* gene that helped define and validate the importance of distant *Sox10* regulatory elements. Over the last two years, the description of two new mouse models further validated the importance of regulatory sequences by identifying the *Fam162b* and *Col6a4* genes (Bergeron et al., 2015; Soret et al., 2015). The first new mouse model, a transgenic line named *TashT*, displays a partially penetrant aganglionic megacolon phenotype shown to result from delayed migration of ENCCs, concomitant with insensitivity towards Gdnf and Edn3. This insertional mutation lies in a gene desert containing multiple highly conserved elements that exhibit repressive activity in reporter assays. RNASeq and 3 C assays revealed the insertion results, at least in part, in loss of repression of the uncharacterized *Fam162b* gene in ENCCs. Interestingly, the partially penetrant aganglionic megacolon phenotype mostly affects males and may through future studies explain the prominent gender bias of 4:1 in males compared to females that is known to occur in human HSCR disease (for review see (Amiel et al., 2008)). The second model named *Holstein*, which was generated in the same genetic screen, led to the altered expression of the *Col6a4* gene (Soret et al., 2015). *Holstein* mice model HSCR and present with delayed colonization of fetal bowel by ENCCs due to slower cell migration. A smaller percentage of enteric glia-fated derivatives, and a larger percentage of undifferentiated ENCCs

were also observed in *Holstein* fetal intestine, suggesting that the mutation slows glial differentiation. The transgene insertion site maps between collagen-6α4 (*Col6a4*) and glycerate kinase (*Glyctk*). RNA-Seq analysis on isolated E12.5 ENCCs revealed that *Col6a4* mRNA is markedly increased (about 250-fold) in *Holstein* mutants; however, collagen VI protein levels are only about 3-fold higher, an observation ascribed to the need to incorporate Col6a4 protein into trimeric collagen monomers that also contain Col6a1 and Col6a2, which are encoded by genes that are not overexpressed in mutant mice.

Other recent work suggests that post-translational regulatory mechanisms also contribute to ENS abnormalities. MicroRNAs are a class of small RNAs that bind to specific mRNA targets, leading to mRNA degradation or translational inhibition. Dicer, an RNase III endonuclease, is one of the critical enzymes for miRNA biosynthesis. Only one Dicer gene (*Dicer1*) exists in the mouse genome (Eppig et al., 2015), which presumably mediates the processing of all miRNAs. In order to reveal miRNA function in neural crest development, a tissue specific Dicer knockout mouse was generated through crosses with Wnt1-Cre (Huang et al., 2010; Nie et al., 2011; Zehir et al., 2010). Neural crest-restricted deletion of *Dicer1* is perinatal lethal with mutants exhibiting severe defects of the craniofacial skeleton as well as the enteric, sensory and sympathetic systems. Deletion of *Dicer1* does not affect neural crest cell migration and target tissue colonization; however, the post-migratory neural crest derivatives are dependent on *Dicer* for survival. In the ENS Dicer and the poorly characterized miRNAs it processes, are required to prevent apoptosis just before and during differentiation of ENCCs. As a result, no defects in ENS development were apparent before E14.5, but loss of Dicer dramatically decreased ENS cell density along the whole length of the fetal intestine from E17. Huang et al. also demonstrated that miRNAs were required for the differentiation and survival of dopaminergic neurons in other parts of the peripheral nervous system, but detailed analysis of how Dicer loss leads to ENS deficits remains to be investigated further. To date, very few studies have investigated the roles of miRNAs in HSCR. To our knowledge, fewer than ten studies (most of them from the same group) have been published on this topic. In 2014, Tang W et al. collected close to 100 serum samples from HSCR cases and matched controls, and an initial screening of genes within which miRNAs reside or downstream regulated mRNAs was performed. Alterations of *Ret*, *Pten*, *Diexf*, CD47/*Cul3*, *Sox9*, and *Nid1* were reported (Lei et al., 2014; Li et al., 2014; Mi et al., 2014; Sharan et al., 2015; Tang et al., 2014; Zhu et al., 2015). However, no specific deletion/mutation of miRNA leading to aganglionosis in mouse models has been reported to date. The exact role of miRNA and Dicer on major ENS players' regulation therefore needs further investigation.

Most recently the effects of environmental factors were explicitly shown to contribute to HSCR susceptibility. To test the hypothesis that certain metabolites of common medicines might increase HSCR risk, Heuckeroth's team treated cell cultures and animal models with several drugs commonly used during early human pregnancy. Retinoic acid, a metabolite of vitamin A, was the first tested molecule shown to influence the proliferation and differentiation of mouse ENCCs *in* vitro (Sato and Heuckeroth, 2008). Subsequently, retinol binding protein 4 ($Rbp4^{-/-}$) mutants depleted of Vitamin A were shown to present with colorectal aganglionosis due to impaired lamellipodia formation and reduced ENCCs migration in response to Gdnf. This led to the suggestion that Vitamin A deficiency may be a non-genetic risk factor that increases HSCR penetrance and expressivity (Fu et al., 2010). Consistent with this possibility, retinaldehyde dehydrogenase (*Raldh2*) mutant mice typically die by E9.5, but viability can be prolonged by exogenous RA supplementation. However even after supplementation *in utero* with Vitamin A $Raldh2^{-/-}$ mutants still lack ENCCs in the fetal intestine, a finding that shows the extreme sensitivity of vagal neural crest to levels of Vitamin A (Niederreither et al., 2003). Combinatorial inactivation of the three Raldh enzymes ($Raldh1^{-/-}$, $Raldh2^{+/-}$, $Raldh3^{+/-}$) identified reduced neuron density and altered ratios of myenteric neurons to glial in the colons of $Raldh1^{KO}$, $Raldh2^{Het}$, $Raldh3^{Het}$ mutants compared to the wild type mice. It was also found that Raldh mutants have altered colonic motility in response to mucosal stimulation. These findings indicate that each of the *Raldh* genes contribute to ENS development and function (Wright-Jin et al., 2013). Generation of conditional knock-outs are now needed to confirm the tissue specific requirement of retinoic acid during ENS development.

The immunosuppressant mycophenolic acid (MPA) was also shown to induce aganglionosis in mice, and enhances the penetrance and phenotype severity of mutations that model HSCR (Lake et al., 2013). In culture, MPA drastically decreases Ret^{+} cell migration out of bowel explants, by decreasing the percentage of migrating ENCCs with lamellipodia, reducing DNA synthesis and by inducing ENCCs apoptosis. *In vivo*, MPA treatment induces bowel aganglionosis and increases the penetrance and severity of aganglionosis in Ret^{9} and $Sox10^{lacZ}$ mutant mice. MPA blocks the rate-limiting step of *de novo* guanine nucleotide synthesis by inhibiting inosine 5' monophosphate dehydrogenase (IMPDH), a ubiquitous metabolic enzyme whose expression is relatively enriched in ENCCs. To further explore the role for this basic metabolic pathway, Lake et al. recently deleted *Impdh2* using a conditional allele crossed with the Wnt1-Cre transgene, and observed defects in multiple neural crest derivatives, including highly penetrant intestinal aganglionosis, agenesis of the craniofacial skeleton, and cardiac outflow tract with great vessel malformations (Lake et al., 2016). ENS defects in mutants lacking *Impdh2* within the neural crest are clearly visible from E13.5 and demonstrate a critical role for de novo guanine nucleotide biosynthesis in ENS development. These exciting findings in mouse models suggest that some cases of HSCR may be preventable through dietary supplementation.

Most recently, Ibuprofen was shown to decrease migration and inhibit bowel colonization by ENCCs in zebrafish, chick and mouse (Schill et al., 2016). Ibuprofen treated ENCCs exhibit reduced migration, fewer lamellipodia and lower levels of active Rac1/cdc42. Additionally, inhibiting ROCK, a RhoA effector and known Rac1 antagonist, reverses Ibuprofen effects on migrating mouse ENCCs in culture. It also inhibits colonization of $Ret^{+/-}$ mouse bowel by ENCCs in vivo, but mice deficient in *Ptgs1* (COX 1) and *Ptgs2* (COX 2) show normal bowel colonization, suggesting COX-independent effects. These findings raise the concern that Ibuprofen may increase HSCR risk in some genetically susceptible families.

In addition to identifying critical molecules for ENS development, study of mouse models has also recently clarified several fundamental developmental processes that occur in ENS formation. The migratory path taken by ENCCs to colonize the gut is one example. Identification of trans-mesenteric migrating cells was revealed by engineered mouse models expressing a photo-convertible reporter that enabled live cell imaging of ENCC by live-cell video microscopy (Nishiyama et al., 2012). Such time-lapse imaging showed that ENCCs reach the hindgut by migrating though the mesentery, effectively cutting across between the midgut and the hindgut, as opposed to migrating along the full length of the fetal intestine around the loop from midgut to cecum and then on to hindgut. The interdependent relationship between vasculature and ENCCs studies also benefited from analysis of mouse models. The vasculature and nervous system indeed share striking similarities in their networked, tree-like architecture and in the way they are super-imposed in mature organs. It has previously been suggested that the intestinal microvasculature network directs the migration of ENCCs along the gut to promote the formation of the

ENS based on experiments in the chick (Nagy et al., 2009). However, fate-mapping and intravascular dye injection recently revealed that in early development both networks form independently of each other and that blood vessel networks are not necessary to guide migrating ENCCs during mouse ENS development (Delalande et al., 2014). As mentioned above, new cell populations contributing to ENS development have also been recently described (Uesaka et al., 2015). Interestingly, Schwann cell precursors (SCPs) give rise to up to 20% of enteric neurons in the large intestine under physiological conditions, and these cells give rise mainly to calretinin-expressing neurons. Another developmental aspect that was clarified is the notion of the "time window" during which the fetal bowel can be colonized. Indeed, grafting experiments previously suggested that if ENCCs have not finished migration by E14.5, aganglionosis will result. However, continued colonization has been observed between E14.5 and E18.5 in *Tcof1* mutants, arguing that the delayed migration is not always predictive or sufficient for the pathogenesis of aganglionosis (Barlow et al., 2012). Rather, a balance between ENCCs proliferation in concert with differentiation and extrinsic gut micro-environmental influences are required to complete ENS formation. Another interpretation of these data could rely on the specific defects observed in *Tcof1* mutants, where unlike most other genes, *Tcof1* haploinsufficiency affects pre-migratory neural crest cells in the neural tube and not during migration. Hence, ENCCs seem to sense their own density and normally have considerable compensatory ability, but ENCCs with mutations in genes affecting proliferation or in maintaining progenitors in an uncommitted state, are unable to increase their rate of proliferation sufficiently to compensate when density falls below critical levels (hypothesized by Obermayr et al., 2013).

4. Gene interactions that impact the ENS

Because ENS development is highly complex, ENCCs must concurrently receive and respond to the activity of multiple factors. Initial evidence from human genetic studies suggested that interactions between variants at the *RET* and *EDNRB* genes in some HSCR patients could influence disease severity of some HSCR patients (Auricchio et al., 1999; Carrasquillo et al., 2002). The initial genetic association studies in human patients were confirmed by subsequent crosses between *Ret* knockout and *Ednrb* hypomorphic mutant mice that resulted in increased severity and penetrance of aganglionosis in *Ret:Ednrb* double mutants (Carrasquillo et al., 2002). Interaction between the Ret and Ednrb pathways in mice was further supported by altered ENS phenotypes in *Edn3:Ret* double mutants relative to single mutant animals (Barlow et al., 2003). Subsequently, combined effects between the ligands Gdnf and Edn3 *in vitro* were documented. While both Gdnf and Edn3 act synergistically to enhance ENCCs proliferation, they have antagonistic roles with respect to differentiation and migration with Edn3 inhibiting Gdnf-mediated effects (Barlow et al., 2003). These studies have illustrated how the coordinated activity between these two pathways is essential, particularly in the cecum where Gdnf is strongly up-regulated at the stage ENCCs begin populating this region of the intestine.

Additional interactions between signaling pathways and molecules that may underlie the variability of aganglionosis seen in HSCR patients have been identified by examining ENS deficits in double mutant offspring produced by crossing distinct single gene mouse mutants (Table 4 and references therein). Not surprisingly given the prominent expression of *Sox10* early in ENCC development, multiple genes have been identified that interact with this transcription factor-encoding gene. Genes that have been found to interact with Sox10 via double mutant crosses include *Ednrb*, *Edn3*, *Sox8*, *Zeb2*, *Itgb1*, *L1Cam, Ets1,* and *Sufu.* Separate analysis in mouse mutants supports interactions between *EdnrB* and *L1Cam*, *Itgb1* and *Cdh2*, as well as *Pax3 and Tcof1* or *Cdx*. Some of these genetic interactions may be the result of direct crosstalk between the individual genes as indicated by in vitro studies showing that Sox10 binds to *Ret* and *Ednrb* regulatory regions (Lang and Epstein, 2003; Zhu et al., 2004). Other gene interactions that lead to increased aganglionosis may be indirect, and are likely mediated by common downstream signaling molecules, such as protein kinase A in the case of *Ret* and *Ednrb* interaction. These types of crosses aid in understanding the coordinated function of each molecule tested, particularly when careful detailed studies of multiple time points in ENCC migration and differentiation are analyzed.

While double mutant crosses can identify genes that exacerbate aganglionosis in HSCR models, they require knowledge of the pathway under investigation and availability of mouse mutants in specific genes. In contrast genome-wide linkage studies have the potential to identify genes that might be missed by candidate gene approaches and are capable of identifying variants that alter transcription, protein levels, or regulatory RNAs. To date there has been only a single modifier screen to identify genomic regions that influence penetrance and severity of aganglionosis in mouse models despite the ready availability of many inbred strains (Owens et al., 2005). Using $Sox10^{Dom}$ mutant mice bred onto distinct inbred genetic backgrounds Southard-Smith's team identified five genomic regions that alter aganglionosis in an unbiased genome-wide survey. Because this effort did not introduce any new mutations into the genome, as is done in ENU mutagenesis, the intervals identified harbor naturally occurring variants that interact with the initial $Sox10^{Dom}$ allele to increase the severity or penetrance of aganglionosis in this HSCR model. Since modifier intervals are so large future fine mapping is needed to determine whether the regions on mouse chromosomes 3, 5, 8, 11 and 14 in this study correspond to previously known HSCR susceptibility genes or are indications of novel genes that remain to be identified. Developmental studies performed by the same group determined that differences in the genetic backgrounds of C57BL6J and C3HeB/FeJ strains not only altered migration of ENCCs in the fetal bowel of $Sox10^{Dom}$ mutants but also affected the developmental potential of ENS progenitors (Walters et al., 2010). While these studies provide insight into the genome regions that influence the $Sox10^{Dom}$ allele, the identified modifier genes will differ depending on the initial HSCR mutation incorporated into crosses (*Sox10* versus *Ednrb* versus *Ret*). As a result such approaches offer fertile ground for identifying naturally occurring variants that influence ENS development and maturation.

A large number of candidate genes that are associated with HSCR disease susceptibility have been identified by comparative genome hybridization (Jiang et al., 2011). A number of these genes, such as the Semaphorins, have not yet been implicated as causative for aganglionosis in mouse models despite the fact that knock down experiments in zebrafish suggest an interaction between *Ret* and *Sema3C/3D* (Jiang et al., 2015). These differences may be the result of lethality in haploinsufficient *Sema* mouse mutants that complicates analysis of vagal ENCCs in double mutant crosses (Feiner et al., 2001). Further investigation with carefully designed mouse alleles that reduce gene expression in specific neural crest lineages will enable validation of the gene interactions detected in HSCR patients and future mechanistic analysis of the gene interactions in double mutant mouse crosses.

5. Conclusion

Mouse models have provided un-precedented insight into the genes and developmental processes that are essential for

formation of the elaborate neural network that makes up the ENS in the bowel. Not only have single gene mutations elaborated our understanding of individual gene function, but double mutant and modifier studies of mouse HSCR models also provide a means to investigate the genetic basis of oligogenic inheritance in HSCR. New HSCR susceptibility genes are likely to emerge from ongoing mutagenesis screens and emerging genetic resources like the collaborative cross and diversity outbred lines (Churchill et al., 2004; Churchill et al., 2012; Threadgill et al., 2002). While important advances have been made in understanding the genetic basis of HSCR, it is clear that mouse models will continue to aid in identifying the genes and developmental mechanisms that lead to this complex disorder.

Disclosures

Authors declare no conflict of interest.

Acknowledgments

The work performed by the N. B. team is supported by INSERM, Agence Nationale de la Recherche (ANR) (ANR-12-BSV2–0019 ENSINTED) and foundation ARC pour la recherche sur le cancer. Research performed by the E. M. S^2 team was supported by National Institutes of Health, USA R01 DK60047 and March of Dimes FY12–450.

References

Akbareian, S.E., Nagy, N., Steiger, C.E., Mably, J.D., Miller, S.A., Hotta, R., Molnar, D., Goldstein, A.M., 2013. Enteric neural crest-derived cells promote their migration by modifying their microenvironment through tenascin-C production. Dev. Biol. 382, 446–456.

Amiel, J., Laudier, B., Attie-Bitach, T., Trang, H., de Pontual, L., Gener, B., Trochet, D., Etchevers, H., Ray, P., Simonneau, M., Vekemans, M., Munnich, A., Gaultier, C., Lyonnet, S., 2003. Polyalanine expansion and frameshift mutations of the paired-like homeobox gene PHOX2B in congenital central hypoventilation syndrome. Nat. Genet. 33, 459–461.

Amiel, J., Sproat-Emison, E., Garcia-Barcelo, M., Lantieri, F., Burzynski, G., Borrego, S., Pelet, A., Arnold, S., Miao, X., Griseri, P., Brooks, A.S., Antinolo, G., de Pontual, L., Clement-Ziza, M., Munnich, A., Kashuk, C., West, K., Wong, K.K., Lyonnet, S., Chakravarti, A., Tam, P.K., Ceccherini, I., Hofstra, R.M., Fernandez, R., Hirschsprung Disease, C., 2008. Hirschsprung disease, associated syndromes and genetics: a review. J. Med. Genet. 45, 1–14.

Anderson, R.B., Turner, K.N., Nikonenko, A.G., Hemperly, J., Schachner, M., Young, H. M., 2006. The cell adhesion molecule l1 is required for chain migration of neural crest cells in the developing mouse gut. Gastroenterology 130, 1221–1232.

Angrist, M., Bolk, S., Halushka, M., Lapchak, P.A., 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.

Antonellis, A., Bennett, W.R., Menheniott, T.R., Prasad, A.B., Lee-Lin, S.Q., Program, N. C.S., Green, E.D., Paisley, D., Kelsh, R.N., Pavan, W.J., Ward, A., 2006. Deletion of long-range sequences at Sox10 compromises developmental expression in a mouse model of Waardenburg-Shah (WS4) syndrome. Hum. Mol. Genet. 15, 259–271.

Asai, N., Fukuda, T., Wu, Z., Enomoto, A., Pachnis, V., Takahashi, M., Costantini, F., 2006. Targeted mutation of serine 697 in the Ret tyrosine kinase causes migration defect of enteric neural crest cells. Development 133, 4507–4516.

Attie, T., Pelet, A., Edery, P., Eng, C., Mulligan, L.M., Amiel, J., Boutrand, L., Beldjord, C., Nihoul-Fekete, C., Munnich, A., et al., 1995. Diversity of RET proto-oncogene mutations in familial and sporadic Hirschsprung disease. Hum. Mol. Genet. 4, 1381–1386.

Attie, T., Pelet, A., Sarda, P., Eng, C., Edery, P., Mulligan, L.M., Ponder, B.A., Munnich, A., Lyonnet, S., 1994. A 7 bp deletion of the RET proto-oncogene in familial Hirschsprung's disease. Hum. Mol. Genet. 3, 1439–1440.

Auricchio, A., Griseri, P., Carpentieri, M.L., Betsos, N., Staiano, A., Tozzi, A., Priolo, M., Thompson, H., Bocciardi, R., Romeo, G., Ballabio, A., Ceccherini, I., 1999. Double heterozygosity for a RET substitution interfering with splicing and an EDNRB missense mutation in Hirschsprung disease. Am. J. Hum. Genet. 64, 1216–1221.

Badner, J.A., Sieber, W.K., Garver, K.L., Chakravarti, A., 1990. A genetic study of Hirschsprung disease. Am. J. Hum. Genet. 46, 568–580.

Barlow, A., de Graaff, E., Pachnis, V., 2003. Enteric nervous system progenitors are coordinately controlled by the G protein-coupled receptor EDNRB and the receptor tyrosine kinase RET. Neuron 40, 905–916.

Barlow, A.J., Dixon, J., Dixon, M., Trainor, P.A., 2013. Tcof1 acts as a modifier of Pax3 during enteric nervous system development and in the pathogenesis of colonic aganglionosis. Hum. Mol. Genet. 22, 1206–1217.

Barlow, A.J., Dixon, J., Dixon, M.J., Trainor, P.A., 2012. Balancing neural crest cell intrinsic processes with those of the microenvironment in Tcof1 haploinsufficient mice enables complete enteric nervous system formation. Hum. Mol. Genet. 21, 1782–1793.

Bates, M.D., Dunagan, D.T., Welch, L.C., Kaul, A., Harvey, R.P., 2006. The Hlx homeobox transcription factor is required early in enteric nervous system development. BMC Dev. Biol. 6, 33.

Baynash, A.G., Hosoda, K., Giaid, A., Richardson, J.A., Emoto, N., Hammer, R.E., Yanagisawa, M., 1994. Interaction of *endo*thelin-3 with *endo*thelin-B receptor is essential for development of epidermal melanocytes and enteric neurons. Cell 79, 1277–1285.

Bergeron, K.F., Cardinal, T., Toure, A.M., Beland, M., Raiwet, D.L., Silversides, D.W., Pilon, N., 2015. Male-biased aganglionic megacolon in the TashT mouse line due to perturbation of silencer elements in a large gene desert of chromosome 10. PLoS Genet. 11, e1005093.

Bergeron, K.F., Silversides, D.W., Pilon, N., 2013. The developmental genetics of Hirschsprung's disease. Clin. Genet. 83, 15–22.

Biau, S., Jin, S., Fan, C.M., 2013. Gastrointestinal defects of the Gas1 mutant involve dysregulated Hedgehog and Ret signaling. Biol. Open. 2, 144–155.

Blaugrund, E., Pham, T.D., Tennyson, V.M., Lo, L., Sommer, L., Anderson, D.J., Gershon, M.D., 1996. Distinct subpopulations of enteric neuronal progenitors defined by time of development, sympathoadrenal lineage markers and Mash-1-dependence. Development 122, 309–320.

Bondurand, N., Natarajan, D., Barlow, A., Thapar, N., Pachnis, V., 2006. Maintenance of mammalian enteric nervous system progenitors by SOX10 and *endo*thelin 3 signalling. Development 133, 2075–2086.

Bondurand, N., Sham, M.H., 2013. The role of SOX10 during enteric nervous system development. Dev. Biol. 382, 330–343.

Breau, M.A., Dahmani, A., Broders-Bondon, F., Thiery, J.P., Dufour, S., 2009. Beta1 integrins are required for the invasion of the caecum and proximal hindgut by enteric neural crest cells. Development 136, 2791–2801.

Breau, M.A., Pietri, T., Eder, O., Blanche, M., Brakebusch, C., Fassler, R., Thiery, J.P., Dufour, S., 2006. Lack of beta1 integrins in enteric neural crest cells leads to a Hirschsprung-like phenotype. Development 133, 1725–1734.

Britsch, S., Goerich, D.E., Riethmacher, D., Peirano, R.I., Rossner, M., Nave, K.A., Birchmeier, C., Wegner, M., 2001. The transcription factor Sox10 is a key regulator of peripheral glial development. Genes Dev. 15, 66–78.

Broders-Bondon, F., Paul-Gilloteaux, P., Carlier, C., Radice, G.L., Dufour, S., 2012. N-cadherin and beta1-integrins cooperate during the development of the enteric nervous system. Dev. Biol. 364, 178–191.

Brooks, A.S., Oostra, B.A., Hofstra, R.M., 2005. Studying the genetics of Hirschsprung's disease: unraveling an oligogenic disorder. Clin. Genet. 67, 6–14.

Brun, P., Giron, M.C., Qesari, M., Porzionato, A., Caputi, V., Zoppellaro, C., Banzato, S., Grillo, A.R., Spagnol, L., De Caro, R., Pizzuti, D., Barbieri, V., Rosato, A., Sturniolo, G.C., Martines, D., Zaninotto, G., Palu, G., Castagliuolo, I., 2013. Toll-like receptor 2 regulates intestinal inflammation by controlling integrity of the enteric nervous system. Gastroenterology 145, 1323–1333.

Burns, A.J., Douarin, N.M., 1998. The sacral neural crest contributes neurons and glia to the post-umbilical gut: spatiotemporal analysis of the development of the enteric nervous system. Development 125, 4335–4347.

Butler Tjaden, N.E., Trainor, P.A., 2013. The developmental etiology and pathogenesis of Hirschsprung disease. Transl. Res. 162, 1–15.

Cacalano, G., Farinas, I., Wang, L.C., Hagler, K., Forgie, A., Moore, M., Armanini, M., Phillips, H., Ryan, A.M., Reichardt, L.F., Hynes, M., Davies, A., Rosenthal, A., 1998. GFRalpha1 is an essential receptor component for GDNF in the developing nervous system and kidney. Neuron 21, 53–62.

Cantrell, V.A., Owens, S.E., Chandler, R.L., Airey, D.C., Bradley, K.M., Smith, J.R., Southard-Smith, E.M., 2004. Interactions between Sox10 and EdnrB modulate penetrance and severity of aganglionosis in the Sox10Dom mouse model of Hirschsprung disease. Hum. Mol. Genet. 13, 2289–2301.

Carniti, C., Belluco, S., Riccardi, E., Cranston, A.N., Mondellini, P., Ponder, B.A., Scanziani, E., Pierotti, M.A., Bongarzone, I., 2006. The Ret(C620R) mutation affects renal and enteric development in a mouse model of Hirschsprung's disease. Am. J. Pathol. 168, 1262–1275.

Carrasquillo, M.M., McCallion, A.S., Puffenberger, E.G., Kashuk, C.S., Nouri, N., Chakravarti, A., 2002. Genome-wide association study and mouse model identify interaction between RET and EDNRB pathways in Hirschsprung disease. Nat. Genet. 32, 237–244.

Chakravarti, A., McCallion, A.S., Lyonnet, S., 2004. Hirschsprung disease, The Metabolic and Molecular Bases of Inherited Disease. McGraw-Hill Co., pp. 1–47.

Chalazonitis, A., D'Autreaux, F., Guha, U., Pham, T.D., Faure, C., Chen, J.J., Roman, D., Kan, L., Rothman, T.P., Kessler, J.A., Gershon, M.D., 2004. Bone morphogenetic protein-2 and -4 limit the number of enteric neurons but promote development of a TrkC-expressing neurotrophin-3-dependent subset. J. Neurosci. 24, 4266–4282.

Chalazonitis, A., D'Autreaux, F., Pham, T.D., Kessler, J.A., Gershon, M.D., 2011a. Bone morphogenetic proteins regulate enteric gliogenesis by modulating ErbB3 signaling. Dev. Biol. 350, 64–79.

Chalazonitis, A., Pham, T.D., Li, Z., Roman, D., Guha, U., Gomes, W., Kan, L., Kessler, J. A., Gershon, M.D., 2008. Bone morphogenetic protein regulation of enteric neuronal phenotypic diversity: relationship to timing of cell cycle exit. J. Comp.

Neurol. 509, 474–492.
Chalazonitis, A., Pham, T.D., Rothman, T.P., DiStefano, P.S., Bothwell, M., Blair-Flynn, J., Tessarollo, L., Gershon, M.D., 2001. Neurotrophin-3 is required for the survival-differentiation of subsets of developing enteric neurons. J. Neurosci. 21, 5620–5636.
Chalazonitis, A., Tang, A.A., Shang, Y., Pham, T.D., Hsieh, I., Setlik, W., Gershon, M.D., Huang, E.J., 2011b. Homeodomain interacting protein kinase 2 regulates postnatal development of enteric dopaminergic neurons and glia via BMP signaling. J. Neurosci. 31, 13746–13757.
Churchill, G.A., Airey, D.C., Allayee, H., Angel, J.M., Attie, A.D., Beatty, J., Beavis, W.D., Belknap, J.K., Bennett, B., Berrettini, W., Bleich, A., Bogue, M., Broman, K.W., Buck, K.J., Buckler, E., Burmeister, M., Chesler, E.J., Cheverud, J.M., Clapcote, S., Cook, M.N., Cox, R.D., Crabbe, J.C., Crusio, W.E., Darvasi, A., Deschepper, C.F., Doerge, R.W., Farber, C.R., Forejt, J., Gaile, D., Garlow, S.J., Geiger, H., Gershenfeld, H., Gordon, T., Gu, J., Gu, W., de Haan, G., Hayes, N.L., Heller, C., Himmelbauer, H., Hitzemann, R., Hunter, K., Hsu, H.C., Iraqi, F.A., Ivandic, B., Jacob, H.J., Jansen, R.C., Jepsen, K.J., Johnson, D.K., Johnson, T.E., Kempermann, G., Kendziorski, C., Kotb, M., Kooy, R.F., Llamas, B., Lammert, F., Lassalle, J.M., Lowenstein, P.R., Lu, L., Lusis, A., Manly, K.F., Marcucio, R., Matthews, D., Medrano, J.F., Miller, D.R., Mittleman, G., Mock, B.A., Mogil, J.S., Montagutelli, X., Morahan, G., Morris, D.G., Mott, R., Nadeau, J.H., Nagase, H., Nowakowski, R.S., O'Hara, B.F., Osadchuk, A.V., Page, G.P., Paigen, B., Paigen, K., Palmer, A.A., Pan, H. J., Peltonen-Palotie, L., Peirce, J., Pomp, D., Pravenec, M., Prows, D.R., Qi, Z., Reeves, R.H., Roder, J., Rosen, G.D., Schadt, E.E., Schalkwyk, L.C., Seltzer, Z., Shimomura, K., Shou, S., Sillanpaa, M.J., Siracusa, L.D., Snoeck, H.W., Spearow, J. L., Svenson, K., Tarantino, L.M., Threadgill, D., Toth, L.A., Valdar, W., de Villena, F. P., Warden, C., Whatley, S., Williams, R.W., Wiltshire, T., Yi, N., Zhang, D., Zhang, M., Zou, F., Complex Trait, C.,, 2004. The Collaborative Cross, a community resource for the genetic analysis of complex traits. Nat. Genet 36, 1133–1137.
Churchill, G.A., Gatti, D.M., Munger, S.C., Svenson, K.L., 2012. The Diversity Outbred mouse population. Mamm. Genome 23, 713–718.
Corpening, J.C., Deal, K.K., Cantrell, V.A., Skelton, S.B., Buehler, D.P., Southard-Smith, E.M., 2011. Isolation and live imaging of enteric progenitors based on Sox10-Histone2BVenus transgene expression. Genesis 49, 599–618.
Coventry, S., Yost, C., Palmiter, R.D., Kapur, R.P., 1994. Migration of ganglion cell precursors in the ileocaca of normal and lethal spotted embryos, a murine model for Hirschsprung disease. Lab. Invest. 71, 82–93.
Crone, S.A., Negro, A., Trumpp, A., Giovannini, M., Lee, K.F., 2003. Colonic epithelial expression of ErbB2 is required for postnatal maintenance of the enteric nervous system. Neuron 37, 29–40.
D'Autreaux, F., Margolis, K.G., Roberts, J., Stevanovic, K., Mawe, G., Li, Z., Karamooz, N., Ahuja, A., Morikawa, Y., Cserjesi, P., Setlick, W., Gershon, M.D., 2011. Expression level of Hand2 affects specification of enteric neurons and gastrointestinal function in mice. Gastroenterology 141, 576–587, 587 e571-576.
de Graaff, E., Srinivas, S., Kilkenny, C., D'Agati, V., Mankoo, B.S., Costantini, F., Pachnis, V., 2001. Differential activities of the RET tyrosine kinase receptor isoforms during mammalian embryogenesis. Genes Dev. 15, 2433–2444.
Delalande, J.M., Natarajan, D., Vernay, B., Finlay, M., Ruhrberg, C., Thapar, N., Burns, A.J., 2014. Vascularisation is not necessary for gut colonisation by enteric neural crest cells. Dev. Biol. 385, 220–229.
Druckenbrod, N.R., Epstein, M.L., 2005. The pattern of neural crest advance in the tenascin-C and colon. Dev. Biol. 287, 125–133.
Druckenbrod, N.R., Epstein, M.L., 2009. Age-dependent changes in the gut environment restrict the invasion of the hindgut by enteric neural progenitors. Development 136, 3195–3203.
Durbec, P., Marcos-Gutierrez, C.V., Kilkenny, C., Grigoriou, M., Wartiowaara, K., Suvanto, P., Smith, D., Ponder, B., Costantini, F., Saarma, M., et al., 1996. GDNF signalling through the Ret receptor tyrosine kinase. Nature 381, 789–793.
Edery, P., Lyonnet, S., Mulligan, L.M., Pelet, A., Dow, E., Abel, L., Holder, S., Nihoul-Fekete, C., Ponder, B.A., Munnich, A., 1994. Mutations of the RET proto-oncogene in Hirschsprung's disease. Nature 367, 378–380.
Eketjall, S., Ibanez, C.F., 2002. Functional characterization of mutations in the GDNF gene of patients with Hirschsprung disease. Hum. Mol. Genet. 11, 325–329.
Emison, E.S., McCallion, A.S., Kashuk, C.S., Bush, R.T., Grice, E., Lin, S., Portnoy, M.E., Cutler, D.J., Green, E.D., Chakravarti, A., 2005. A common sex-dependent mutation in a RET enhancer underlies Hirschsprung disease risk. Nature 434, 857–863.
Enomoto, H., Araki, T., Jackman, A., Heuckeroth, R.O., Snider, W.D., Johnson Jr., E.M., Milbrandt, J., 1998. GFR alpha1-deficient mice have deficits in the enteric nervous system and kidneys. Neuron 21, 317–324.
Eppig, J.T., Blake, J.A., Bult, C.J., Kadin, J.A., Richardson, J.E., Mouse Genome Database, G., 2015. The Mouse Genome Database (MGD): facilitating mouse as a model for human biology and disease. Nucleic Acids Res. 43, D726–D736.
Feiner, L., Webber, A.L., Brown, C.B., Lu, M.M., Jia, L., Feinstein, P., Mombaerts, P., Epstein, J.A., Raper, J.A., 2001. Targeted disruption of semaphorin 3C leads to persistent truncus arteriosus and aortic arch interruption. Development 128, 3061–3070.
Fu, M., Landreville, S., Agapova, O.A., Wiley, L.A., Shoykhet, M., Harbour, J.W., Heuckeroth, R.O., 2013. Retinoblastoma protein prevents enteric nervous system defects and intestinal pseudo-obstruction. J. Clin. Invest. 123, 5152–5164.
Fu, M., Sato, Y., Lyons-Warren, A., Zhang, B., Kane, M.A., Napoli, J.L., Heuckeroth, R. O., 2010. Vitamin A facilitates enteric nervous system precursor migration by reducing Pten accumulation. Development 137, 631–640.
Fuchs, S., Herzog, D., Sumara, G., Buchmann-Moller, S., Civenni, G., Wu, X., Chrostek-Grashoff, A., Suter, U., Ricci, R., Relvas, J.B., Brakebusch, C., Sommer, L., 2009. Stage-specific control of neural crest stem cell proliferation by the small rho GTPases Cdc42 and Rac1. Cell Stem Cell 4, 236–247.
Fujimoto, T., 1988. Natural history and pathophysiology of enterocolitis in the piebald lethal mouse model of Hirschsprung's disease. J. Pediatr. Surg. 23, 237–242.
Furness, J.B., 2012. The enteric nervous system and neurogastroenterology. Nat. Rev. Gastroenterol. Hepatol. 9, 286–294.
Gianino, S., Grider, J.R., Cresswell, J., Enomoto, H., Heuckeroth, R.O., 2003. GDNF availability determines enteric neuron number by controlling precursor proliferation. Development 130, 2187–2198.
Goldstein, A.M., Hofstra, R.M., Burns, A.J., 2013. Building a brain in the gut: development of the enteric nervous system. Clin. Genet. 83, 307–316.
Griseri, P., Lantieri, F., Puppo, F., Bachetti, T., Di Duca, M., Ravazzolo, R., Ceccherini, I., 2007. A common variant located in the 3'UTR of the RET gene is associated with protection from Hirschsprung disease. Hum. Mutat. 28, 168–176.
Hagl, C.I., Klotz, M., Wink, E., Kranzle, K., Holland-Cunz, S., Gretz, N., Diener, M., Schafer, K.H., 2008. Temporal and regional morphological differences as a consequence of FGF-2 deficiency are mirrored in the myenteric proteome. Pediatr. Surg. Int. 24, 49–60.
Hagl, C.I., Wink, E., Scherf, S., Heumuller-Klug, S., Hausott, B., Schafer, K.H., 2013. FGF2 deficit during development leads to specific neuronal cell loss in the enteric nervous system. Histochem Cell Biol. 139, 47–57.
Hansen, C., Li, J.Y., 2012. Beyond alpha-*syn*uclein transfer: pathology propagation in Parkinson's disease. Trends Mol. Med 18, 248–255.
Harrison, C., Shepherd, I.T., 2013. Choices choices: regulation of precursor differentiation during enteric nervous system development. Neurogastroenterol. Motil. 25, 554–562.
Hatano, M., Aoki, T., Dezawa, M., Yusa, S., Iitsuka, Y., Koseki, H., Taniguchi, M., Tokuhisa, T., 1997. A novel pathogenesis of megacolon in Ncx/Hox11L1 deficient mice. J. Clin. Invest. 100, 795–801.
Hearn, C.J., Murphy, M., Newgreen, D., 1998. GDNF and ET-3 differentially modulate the numbers of avian enteric neural crest cells and enteric neurons in vitro. Dev. Biol. 197, 93–105.
Hendershot, T.J., Liu, H., Sarkar, A.A., Giovannucci, D.R., Clouthier, D.E., Abe, M., Howard, M.J., 2007. Expression of Hand2 is sufficient for neurogenesis and cell type-specific gene expression in the enteric nervous system. Dev. Dyn. 236, 93–105.
Herbarth, B., Pingault, V., Bondurand, N., Kuhlbrodt, K., Hermans-Borgmeyer, I., Puliti, A., Lemort, N., Goossens, M., Wegner, M., 1998. Mutation of the Sry-related Sox10 gene in Dominant megacolon, a mouse model for human Hirschsprung disease. Proc. Natl. Acad. Sci. USA 95, 5161–5165.
Heuckeroth, R.O., Enomoto, H., Grider, J.R., Golden, J.P., Hanke, J.A., Jackman, A., Molliver, D.C., Bardgett, M.E., Snider, W.D., Johnson Jr., E.M., Milbrandt, J., 1999. Gene targeting reveals a critical role for neurturin in the development and maintenance of enteric, sensory, and parasympathetic neurons. Neuron 22, 253–263.
Heuckeroth, R.O., Schafer, K.H., 2016. Gene-environment interactions and the enteric nervous system: Neural plasticity and Hirschsprung disease prevention. Dev. Biol.
Hofstra, R.M., Osinga, J., Tan-Sindhunata, G., Wu, Y., Kamsteeg, E.J., Stulp, R.P., van Ravenswaaij-Arts, C., Majoor-Krakauer, D., Angrist, M., Chakravarti, A., Meijers, C., Buys, C.H., 1996. A homozygous mutation in the *endo*thelin-3 gene associated with a combined Waardenburg type 2 and Hirschsprung phenotype (Shah-Waardenburg syndrome). Nat. Genet. 12, 445–447.
Hofstra, R.M., Valdenaire, O., Arch, E., Osinga, J., Kroes, H., Loffler, B.M., Hamosh, A., Meijers, C., Buys, C.H., 1999. A loss-of-function mutation in the *endo*thelin-converting enzyme 1 (ECE-1) associated with Hirschsprung disease, cardiac defects, and autonomic dysfunction. Am. J. Hum. Genet. 64, 304–308.
Hosoda, K., Hammer, R.E., Richardson, J.A., Baynash, A.G., Cheung, J.C., Giaid, A., Yanagisawa, M., 1994. Targeted and natural (piebald-lethal) mutations of endothelin-B receptor gene produce megacolon associated with spotted coat color in mice. Cell 79, 1267–1276.
Huang, T., Liu, Y., Huang, M., Zhao, X., Cheng, L., 2010. Wnt1-cre-mediated conditional loss of Dicer results in malformation of the midbrain and cerebellum and failure of neural crest and dopaminergic differentiation in mice. J. Mol. Cell Biol. 2, 152–163.
Ivanchuk, S.M., Myers, S.M., Eng, C., Mulligan, L.M., 1996. De novo mutation of GDNF, ligand for the RET/GDNFR-alpha receptor complex, in Hirschsprung disease. Hum. Mol. Genet. 5, 2023–2026.
Jain, S., Naughton, C.K., Yang, M., Strickland, A., Vij, K., Encinas, M., Golden, J., Gupta, A., Heuckeroth, R., Johnson Jr., E.M., Milbrandt, J., 2004. Mice expressing a dominant-negative Ret mutation phenocopy human Hirschsprung disease and delineate a direct role of Ret in spermatogenesis. Development 131, 5503–5513.
Jannot, A.S., Pelet, A., Henrion-Caude, A., Chaoui, A., Masse-Morel, M., Arnold, S., Sanlaville, D., Ceccherini, I., Borrego, S., Hofstra, R.M., Munnich, A., Bondurand, N., Chakravarti, A., Clerget-Darpoux, F., Amiel, J., Lyonnet, S., 2013. Chromosome 21 scan in Down syndrome reveals DSCAM as a predisposing locus in Hirschsprung disease. PLoS One 8, e62519.
Jiang, Q., Arnold, S., Heanue, T., Kilambi, K.P., Doan, B., Kapoor, A., Ling, A.Y., Sosa, M. X., Guy, M., Jiang, Q., Burzynski, G., West, K., Bessling, S., Griseri, P., Amiel, J., Fernandez, R.M., Verheij, J.B., Hofstra, R.M., Borrego, S., Lyonnet, S., Ceccherini, I., Gray, J.J., Pachnis, V., McCallion, A.S., Chakravarti, A., 2015. Functional loss of semaphorin 3C and/or semaphorin 3D and their epistatic interaction with ret are critical to Hirschsprung disease liability. Am. J. Hum. Genet. 96, 581–596.
Jiang, Q., Ho, Y.Y., Hao, L., Nichols Berrios, C., Chakravarti, A., 2011. Copy number variants in candidate genes are genetic modifiers of Hirschsprung disease. PLoS One 6, e21219.

Jin, S., Martinelli, D.C., Zheng, X., Tessier-Lavigne, M., Fan, C.M., 2015. Gas1 is a receptor for sonic hedgehog to repel enteric axons. Proc. Natl. Acad. Sci. USA 112, E73–E80.
Kapur, R.P., 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.
Kapur, R.P., 2000. Colonization of the murine hindgut by sacral crest-derived neural precursors: experimental support for an evolutionarily conserved model. Dev. Biol. 227, 146–155.
Kim, J., Lo, L., Dormand, E., Anderson, D.J., 2003. SOX10 maintains multipotency and inhibits neuronal differentiation of neural crest stem cells. Neuron 38, 17–31.
Lake, J.I., Avetisyan, M., Zimmermann, A.G., Heuckeroth, R.O., 2016. Neural crest requires Impdh2 for development of the enteric nervous system, great vessels, and craniofacial skeleton. Dev. Biol. 409, 152–165.
Lake, J.I., Heuckeroth, R.O., 2013. Enteric nervous system development: migration, differentiation, and disease. Am. J. Physiol. Gastrointest. Liver Physiol. 305, G1–24.
Lake, J.I., Tusheva, O.A., Graham, B.L., Heuckeroth, R.O., 2013. Hirschsprung-like disease is exacerbated by reduced de novo GMP synthesis. J. Clin. Invest. 123, 4875–4887.
Lang, D., Chen, F., Milewski, R., Li, J., Lu, M.M., Epstein, J.A., 2000. Pax3 is required for enteric ganglia formation and functions with Sox10 to modulate expression of c-ret. J. Clin. Invest. 106, 963–971.
Lang, D., Epstein, J.A., 2003. Sox10 and Pax3 physically interact to mediate activation of a conserved c-RET enhancer. Hum. Mol. Genet. 12, 937–945.
Le Douarin, N.M., Teillet, M.A., 1974. Experimental analysis of the migration and differentiation of neuroblasts of the autonomic nervous system and of neurectodermal mesenchymal derivatives, using a biological cell marking technique. Dev. Biol. 41, 162–184.
Lecerf, L., Kavo, A., Ruiz-Ferrer, M., Baral, V., Watanabe, Y., Chaoui, A., Pingault, V., Borrego, S., Bondurand, N., 2014. An impairment of long distance SOX10 regulatory elements underlies isolated Hirschsprung disease. Hum. Mutat. 35, 303–307.
Lei, H., Tang, J., Li, H., Zhang, H., Lu, C., Chen, H., Li, W., Xia, Y., Tang, W., 2014. MiR-195 affects cell migration and cell proliferation by down-regulating DIEXF in Hirschsprung's disease. BMC Gastroenterol. 14, 123.
Lei, J., Howard, M.J., 2011. Targeted deletion of Hand2 in enteric neural precursor cells affects its functions in neurogenesis, neurotransmitter specification and gangliogenesis, causing functional aganglionosis. Development 138, 4789–4800.
Li, H., Tang, J., Lei, H., Cai, P., Zhu, H., Li, B., Xu, X., Xia, Y., Tang, W., 2014. Decreased MiR-200a/141 suppress cell migration and proliferation by targeting PTEN in Hirschsprung's disease. Cell Physiol. Biochem. 34, 543–553.
Li, Z., Caron, M.G., Blakely, R.D., Margolis, K.G., Gershon, M.D., 2010. Dependence of serotonergic and other nonadrenergic enteric neurons on norepinephrine transporter expression. J. Neurosci. 30, 16730–16740.
Li, Z., Chalazonitis, A., Huang, Y.Y., Mann, J.J., Margolis, K.G., Yang, Q.M., Kim, D.O., Cote, F., Mallet, J., Gershon, M.D., 2011. Essential roles of enteric neuronal serotonin in gastrointestinal motility and the development/survival of enteric dopaminergic neurons. J. Neurosci. 31, 8998–9009.
Liu, J.A., Lai, F.P., Gui, H.S., Sham, M.H., Tam, P.K., Garcia-Barcelo, M.M., Hui, C.C., Ngan, E.S., 2015. Identification of GLI mutations in patients with hirschsprung disease that disrupt enteric nervous system development in mice. Gastroenterology 149, 1837–1848, e1835.
Lui, V.C., Cheng, W.W., Leon, T.Y., Lau, D.K., Garcia-Barcelo, M.M., Miao, X.P., Kam, M. K., So, M.T., Chen, Y., Wall, N.A., Sham, M.H., Tam, P.K., 2008. Perturbation of hoxb5 signaling in vagal neural crests down-regulates ret leading to intestinal hypoganglionosis in mice. Gastroenterology 134, 1104–1115.
Maka, M., Stolt, C.C., Wegner, M., 2005. Identification of Sox8 as a modifier gene in a mouse model of Hirschsprung disease reveals underlying molecular defect. Dev. Biol. 277, 155–169.
McCallion, A.S., Stames, E., Conlon, R.A., Chakravarti, A., 2003. Phenotype variation in two-locus mouse models of Hirschsprung disease: tissue-specific interaction between Ret and Ednrb. Proc. Natl. Acad. Sci. USA 100, 1826–1831.
McKeown, S.J., Stamp, L., Hao, M.M., Young, H.M., 2013. Hirschsprung disease: a developmental disorder of the enteric nervous system. Wiley Interdiscip. Rev. Dev. Biol. 2, 113–129.
Mi, J., Chen, D., Wu, M., Wang, W., Gao, H., 2014. Study of the effect of miR124 and the SOX9 target gene in Hirschsprung's disease. Mol. Med Rep. 9, 1839–1843.
Moore, M.W., Klein, R.D., Farinas, I., Sauer, H., Armanini, M., Phillips, H., Reichardt, L. F., Ryan, A.M., Carver-Moore, K., Rosenthal, A., 1996. Renal and neuronal abnormalities in mice lacking GDNF. Nature 382, 76–79.
Morikawa, Y., D'Autreaux, F., Gershon, M.D., Cserjesi, P., 2007. Hand2 determines the noradrenergic phenotype in the mouse sympathetic nervous system. Dev. Biol. 307, 114–126.
Mundell, N.A., Plank, J.L., LeGrone, A.W., Frist, A.Y., Zhu, L., Shin, M.K., Southard-Smith, E.M., Labosky, P.A., 2012. Enteric nervous system specific deletion of Foxd3 disrupts glial cell differentiation and activates compensatory enteric progenitors. Dev. Biol. 363, 373–387.
Musser, M.A., Correa, H., Southard-Smith, E.M., 2015. Enteric neuron imbalance and proximal dysmotility in ganglionated intestine of the Hirschsprung mouse model. Cell Mol. Gastroenterol. Hepatol. 1, 87–101.
Musser, M.A., Southard-Smith, E.M., 2013. Balancing on the crest - Evidence for disruption of the enteric ganglia via inappropriate lineage segregation and consequences for gastrointestinal function. Dev. Biol. 382, 356–364.
Nagashimada, M., Ohta, H., Li, C., Nakao, K., Uesaka, T., Brunet, J.F., Amiel, J., Trochet, D., Wakayama, T., Enomoto, H., 2012. Autonomic neurocristopathy-associated mutations in PHOX2B dysregulate Sox10 expression. J. Clin. Invest. 122, 3145–3158.
Nagy, N., Goldstein, A.M., 2006. Endothelin-3 regulates neural crest cell proliferation and differentiation in the hindgut enteric nervous system. Dev. Biol. 293, 203–217.
Nagy, N., Mwizerwa, O., Yaniv, K., Carmel, L., Pieretti-Vanmarcke, R., Weinstein, B. M., Goldstein, A.M., 2009. Endothelial cells promote migration and proliferation of enteric neural crest cells via beta1 integrin signaling. Dev. Biol. 330, 263–272.
Ngan, E.S., Garcia-Barcelo, M.M., Yip, B.H., Poon, H.C., Lau, S.T., Kwok, C.K., Sat, E., Sham, M.H., Wong, K.K., Wainwright, B.J., Cherny, S.S., Hui, C.C., Sham, P.C., Lui, V.C., Tam, P.K., 2011. Hedgehog/Notch-induced premature gliogenesis represents a new disease mechanism for Hirschsprung disease in mice and humans. J. Clin. Invest. 121, 3467–3478.
Nie, X., Wang, Q., Jiao, K., 2011. Dicer activity in neural crest cells is essential for craniofacial organogenesis and pharyngeal arch artery morphogenesis. Mech. Dev. 128, 200–207.
Niederreither, K., Vermot, J., Le Roux, I., Schuhbaur, B., Chambon, P., Dolle, P., 2003. The regional pattern of retinoic acid synthesis by RALDH2 is essential for the development of posterior pharyngeal arches and the enteric nervous system. Development 130, 2525–2534.
Nishiyama, C., Uesaka, T., Manabe, T., Yonekura, Y., Nagasawa, T., Newgreen, D.F., Young, H.M., Enomoto, H., 2012. Trans-mesenteric neural crest cells are the principal source of the colonic enteric nervous system. Nat. Neurosci. 15, 1211–1218.
Obermayr, F., Hotta, R., Enomoto, H., Young, H.M., 2013. Development and developmental disorders of the enteric nervous system. Nat. Rev. Gastroenterol. Hepatol. 10, 43–57.
Okamura, Y., Saga, Y., 2008. Notch signaling is required for the maintenance of enteric neural crest progenitors. Development 135, 3555–3565.
Owens, S.E., Broman, K.W., Wiltshire, T., Elmore, J.B., Bradley, K.M., Smith, J.R., Southard-Smith, EM., 2005. Genome-wide linkage identifies novel modifier loci of aganglionosis in the Sox10Dom model of Hirschsprung disease. Hum. Mol. Genet. 14 (11), 1549–1558.
Paratore, C., Eichenberger, C., Suter, U., Sommer, L., 2002. Sox10 haploinsufficiency affects maintenance of progenitor cells in a mouse model of Hirschsprung disease. Hum. Mol. Genet. 11, 3075–3085.
Paratore, C., Goerich, D.E., Suter, U., Wegner, M., Sommer, L., 2001. Survival and glial fate acquisition of neural crest cells are regulated by an interplay between the transcription factor Sox10 and extrinsic combinatorial signaling. Development 128, 3949–3961.
Pattyn, A., Morin, X., Cremer, H., Goridis, C., Brunet, J.F., 1999. The homeobox gene Phox2b is essential for the development of autonomic neural crest derivatives. Nature 399, 366–370.
Pham, T.D., Gershon, M.D., Rothman, T.P., 1991. Time of origin of neurons in the murine enteric nervous system: sequence in relation to phenotype. J. Comp. Neurol. 314, 789–798.
Pichel, J.G., Shen, L., Sheng, H.Z., Granholm, A.C., Drago, J., Grinberg, A., Lee, E.J., Huang, S.P., Saarma, M., Hoffer, B.J., Sariola, H., Westphal, H., 1996a. Defects in enteric innervation and kidney development in mice lacking GDNF. Nature 382, 73–76.
Pichel, J.G., Shen, L., Sheng, H.Z., Granholm, A.C., Drago, J., Grinberg, A., Lee, E.J., Huang, S.P., Saarma, M., Hoffer, B.J., Sariola, H., Westphal, H., 1996b. GDNF is required for kidney development and enteric innervation. Cold Spring Harb. Symp. Quant. Biol. 61, 445–457.
Pingault, V., Bondurand, N., Kuhlbrodt, K., Goerich, D.E., Prehu, M.O., Puliti, A., Herbarth, B., Hermans-Borgmeyer, I., Legius, E., Matthijs, G., Amiel, J., Lyonnet, S., Ceccherini, I., Romeo, G., Smith, J.C., Read, A.P., Wegner, M., Goossens, M., 1998. SOX10 mutations in patients with Waardenburg-Hirschsprung disease. Nat. Genet. 18, 171–173.
Pingault, V., Ente, D., Dastot-Le Moal, F., Goossens, M., Marlin, S., Bondurand, N., 2010. Review and update of mutations causing Waardenburg syndrome. Hum. Mutat. 31, 391–406.
Pritchard, C.A., Bolin, L., Slattery, R., Murray, R., McMahon, M., 1996. Post-natal lethality and neurological and gastrointestinal defects in mice with targeted disruption of the A-Raf protein kinase gene. Curr. Biol. 6, 614–617.
Puffenberger, E.G., Kauffman, E.R., Bolk, S., Matise, T.C., Washington, S.S., Angrist, M., Weissenbach, J., Garver, K.L., Mascari, M., Ladda, R., et al., 1994. Identity-by-descent and association mapping of a recessive gene for Hirschsprung disease on human chromosome 13q22. Hum. Mol. Genet. 3, 1217–1225.
Puig, I., Champeval, D., De Santa Barbara, P., Jaubert, F., Lyonnet, S., Larue, L., 2009. Deletion of Pten in the mouse enteric nervous system induces ganglioneuromatosis and mimics intestinal pseudoobstruction. J. Clin. Invest. 119, 3586–3596.
Qiu, M., Bulfone, A., Martinez, S., Meneses, J.J., Shimamura, K., Pedersen, R.A., Rubenstein, J.L., 1995. Null mutation of Dlx-2 results in abnormal morphogenesis of proximal first and second branchial arch derivatives and abnormal differentiation in the forebrain. Genes Dev. 9, 2523–2538.
Ramalho-Santos, M., Melton, D.A., McMahon, A.P., 2000. Hedgehog signals regulate multiple aspects of gastrointestinal development. Development 127, 2763–2772.
Riethmacher, D., Sonnenberg-Riethmacher, E., Brinkmann, V., Yamaai, T., Lewin, G. R., Birchmeier, C., 1997. Severe neuropathies in mice with targeted mutations in the ErbB3 receptor. Nature 389, 725–730.
Roberts, R.R., Bornstein, J.C., Bergner, A.J., Young, H.M., 2008. Disturbances of colonic motility in mouse models of Hirschsprung's disease. Am. J. Physiol.

Gastrointest. Liver Physiol. 294, G996–G1008.
Romeo, G., Ronchetto, P., Luo, Y., Barone, V., Seri, M., Ceccherini, I., Pasini, B., Bocciardi, R., Lerone, M., Kaariainen, H., et al., 1994. Point mutations affecting the tyrosine kinase domain of the RET proto-oncogene in Hirschsprung's disease. Nature 367, 377–378.
Rossi, J., Herzig, K.H., Voikar, V., Hiltunen, P.H., Segerstrale, M., Airaksinen, M.S., 2003. Alimentary tract innervation deficits and dysfunction in mice lacking GDNF family receptor alpha2. J. Clin. Invest. 112, 707–716.
Rossi, J., Luukko, K., Poteryaev, D., Laurikainen, A., Sun, Y.F., Laakso, T., Eerikainen, S., Tuominen, R., Lakso, M., Rauvala, H., Arumae, U., Pasternack, M., Saarma, M., Airaksinen, M.S., 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.
Rothman, T.P., Gershon, M.D., 1984. Regionally defective colonization of the terminal bowel by the precursors of enteric neurons in lethal spotted mutant mice. Neuroscience 12, 1293–1311.
Saldana-Caboverde, A., Perera, E.M., Watkins-Chow, D.E., Hansen, N.F., Vemulapalli, M., Mullikin, J.C., Program, N.C.S., Pavan, W.J., Kos, L., 2015. The transcription factors Ets1 and Sox10 interact during murine melanocyte development. Dev. Biol. 407, 300–312.
Sanchez, M.P., Silos-Santiago, I., Frisen, J., He, B., Lira, S.A., Barbacid, M., 1996. Renal agenesis and the absence of enteric neurons in mice lacking GDNF. Nature 382, 70–73.
Sanchez-Ferras, O., Coutaud, B., Djavanbakht Samani, T., Tremblay, I., Souchkova, O., Pilon, N., 2012. Caudal-related homeobox (Cdx) protein-dependent integration of canonical Wnt signaling on paired-box 3 (Pax3) neural crest enhancer. J Biol Chem 287, 16623–16635.
Sanchez-Ferras, O., Bernas, G., Farnos, O., Toure, A.M., Souchkova, O., Pilon, N., 2016. A direct role for murine Cdx proteins in the trunk neural crest gene regulatory network. Development 143, 1363–1374.
Sasselli, V., Boesmans, W., Vanden Berghe, P., Tissir, F., Goffinet, A.M., Pachnis, V., 2013. Planar cell polarity genes control the connectivity of enteric neurons. J. Clin. Invest. 123, 1763–1772.
Sato, Y., Heuckeroth, R.O., 2008. Retinoic acid regulates murine enteric nervous system precursor proliferation, enhances neuronal precursor differentiation, and reduces neurite growth in vitro. Dev. Biol. 320, 185–198.
Schill, E.M., Lake, J.I., Tusheva, O.A., Nagy, N., Bery, S.K., Foster, L., Avetisyan, M., Johnson, S.L., Stenson, W.F., Goldstein, A.M., Heuckeroth, R.O., 2016. Ibuprofen slows migration and inhibits bowel colonization by enteric nervous system precursors in zebrafish, chick and mouse. Dev. Biol. 409, 473–488.
Schuchardt, A., D'Agati, V., Larsson-Blomberg, L., Costantini, F., Pachnis, V., 1994. Defects in the kidney and enteric nervous system of mice lacking the tyrosine kinase receptor Ret. Nature 367, 380–383.
Sharan, A., Zhu, H., Xie, H., Li, H., Tang, J., Tang, W., Zhang, H., Xia, Y., 2015. Down-regulation of miR-206 is associated with Hirschsprung disease and suppresses cell migration and proliferation in cell models. Sci. Rep. 5, 9302.
Shen, L., Pichel, J.G., Mayeli, T., Sariola, H., Lu, B., Westphal, H., 2002. Gdnf haploinsufficiency causes Hirschsprung-like intestinal obstruction and early-onset lethality in mice. Am. J. Hum. Genet 70, 435–447.
Shirasawa, S., Yunker, A.M., Roth, K.A., Brown, G.A., Horning, S., Korsmeyer, S.J., 1997. Enx (Hox11L1)-deficient mice develop myenteric neuronal hyperplasia and megacolon. Nat. Med. 3, 646–650.
Soret, R., Mennetrey, M., Bergeron, K.F., Dariel, A., Neunlist, M., Grunder, F., Faure, C., Silversides, D.W., Pilon, N., Ente-Hirsch Study, G., 2015. A collagen VI-dependent pathogenic mechanism for Hirschsprung's disease. J. Clin. Invest. 125, 4483–4496.
Southard-Smith, E.M., Kos, L., Pavan, W.J., 1998. Sox10 mutation disrupts neural crest development in Dom Hirschsprung mouse model. Nat. Genet. 18, 60–64.
Spouge, D., Baird, P.A., 1985. Hirschsprung disease in a large birth cohort. Teratology 32, 171–177.
Sribudiani, Y., Metzger, M., Osinga, J., Rey, A., Burns, A.J., Thapar, N., Hofstra, R.M., 2011. Variants in RET associated with Hirschsprung's disease affect binding of transcription factors and gene expression. Gastroenterology 140, 572–582, e572.
Stanchina, L., Baral, V., Robert, F., Pingault, V., Lemort, N., Pachnis, V., Goossens, M., Bondurand, N., 2006. Interactions between Sox10, Edn3 and Ednrb during enteric nervous system and melanocyte development. Dev. Biol. 295, 232–249.
Stanchina, L., Van de Putte, T., Goossens, M., Huylebroeck, D., Bondurand, N., 2010. Genetic interaction between Sox10 and Zfhx1b during enteric nervous system development. Dev. Biol. 341, 416–428.
Taketomi, T., Yoshiga, D., Taniguchi, K., Kobayashi, T., Nonami, A., Kato, R., Sasaki, M., Sasaki, A., Ishibashi, H., Moriyama, M., Nakamura, K., Nishimura, J., Yoshimura, A., 2005. Loss of mammalian Sprouty2 leads to enteric neuronal hyperplasia and esophageal achalasia. Nat. Neurosci. 8, 855–857.
Tang, W., Li, H., Tang, J., Wu, W., Qin, J., Lei, H., Cai, P., Huo, W., Li, B., Rehan, V., Xu, X., Geng, Q., Zhang, H., Xia, Y., 2014. Specific serum microRNA profile in the molecular diagnosis of Hirschsprung's disease. J. Cell Mol. Med. 18, 1580–1587.
Teng, L., Mundell, N.A., Frist, A.Y., Wang, Q., Labosky, P.A., 2008. Requirement for Foxd3 in the maintenance of neural crest progenitors. Development 135, 1615–1624.
Threadgill, D.W., Hunter, K.W., Williams, R.W., 2002. Genetic dissection of complex and quantitative traits: from fantasy to reality via a community effort. Mamm. Genome 13, 175–178.
Uesaka, T., Enomoto, H., 2010. Neural precursor death is central to the pathogenesis of intestinal aganglionosis in Ret hypomorphic mice. J. Neurosci. 30, 5211–5218.
Uesaka, T., Jain, S., Yonemura, S., Uchiyama, Y., Milbrandt, J., Enomoto, H., 2007. Conditional ablation of GFRalpha1 in postmigratory enteric neurons triggers unconventional neuronal death in the colon and causes a Hirschsprung's disease phenotype. Development 134, 2171–2181.
Uesaka, T., Nagashimada, M., Enomoto, H., 2015. Neuronal differentiation in schwann cell lineage underlies postnatal neurogenesis in the enteric nervous system. J. Neurosci. 35, 9879–9888.
Uesaka, T., Nagashimada, M., Yonemura, S., Enomoto, H., 2008. Diminished Ret expression compromises neuronal survival in the colon and causes intestinal aganglionosis in mice. J. Clin. Invest. 118, 1890–1898.
Uesaka, T., Young, H.M., Pachnis, V., Enomoto, H., 2016. Development of the intrinsic and extrinsic innervation of the gut. Dev. Biol.
Van de Putte, T., Francis, A., Nelles, L., van Grunsven, L.A., Huylebroeck, D., 2007. Neural crest-specific removal of Zfhx1b in mouse leads to a wide range of neurocristopathies reminiscent of Mowat-Wilson syndrome. Hum. Mol. Genet 16, 1423–1436.
Van de Putte, T., Maruhashi, M., Francis, A., Nelles, L., Kondoh, H., Huylebroeck, D., Higashi, Y., 2003. Mice lacking ZFHX1B, the gene that codes for Smad-interacting protein-1, reveal a role for multiple neural crest cell defects in the etiology of Hirschsprung disease-mental retardation syndrome. Am. J. Hum. Genet. 72, 465–470.
Viader, A., Wright-Jin, E.C., Vohra, B.P., Heuckeroth, R.O., Milbrandt, J., 2011. Differential regional and subtype-specific vulnerability of enteric neurons to mitochondrial dysfunction. PLoS One 6, e27727.
Wahlbuhl, M., Reiprich, S., Vogl, M.R., Bosl, M.R., Wegner, M., 2012. Transcription factor Sox10 orchestrates activity of a neural crest-specific enhancer in the vicinity of its gene. Nucleic Acids Res. 40, 88–101.
Wakamatsu, N., Yamada, Y., Yamada, K., Ono, T., Nomura, N., Taniguchi, H., Kitoh, H., Mutoh, N., Yamanaka, T., Mushiake, K., Kato, K., Sonta, S., Nagaya, M., 2001. Mutations in SIP1, encoding Smad interacting protein-1, cause a form of Hirschsprung disease. Nat. Genet 27, 369–370.
Wallace, A.S., Barlow, A.J., Navaratne, L., Delalande, J.M., Tauszig-Delamasure, S., Corset, V., Thapar, N., Burns, A.J., 2009. Inhibition of cell death results in hyperganglionosis: implications for enteric nervous system development. Neurogastroenterol. Motil. 21, 768-e749.
Wallace, A.S., Tan, M.X., Schachner, M., Anderson, R.B., 2011. L1cam acts as a modifier gene for members of the endothelin signalling pathway during enteric nervous system development. Neurogastroenterol. Motil. 23, e510–e522.
Walters, L.C., Cantrell, V.A., Weller, K.P., Mosher, J.T., Southard-Smith, E.M., 2010. Genetic background impacts developmental potential of enteric neural crest-derived progenitors in the Sox10Dom model of Hirschsprung disease. Hum. Mol. Genet 19, 4353–4372.
Wang, H., Hughes, I., Planer, W., Parsadanian, A., Grider, J.R., Vohra, B.P., Keller-Peck, C., Heuckeroth, R.O., 2010. The timing and location of glial cell line-derived neurotrophic factor expression determine enteric nervous system structure and function. J. Neurosci. 30, 1523–1538.
Wang, X., Chan, A.K., Sham, M.H., Burns, A.J., Chan, W.Y., 2011. Analysis of the sacral neural crest cell contribution to the hindgut enteric nervous system in the mouse embryo. Gastroenterology 141 (992–1002), e1001–e1006.
Watanabe, Y., Broders-Bondon, F., Baral, V., Paul-Gilloteaux, P., Pingault, V., Dufour, S., Bondurand, N., 2013. Sox10 and Itgb1 interaction in enteric neural crest cell migration. Dev. Biol. 379, 92–106.
Webster, W., 1973. Embryogenesis of the enteric ganglia in normal mice and in mice that develop congenital aganglionic megacolon. J. Embryol. Exp. Morphol. 30, 573–585.
Wright-Jin, E.C., Grider, J.R., Duester, G., Heuckeroth, R.O., 2013. Retinaldehyde dehydrogenase enzymes regulate colon enteric nervous system structure and function. Dev. Biol. 381, 28–37.
Wu, J.J., Chen, J.X., Rothman, T.P., Gershon, M.D., 1999. Inhibition of in vitro enteric neuronal development by *endo*thelin-3: mediation by endothelin B receptors. Development 126, 1161–1173.
Yamada, T., Ohtani, S., Sakurai, T., Tsuji, T., Kunieda, T., Yanagisawa, M., 2006. Reduced expression of the *endo*thelin receptor type B gene in piebald mice caused by insertion of a retroposon-like element in intron 1. J. Biol. Chem. 281, 10799–10807.
Yan, H., Bergner, A.J., Enomoto, H., Milbrandt, J., Newgreen, D.F., Young, H.M., 2004. Neural cells in the esophagus respond to glial cell line-derived neurotrophic factor and neurturin, and are RET-dependent. Dev. Biol. 272, 118–133.
Yanagisawa, H., Yanagisawa, M., Kapur, R.P., Richardson, J.A., Williams, S.C., Clouthier, D.E., de Wit, D., Emoto, N., Hammer, R.E., 1998. Dual genetic pathways of endothelin-mediated intercellular signaling revealed by targeted disruption of endothelin converting enzyme-1 gene. Development 125, 825–836.
Yang, J.T., Liu, C.Z., Villavicencio, E.H., Yoon, J.W., Walterhouse, D., Iannaccone, P.M., 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.
Young, H.M., 2012. Physiology of the Gastrointestinal Tract, 5th ed. Elsevier,, London, UK.
Young, H.M., Bergner, A.J., Simpson, M.J., McKeown, S.J., Hao, M.M., Anderson, C.R., Enomoto, H., 2014. Colonizing while migrating: how do individual enteric neural crest cells behave? BMC Biol. 12, 23.
Young, H.M., Hearn, C.J., Ciampoli, D., Southwell, B.R., Brunet, J.F., Newgreen, D.F., 1998. A single rostrocaudal colonization of the rodent intestine by enteric neuron precursors is revealed by the expression of Phox2b, Ret, and p75 and by explants grown under the kidney capsule or in organ culture. Dev. Biol. 202, 67–84.
Zehir, A., Hua, L.L., Maska, E.L., Morikawa, Y., Cserjesi, P., 2010. Dicer is required for survival of differentiating neural crest cells. Dev. Biol. 340, 459–467.

Zhang, B., Jain, S., Song, H., Fu, M., Heuckeroth, R.O., Erlich, J.M., Jay, P.Y., Milbrandt, J., 2007. Mice lacking sister chromatid cohesion protein PDS5B exhibit developmental abnormalities reminiscent of Cornelia de Lange syndrome. Development 134, 3191–3201.
Zhang, Y., Kim, T.H., Niswander, L., 2012. Phactr4 regulates directional migration of enteric neural crest through PP1, integrin signaling, and cofilin activity. Genes Dev. 26, 69–81.
Zhang, Y., Niswander, L., 2012. Phactr4: a new integrin modulator required for directional migration of enteric neural crest cells. Cell Adh Migr. 6, 419–423.
Zhang, Y., Niswander, L., 2013. Zic2 is required for enteric nervous system development and neurite outgrowth: a mouse model of enteric hyperplasia and dysplasia. Neurogastroenterol. Motil. 25, 538–541.
Zhou, R., Niwa, S., Homma, N., Takei, Y., Hirokawa, N., 2009. KIF26A is an unconventional kinesin and regulates GDNF-Ret signaling in enteric neuronal development. Cell 139, 802–813.
Zhu, D., Xie, H., Li, H., Cai, P., Zhu, H., Xu, C., Chen, P., Sharan, A., Xia, Y., Tang, W., 2015. Nidogen-1 is a common target of microRNAs MiR-192/215 in the pathogenesis of Hirschsprung's disease. J. Neurochem 134, 39–46.
Zhu, L., Lee, H.O., Jordan, C.S., Cantrell, V.A., Southard-Smith, E.M., Shin, M.K., 2004. Spatiotemporal regulation of *endo*thelin receptor-B by SOX10 in neural crest-derived enteric neuron precursors. Nat. Genet. 36, 732–737.
Zimmer, J., Puri, P., 2015. Knockout mouse models of Hirschsprung's disease. Pediatr. Surg. Int. 31, 787–794.