Literature review: enteric nervous system development, genetic and epigenetic regulation in the etiology of Hirschsprung's disease

R. Diposarosa, N.A. Bustama, Edhyana Sahiratmadja, P.S. Susanto, Y. Sribudiani

Department of Surgery, Division of Pediatric Surgery, Dr. Hasan Sadikin General Hospital, Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia
Department of Biomedical Sciences, Division of Biochemistry and Molecular Biology, Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia
Research Center of Medical Genetics, Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia

ARTICLE INFO

Keywords:
- Enteric nervous system
- Genetic
- Epigenetic
- Hirschsprung
- miRNA

ABSTRACT

Hirschsprung's disease (HSCR) is a developmental disorder of the enteric nervous system (ENS) derived from neural crest cells (NCCs), which affects their migration, proliferation, differentiation, or preservation in the digestive tract, resulting in aganglionosis in the distal intestine. The regulation of both NCCs and the surrounding environment involves various genes, signaling pathways, transcription factors, and morphogens. Therefore, changes in gene expression during the development of the ENS may contribute to the pathogenesis of HSCR.

This review discusses several mechanisms involved in the development of ENS, confirming that deviant genetic and epigenetic patterns, such as DNA methylation, histone modification, and microRNA (miRNA) regulation, can contribute to the development of neurocristopathy. Specifically, the epigenetic regulation of miRNA expression and its relationship to cellular interactions and gene activation through various major pathways in Hirschsprung's disease will be discussed.

1. Introduction

Hirschsprung's disease (HSCR) is a developmental failure of the enteric nervous system (ENS) derived from neural crest cells (NCCs) which affects their migration, proliferation, differentiation, or preservation in the digestive tract. This alteration causes aganglionosis in the distal intestine, resulting in intestinal obstruction. Various genes and signaling pathways are tightly regulated during the development of the ENS by controlling both NCCs and their surrounding environment [1].

The development of HSCR is a complex process that is influenced by various factors, with 20% of HSCR cases being familial, and the rest are sporadic. At least 20 genes are known to have a role in the pathogenesis of HSCR including rearranged during transfection (RET), glial cell-derived neurotrophic factor (GDNF), GDNF family receptor alpha 1 (GFRα1), neurturin (NRTN), endothelin receptor type B (EDNRB), endothelin 3 (ET3), zinc finger homeobox 1B (ZFHX1B) or ZEB2, paired-like homeobox 2b (PHOX2B), SRY-box 10 (SOX10), Indian Hedgehog (IHH) and Sonic Hedgehog (SHH). These genes encode receptors, ligands, and transcription factors. It has been established that RET is the major gene in the etiology of HSCR disease, with other known genes having a minor contribution accounting for about 30% of all HSCR cases [2, 3, 4, 5, 6].

2. Hirschsprung’s disease

2.1. Definition and incidence

Hirschsprung’s disease (HSCR) is a congenital abnormality characterized by loss of ganglion cells in the wall of the distal intestine...
The clinical characteristics of HSCR are a distended abdomen, delayed symptom presentation to diagnosis is nine days old (age range 1 to 176 days), however, the penetrance of the RET mutation is 72% in men and 51% in women. There are no nationwide data regarding the incidence of HSCR in Indonesia. However, based on the database of Dr. Hasan Sadikin Hospital, the referral hospital for the West Java province in Indonesia, there have been 298 HSCR cases in last three years.

2. Signs and symptoms

Patients with HPCR present a history of constipation since the neonatal period. The median age at presentation of the symptoms is two days old (age range 1–159 days old), and the median age at diagnosis is nine days old (age range 1–177 days old) with the median time from symptom presentation to diagnosis is five days (range 0–176 days). The clinical characteristics of HPCR are a distended abdomen, delayed meconium discharge over the first 24 h, and vomiting, however, only 26.2% of patients show this classic triad.

The first defecation occurs within 24 h after birth in 94% of full-term babies, and within 48 h in 98.5% of full-term babies. A previous study shows that less than 10% of infants with HPCR release meconium within 24 h after birth. Another study has found that 24% of HPCR patients excrete meconium within 24 h and 44% within 48 h. The National Institute for Health and Care Excellence (NICE) guideline regarding constipation in children and adolescents now includes delays in meconium excretion more than 48 h in full-term infants as a red flag and should be referred immediately to rule out the possibility of HPCR.

Older children at diagnosis tend to show symptoms of chronic constipation, distended abdomen, vomiting, and failure to thrive. In 10% of cases, enterocolitis, fever, and septicemia can occur.

3. Enteric nervous system (ENS)

3.1. Development of the ENS

The mammalian gastrointestinal tract consists of cells derived from all three germ layers, with the epithelial lining of the gastrointestinal tract, liver parenchyma, and pancreatic cells all originating from the endoderm. The mesoderm layer forms mesenchymal elements, including smooth muscle and stromal cells. The juxtaposition of the ectoderm with the neural plate induces the neural crest which in turn generates NCCs, the progenitors of the enteric nervous system. Vagally derived ENCCs, the progenitors of the enteric nervous system, mediate cell proliferation, regulated differentiation, survival, or apoptosis of normal NCCs. Three important aspects of NCCs to develop ENS are migration, proliferation, and differentiation.

3.1.1. Migration

Previous studies on NCCs ablation in animals have shown that neurons and glia of the ENS originated from the vagal and sacral segments of NCCs. The vagal crest (somite level 1–7) provides most of the precursors of enteric neurons and glia and colonization of the entire intestine. These cells are known as enteric neural crest derived cells (ENCCs), the progenitors of the enteric nervous system. Vagally derived NCCs migrate and enter the foregut mesenchyme at human embryonic age 4–5 weeks. The cells migrate rostrocaudally along the gastrointestinal tract to reach the distal ileum at the 7th week, mid-colon at the 8th week, and distal rectum at the 12th week of gestation. By the 10th week, ENCCs reach the distal intestine and intertwine into the myenteric plexus. The ENS in the intestinal segment below the umbilicus is derived from the sacral crest cells (caudal to somite 28). Vagal and sacral ENCCs appear to be different distinct cell populations, regarding not only their origin but also their migration behavior. The direction of sacral ENCCs migration is opposite of that of the vagal ENCCs pool, while vagal derived ENCCs migrate rostrocaudally, the sacral derived ENCCs migrate caudorostrally. Vagal ENCCs are more invasive as they colonize almost the entire gut but sacral ENCCs only colonize a small end-part of gastrointestinal tract. When Vagal NCCs are grafted into sacral region, their intrinsic entities are still the same and they are still able to colonize further than do sacral NCCs.

The enteric ganglia are interconnected to form two plexuses that extend along the intestine with the myenteric plexus (Auerbach) in the outer layer and the submucosal plexus (Meissner) in the inner layer. The myenteric plexus that develops early on lies between the layers of the...
ENCCs, such as RET/GDNF, EDNRB/EDN3 and several morphogens involved in regulating migration, proliferation, and differentiation of colonization of the hindgut [13].

Migration of ENCCs is not only a mere movement toward the anus but a dynamic process that involves complex interactions between migrating ENCCs and developing intestines [26]. Different populations of ENCCs exhibit different migration behavior pattern, depending on their position along the migration trail. Cells in the migration wavefront show a significant caudal expansion, while cells in the trailing wave tend to be limited, therefore, the ENCCs in the wavefront are responsible for the colonization of the hindgut [13].

Previous studies have found many molecular signaling mechanisms involved in regulating migration, proliferation, and differentiation of ENCCs, such as RET/GDNF, EDNRB/EDN3 and several morphogens such as Netrin, SHH [26, 30, 31]. It has also been shown that RET/GDNF signals are crucial for the survival and proliferation of ENCCs (Figure 1) [18].

3.1.2. Proliferation

The size of the ENCC population determine the normal speed of cells and completion of cell invasion in the gut [32]. Reduction of vagal NCCs by partial ablation in vivo before migration in avians leads to Hirschsprung-like aganglionosis [21, 29, 33] It is presumed that if the remaining ENCCs are given sufficient time to colonize the gut, eventually complete cell colonization in the gut will occur. However, if complete cell colonization in the gut is required in a particular time frame, then the reduction of initial ENCCs will lead to failure of complete cell colonization [21, 34].

Simpson, et al. in 2007 developed a mathematical model to predict cell invasion in the gut by incorporating two basic cell functions as parameters, cell motility and proliferation. Their study showed that cell proliferation in the wavefront is critical in driving the invasive process [39]. Different neuronal-progenitor cells exit the cell cycle at different time points, with cells expressing early neuron markers such as Tuj1 and HuC/HuD appearing in the ENS soon after colonization begins, although most ENCCs, particularly in the migrating wavefront, remain undifferentiated. The earliest formed neurons are serotonin (5-HT) neurons arising at day 8–14 (peak at day 10), followed by cholinergic neurons which produce choline acetyltransferase (peak at day 12), and enkephalin (peak at day 14) [40].

Sox10 and Edn3 are both important in the maintenance of ENCCs in mammals, however, Sox10 is not sufficient to maintain ENCCs in an undifferentiated state as glial cells also express this gene [41]. Notch in...
mice is required to prevent premature differentiation and depletion of undifferentiated ENCCs. Transcriptomics studies on gut tissue and animal study performed by Nishino et al. (2010) showed that Lgi4 is required for the differentiation of glial cells [42]. BMP signaling involved in the cell cycle exit of the ENCCs and induces glial differentiation in the ENS, subsequently, developing glial cells are dependent on glial growth factors such as GGF2 and NRG1. Enteric glial cells start to form from day 12 onward in rat and continue to mature to 4 weeks postnatal [40, 43].

The neurons are grouped into ganglia, with each ganglion containing glial cells and many different types of neurons such as motor neurons, sensory neurons, interneurons, secretomotor neurons, and vasomotor neurons [38]. Many signaling pathways, including BMP2 and BMP4, NOTCH, SHH, SEMA3A, NRG1, and Neurturin play a role in plexus patterning [31]. The balance between proliferation and differentiation is necessary to maintain sufficient progenitor cell collections to ensure total colonization of the ENS [13, 19].

3.2. Genetic aspects of the development of the ENS

ENCCs are transient and multipotent progenitors that produce a variety of cell types, including neural, endocrine, pigment, craniofacial, and conotruncal cardiac cells. Neurocrystopathy is a diverse class of pathologies that may arise from developmental defects of NCCs [13, 25]. Conditions that cause interference with the migration of enteric neuroblasts can also affect migration, differentiation, or preservation of other cells that are also derived from NCCs. Disruption of genetic sequences that produce changes in the function of any gene responsible for migration, proliferation, differentiation, preservation, or changing the permissive environment for migration of NCCs has the potential to cause the failure of the development of the ENS [20].

Mutations in target genes alter the molecules involved in intestinal colonization by ENCC, including factors secreted by intestinal mesenchyme acting on receptors expressed by ENCCs, transcription factors, morphogens, proteins that send signals from the cells membranes to the nucleus, and adhesion molecules. Mutations in genes that encode many of these components have been linked to the occurrence of HSCR in humans and most of these factors are known to affect many cellular processes during development [44].

Many genes contribute to the normal migration, proliferation and differentiation of ENCCs which eventually form a functional ENS, including RET, GDNF, NRTN, EDNRB, EDN3, ECE1, PHOX2B, SOX10, PAX3, SHH, IHH, GLI, and ZEB2 (SIP1, ZFHX1B). Mutations can be in the form of nonsense mutations, missense mutations, small deletions, and insertions. Three major contributing genes to the pathogenesis of HSCR are RET, EDNRB, and GDNF [12, 45, 46]. Each of these complex cellular events must be guided by certain molecular signaling pathways such as RET/GDNF and EDNBR/EDN3, transcription factors such as SOX10, PAX3, PHOX2B, ZFHX1B, TTF-1 or even among several morphogens like Hedgehog (HH), Netrins, or Semaphorins [7, 26, 47, 48, 49].

3.2.1. RET/GDNF pathways

The RET proto-oncogene is a major gene that causes HSCR and RET mutations have been identified in 50% of familial cases and 15–35% of sporadic cases of HSCR. In total, at least 20% of all HSCR cases are caused by the RET mutation, hence this gene is the main gene involved in HSCR etiology [2, 13, 18, 50].

The RET proto-oncogene is located on chromosome 10 band q11.2 and encodes a tyrosine kinase transmembrane receptor. Its expression in ENCCs requires SOX10 and PHOX2B transcription factors, with the loss of either factor leading to intestinal aganglionosis. The RET protein has a large extracellular domain, a transmembrane region, and an intracellular kinase domain. It functions as a signaling receptor and has four ligands, namely GDNF, NRTN (neurturin), ARTN (artemisin), and PSPN (persephin) [51, 52, 53, 54].

These ligands activate RET by binding to their respective GPI (glycosylphosphatidylinositol)-linked GDNF family of receptors (GFRα1-4) to form a ligand-co-receptor complex, which binds to RET and induces its dimerization and autophosphorylation of the tyrosine residue in the intracellular domain [55]. These tyrosine residues act as docking sites for adapters and signaling proteins to stimulate multiple downstream pathways [26]. These activated downstream effector molecules are respectively RAS/mitogen-activated protein kinase (MAPK) pathway, Jun-associated N-terminal kinase (JNK), and phosphatidylinositol-3 kinase (PI3K-AKT), JAK-STAT, ERK, and PKC which support cell growth, proliferation, preservation, and differentiation of cells [56].

The GDNF gene consists of two exons (151 and 485 base pairs) located on chromosome 5. The GDNF protein consists of 134 amino acids with a molecular weight of 32–42 kDa (kd) in the form of dimers, which act as ligands for the multi-subunit glycosylphosphatidylinositol (GPI)-anchored co-receptor GFR-α [57]. This complex binds to RET providing signaling components that are involved in and support migration, proliferation, differentiation, and survival of neurons in the ENS [58, 59, 60].

GDNF is a chemoattractant signal for ENCCs and when the wavefront of ENCCs reaches the esophagus, GDNF is expressed in the stomach, then increases again in the cecum as ENCCs approach the distal small intestine. This suggests that GDNF attracts ENCCs that express RET and GFRα to the right location [60]. Thus, aganglionosis can occur by the absence of the RET-GFRα1-GDNF complex caused by a mutation in either one of those encoded genes [6, 45, 61, 62, 63, 64, 65]. Functional tests show that the absence of either GDNF or GFR-α decreases or eliminates RET/GDNF signaling [19].

Pathology examination revealed that GDNF immunoreactivity is found in the ganglion in the myenteric and submucosal plexus. In the hypoganglionic segment, GDNF immunoreactivity was found to decrease and GDNF immunoreactivity was absent in the aganglionic segment [25]. The GDNF mutation has been identified in limited cases, therefore, GDNF is considered a rare susceptibility gene for HSCR [6, 64, 66]. Recently, Sridhuliani et al (2018) identified multiple mutations in known HSCR genes in a large family with HSCR, one of which was an in-frame deletion of GDNF. Functional studies in vitro showed that this mutation prevented secretion of its product reducing RET activation [6].

Another role of RET is the regulation of apoptosis. When there are ligands, RET produces a positive control signal (stimulation) to maintain cell development and survival, whereas, in the absence of ligands, RET produces a negative control signal (inhibition) and apoptosis occurs. This apoptotic effect can be inhibited in the presence of GDNF [67].

3.2.2. EDNRB/EDN3 pathways

Endothelin receptor type B (EDNRB) gene is located on chromosome 13q22 and encodes heptahelical proteins, known as G-protein-coupled receptors that contain seven transmembrane domains expressed by the neural crest derivatives. The extracellular and transmembrane regions are involved in ligand binding, whereas the intracellular domains are involved in intracellular signaling pathways that are mediated by G proteins [67]. Endothelin 3 (EDN3) is a ligand of EDNRB and Endothelin converting enzyme 1 (ECE1) converts the inactive EDN3 precursor to the active form [30, 66, 67].

HSCR patients appear to have mutations in EDNRB, EDN3, and ECE1 in 5% of cases. A study in a mouse model found that EDNRB/EDN3 signals are involved in ENCC migration as the EDNRB is expressed by ENCCs that are migrating while EDN3 is expressed in the migdut and hindgut mesoderm. Other studies have found a role for EDNRB/EDN3 signals in the development of the ENS, EDN3 activation of EDNRB induces ENCCs to proliferate, maintains precursor status, and prevents premature differentiation [30, 31].

Mutation in the EDNRB/EDN3 gene tends to cause short-segment HSCR (S-HSCR), whereas RET mutations play a role in both short- and long-segment-HSCR (L-HSCR). This may be due to the dysregulation of the EDNRB/EDN3 and RET/GDNF signaling impacting the NCCs at different times and location during ENCCs migration in the gut [65, 67, 68].
3.2.3. Semaphorin pathways

Semaphorin is a transmembrane protein or GPI-linked protein involved in neuronal migration, proliferation, survival, and axonal guidance. These proteins are grouped into various classes and subgroups [67]. Their main receptors are the Plexin family receptors. Plexin causes activation of Plexin-associated tyrosine kinase and inhibits integrin-mediated cell adhesion and events downstream of its activation pathway. Semaphorin was originally known as a protein that plays a role in the formation of neural circuits and mediates inhibition of cell motility, thereby interfering with ENCC migration [7, 69].

In the developing cecum and colon, Semaphorin3A (SEMA3A) is expressed by the mesenchyme. In mice, Sem3a, Sem3c, and Sem3d are expressed by the ENS. sema3c and sema3d are also expressed in zebrafish and their knockdown resulted in decreased migration of ENS precursors [69]. In the aganglionic colonic segment of HSCR patients, SEMA3A was found to be expressed at a higher level than in the ganglionic segment of HSCR or of control patients, therefore, increased SEMA3A expression could be a risk factor for HSCR [70]. Recently, the signaling pathway of Semaphorin3C/3D has been considered a regulator in the development of the ENS [49]. Deviant expression of SEMA3D has been found in HSCR patients [48].

3.2.4. Transcription factors

Mutation in the transcription factor of RET such as the SOX10 gene has been reported to cause a high percentage of Wardenburg syndrome type IV (WS4), a congenital disorder characterized by hearing loss, pigmentary abnormalities, and HSCR [71]. In 2010, Sanchez Mejias et al. identified for the first time the SOX10 mutation in isolated HSCR cases. Mutation in PHOX2B has been identified in both isolated and syndromic HSCR cases, such as congenital central hypothyroidism syndrome (CCHS) [72, 73]. An in vitro study showed that poly-alanine contraction at the 3'UTR of PHOX2B reduced the transcription activity, hence, it is likely to down-regulate RET expression and lead to HSCR [72]. Garcia-Barcello et al. (2005) reported that the expression of thyroid transcription factor 1 (TTF1-1) overlapped with p75NTR, a marker for enteric neural crest progenitor cells in humans. TTF1-1 trans-regulates RET expression by binding to the HSCR-associated locus region in the RET promoter [74]. The same group also identified a mutation in the TTF1 gene in Chinese and Caucasian cohorts, with the frequency of the TTF1-1 mutation in Caucasians ~2.85% [75].

3.2.5. Hedgehog (HH) signaling

Glioma-associated oncogene homolog 1 (GLI), a zinc finger protein is a downstream effector of SHH and IHH in the Hedgehog signaling pathway. A mutation in GLI1 was first identified in a Chinese HSCR patient (~25%) in 2015. Subsequently, a functional study in vitro showed that this mutation leads to an increase in SOX10 expression which is known to play a role in regulating neuronal-glial differentiation and migration. The high expression of GLI due to loss of Sufu leads to defective axonal fasciculation and delayed cell colonization in the mouse gut, resulting in hypoganglionosis [76]. Sribudiani et al. (2018) identified a missense mutation in exon 1 of IHH (p.Gln51Lys) and a missense mutation in exon 1 of GLI3 (p.Pro707Ser) in a large family with HSCR. A functional study on the IHH mutation in vitro showed that the mutation leads to reduced expression of GLI1 protein. Injection of morpholinos which target ihh in zebrafish reduce the number of neurons by 13% compared to control [6].

About 30% of HSCR patients show mutations in genes organized into four categories: (a) genes that affect the RET/GDNF pathway; (b) genes involved in the EDNRB/EDN3 pathway; (c) transcription factors; and (d) other genes or cell elements involved in the development of ENS. Although RET mutation is known to be a major risk factor for HSCR, mutations in other additional genes have also been linked to HSCR (Figure 2).

Table 1 summarizes some of the known HSCR genes, their function, and their role in ENS development. These genes do not act independently but also interact with each other. Initially, no connection was found between the RET/GDNF and EDNRB/EDN3 signaling pathway, so these pathways were assumed to operate separately. However, there is an interaction between the RET and EDNRB signaling pathways that regulates the development of the ENS.

EDNRB activation specifically enhances the effect of RET signaling for ENS progenitor proliferation, as well as modifies the migration response of NCCs. EDN3 and GDNF appear to have a synergistic effect on the proliferation of undifferentiated progenitors and an antagonistic effect on the migration of ENCCs. The interaction between the RET/GDNF and EDNRB/EDN3 signaling pathways has been found to regulate ENS development throughout the gut, providing evidence that there is a coordinated interaction between these signaling pathways [67].

3.3. Epigenetic aspects of ENS development

The term epigenetics was first introduced by Conrad Waddington in 1975 as a branch of biology that studies the causal interactions between genes and their products that form phenotypes. Epigenetic features are phenotypes that result from chromosome changes without changes in the order of deoxyribonucleic acid (DNA) [8, 128].

![Figure 2](image-url) Genes and major signaling pathways (e.g. RET/GDNF; EDNRB/EDN3) involved in the pathogenesis of Hirschsprung disease (modified from Alves, 2013 [2]). NCSC: neural crest stem cell.
Epigenetics are involved in many normal cellular processes. Human cells have the same DNA but the body contains many different types of cells, such as neurons, liver cells, pancreatic cells, inflammatory cells, and others [129, 130]. Cells, tissues, and organs are different because

Table 1. The genes involved in HSCR, the accompanying conditions, and their function during ENS development.

| Gene                                | Abbreviation | Mutation | Underlying Condition | Function in ENS Development |
|-------------------------------------|--------------|----------|----------------------|------------------------------|
| Receptor tyrosine kinase            | RET          | Identified in 50% of familial cases [64, 77, 78] and 15–35% of sporadic cases [64, 79] | Isolated HSCR, Multiple endocrine neoplasia type II A (MEN2A) syndrome, type IIB (MEN2B), and medullary thyroid carcinoma | Expressed by ENCCs [86] Supports proliferation, migration, survival and differentiation of ENCCs [81] |
| Neuregulin 1                        | NRG1         | Mutation identified in familial and sporadic HSCR cases [77, 79] | Isolated HSCR cases | Suppress GDNF-induced neuronal differentiation. Survival of postnatal enteric neurons [82, 83] |
| Glial cell-line derived neurotrophic factor family receptor α | GFRα         | -        | -                    | Binding interaction with GDNF [57] Support survival of ENCCs [60, 84] |
| Ligands of RET:                     |              |          |          |                                    |
| glial cell-derived neurotrophic factor, Neuritin, Artemin, Persephin | GDNF, NRTN, ARTN, PSPN | 2.17% [85], <5% [86], 1.40% [85], 1.40% [85], 0.9% [85] | Isolated HSCR | GDNF family RET ligand produced by intestinal mesenchyme [59, 60, 86] Proliferation of undifferentiated ENCCs, promotes axon projections from excitatory motor neurons [87, 88, 89] Promote ENCCs proliferation, migration and differentiation [85, 90] |
| Endothelin B receptor                | EDNRB        | Identified in ~5% [91] | Waardenburg-Shah Syndrome (WS4) | Expressed by ENCCs, maintains ENCCs in an undifferentiated state, EDNRB expression is regulated by SOX10 [92, 93, 94] |
| Endothelin 3                         | EDN3         | Identified in <5% [95, 96, 97] | Waardenburg-Shah Syndrome (WS2), Isolated HSCR | EDNRB produced by intestinal mesenchymes especially the caecum, and its interaction with EDNRB allows distal bowel colonization [41, 92] |
| Endothelin-converting enzyme 1      | ECE-1        | 1 case reported [98] | | Protolytic conversion of endothelin-3 precursors into their active form [99] |
| SHY-related HMG-box 10              | SOX10        | Waardenburg-Shah Syndrome (WS4) [71, 99] | Hearing loss, HSCR, pigmentary abnormalities | Expressed by ENCCs, maintains ENCCs in an undifferentiated state, cell fate, and glial cell differentiation; activates RET transcription and interacts with EDNRB [100, 101, 102, 103, 104] |
| Pairedlike homeobox 2B              | PHOX2B       | Mutation identified in syndromic and isolated HSCR cases [105, 106, 107] | Neuroblastoma congenital central hypoventilation syndrome (CCHS) and HSCR | Expressed by ENCCs; important for the development of derivatives of NCCs. PHOX2B is required for RET expression [108, 109] |
| Zinc finger homeobox 1B             | ZFHX1B (ZEB2) | No mutations were found in isolated HSCR [110] | Mowat Wilson Syndrome | Expressed by ENCCs; important for the formation of vagal NCCs [111, 112] |
| Thyroid Transcription Factor-1      | TITF1        | Mutation identified in Chinese and in Caucasian (~2.85%) [74, 75] | Isolated HSCR cases | Express in myenteric and submucosal plexus; TITF-1 trans-activates RET gene expression [74] |
| Kinesin Binding Protein             | KBP          | Mutation identified in syndromic HSCR cases [113] | Goldberg-Shprintzen Megacolon syndrome (GOSH) | KBP binds to SCG10 to regulate axon growth and maintain neuronal development in the central and enteric nervous systems [114, 115] |
| L1 Cell Adhesion Molecule          | L1CAM        | No mutation was found in isolated HSCR [116, 117, 118] | X-linked hydrocephalus (XLH) and HSCR | Part of the neural cell adhesion molecule promotes ENCCs migration and differentiation [119, 120] |
| Semaphorins                         | SEMA3A       | Mutation identified in HSCR cohorts [48, 69, 70, 121] | | Delays entry of axons and sacral ENCCs into the hindgut. Control neurons connectivity and migration [122, 123, 124] |
| Hedgehogs Genes                     | IHH, GLI     | Mutation identified in familial and sporadic HSCR cases [5, 76] | Isolated HSCR cases | Promote ENCC differentiation into neurons, proliferation and concentric patterning, as well as the survival of a subpopulation of [23, 125, 126, 127] |
they have certain sets of genes that are "turned-on" or expressed, and other sets that are "turned-off" or inhibited. Epigenetic silencing is one way to turn off genes and can contribute to differential expression. Three systems can interact with each other in the process of gene silencing, DNA methylation, histone modification, and microRNA (miRNA) [129, 130, 131].

### 3.3.1. DNA methylation

DNA methylation is a chemical process that adds a methyl group to DNA. This is a very specific process and takes place in the area of the cytosine nucleotide located next to the guanine nucleotide and is connected by a phosphate called the CpG site. The CpG site is methylated by DNA methyltransferases (DNMTs) enzymes. Inserting a methyl group alters the appearance and structure of DNA and modifies gene interactions with other components in the cell nucleus required for transcription [5, 129]. This process is mediated by the family of DNA methyltransferases, DNMT1, DNMT3A, and DNMT3B. Based on its function, DNMT1 represents the maintained methyltransferase and methyltransferases, DNMT1, DNMT3A, and DNMT3B act as de novo methyltransferases [1].

Hypermethylation causes silencing of genes or decreases gene expression, while hypomethylation causes increased transcription activity thereby increasing gene expression (Figure 3) [130]. The RET promoter has a 5′CpG3 rich region that is easily influenced by methylation, so the degree of methylation regulates RET gene expression. Epigenetic inactivation through hypermethylation of RET promoters has been investigated [42]. The expression of GDNF (ligand of RET) is decreased in some HSCR patients due to hypermethylation of the promoter region, whereas GDNF demethylation increases cell proliferation and viability, cell cycle development, and cell invasion in vitro studies of cells derived from HSCR tissue [130].

### 3.3.2. Histone modification

Histones are the main protein component of chromatin, a complex of DNA and proteins which can form nucleosomes. The histone acts as a spool where DNA can spin [129, 131]. Histones condense DNA strands into structural components called nucleosomes, with each nucleosome containing eight histones, two of each nucleus histones (H2A, H2B, H3 and H4), forming an octameric structure called the nucleosome nucleus, in which DNA is wrapped in an unstructured tail [130, 132].

When histones are modified, this can affect the arrangement of chromatin determining whether the associated chromosomal DNA will be transcribed. If chromatin is less condensed (euchromatin), it is in the active phase and the related DNA can be transcribed, whereas if chromatin is more condensed (creates a complex called heterochromatin), then chromatin is inactive and DNA transcription does not occur [129]. The histone core protein is highly conserved throughout evolution and the tail undergoes post-translational modifications such as methylation, acetylation, deacetylation, phosphorylation, ubiquitination, and sumoylation (Figure 4). So far, only methylation, acetylation and histone deacetylation have been described to play a role in the development of NCCs and neuroectodermal onset [130].

Various mechanisms of histone methylation and acetylation have been associated with the development of NCCs, although their implications for HSCR are unknown, hence their potential role in the pathogenesis of HSCR should be investigated. To date, the only histone modulator factor that has been associated with HSCR is MECP2 (Methyl-CpG binding protein 2). The finding of decreased MECP2 expression level in HSCR patients suggests that changes in MECP2 expression level may be relevant in the etiology of HSCR through regulation of histone modification [133].

### 3.3.3. MicroRNA

MicroRNA (miRNA) is a non-coding RNA 19–25 (~22) nucleotides in length, which binds to the 3′ untranslated region (3′-UTR) messenger RNA (mRNA) target of the gene that encodes the protein [44, 134]. Biogenesis of miRNA starts from the transcription of the miRNA gene by RNA polymerase II which produces primary miRNAs (pri-miRNAs), followed by the breakdown by dsRNA-specific ribonuclease Drosophila-Pasha, which breaks down pri-miRNAs from hundreds to thousands of base pairs long into precursor miRNAs (pre-miRNAs) with ~70 base pairs [135]. This process is known as "cropping" [136]. Pre-miRNA, in the form of a hairpin, is transported from the nucleus to the cytoplasm, which is then broken down by RNAse III Dicer into a miRNA duplex of ~22 nucleotides consisting of 2 strands, the "guide" strand and "passenger" (Figure 5) [136]. The "passenger" strand is degraded, and the "guide" strand is incorporated into the RNA-induced silencing complex (RISC) and acts as a functional mature miRNA, working via different mechanisms based on complementarity with the target mRNA [137, 138].

This is achieved through a one-to-many or many-to-one pattern in regulating gene expression [139, 140, 141]. MiRNA guides the miRNA-induced silencing complex (miRISC) to recognize mRNA and decreases regulation of gene expression through post-transcriptional mechanisms, i.e. inhibition of translation and break down of mRNA [9]. If miRNA is almost completely complementary to the target mRNA, there will be degradation of mRNA but if the complementarity is only partial, translational inhibition will occur (Figure 6) [142].

Since miRNA was found as a potential target for HSCR treatment in the future, identification of miRNA and its target genes has become very important. Sergi et al. summarize the miRNA studies that have been conducted to date related to the involvement of miRNA in the pathogenesis of HSCR. Several miRNA target genes have been linked to HSCR, which are mostly involved in cell migration and proliferation. A previous study showed that miR-34b, miR-146a, miR-196a2, miR-200a, miR141,
and miR-192 are downregulated, while miR-195, miR206, and miR-218-1 are upregulated in HSCR; these miRNAs changes alter the expression of genes involved in the pathogenesis of HSCR [49].

One study in China analyzed the miRNA expression in a profile of colon tissue in HSCR patients, finding the increased expression of 73 miRNAs (miR-141-3p, miR-200a-3p, miR-345-5p, and miR-194-5p) and decreased expression of 89 miRNAs (miR-1228-5p, miR-143-5p, miR-30a-3p, and miR3180) [143].

Other studies describing miRNA profiles of colon tissue of HSCR patients found 168 expressed miRNAs (104 upregulated and 64 downregulated), including miR-142-3p, miR-142-5p, miR-146b-5p, miR-369-3p, and miR-429 which represent the main targets of miRNA dysregulation; these miRNAs are significantly upregulated in the aganglionic colon segment of HSCR patients (p < 0.05) and their target genes encoded proteins are involved in the regulation of cell proliferation and migration through RET pathways and other related signaling pathways such as MAPK and PI3K/Akt [140].

Analysis of miRNA-214 expression with the pleiomorphic adenoma gene-like zinc finger 2 (PLAGL2) gene target from the colon tissue of HSCR patients has also been investigated, showing increased levels of miR-214 expression in the aganglionic colon segment, which inhibits cell migration and proliferation directly by deregulating the expression of the target gene PLAGL2 [144]. In other studies, it has been found that the level of miR-369-3p expression in the aganglionic colon segment increased compared with the normal colon. The SRY-box 4 (SOX4) target gene is decreased in both mRNA and protein encoded by SOX4, significantly suppresses cell migration and proliferation [145].

Research on miR-206 expression targeting the Fibronectin-1 (FN1) gene in an HSCR patient population found that miR-206 expression levels were upregulated or two-fold higher in the ganglionic colon segment, and downregulated 0.5 times in the aganglionic colon segment [146]. This result is in contrast to other studies that found a downregulation or decreased level of miR-206 expression results in overexpression of the target serum deprivation response (SDPR) gene. This causes deformation of neural crest cell caveolae, thus interfering with signal transduction in cell differentiation and migration [147]. A summary of studies relating to the involvement of miRNA in the pathogenesis of HSCR is provided in Table 2.

A summary of studies relating to the involvement of miRNA in the pathogenesis of HSCR is summarized in Table 2.

Previous studies have identified several miRNAs that are regulated and expressed differently in the stenotic colonic tissue of HSCR patients.
Table 2. Research on miRNA and how it relates to HSCR.

| No | Researcher | Years | Sample | Method | Result | Conclusion |
|----|------------|-------|--------|--------|--------|------------|
| 1  | Tang et al. [148] | 2013 | 70 HSCR colon and 60 controls | RT-PCR, western blot, MTT assay and flow cytometry | \( \text{miR-141-1, } \text{CD47, } \text{CUL3 in HSCR} \) (p < 0.05) | An aberrant drop of miR-141 plays a role in pathogenesis of HSCR with inhibits cell migration and cell proliferation |
| 2  | Zhou et al. [133] | 2013 | 73 pairs of colon/rectal tissue specimens, including HSCR stenotic, HSCR dilatation, and normal tissue | qRT-PCR, Western blot | \( \text{MeCP2} \) in a HSCR tissue. miRNA-44b expression not affected (p < 0.05) | MeCP2 expression level may play an important role in the pathogenesis of HSCR by decreasing proliferation |
| 3  | Tang et al. [141] | 2014 | 95 HSCR serum and 104 controls | Taqman low density array, qRT-PCR | \( \text{miR-133a, miR-218-1, miR-92a, miR-25, miR-483-5p} \) (p < 0.05) | 5 types of miRNAs in the serum as HSCR markers have the potential to be a non-invasive diagnostic tool for initial HSCR screening |
| 4  | Mi et al. [149] | 2014 | 50 HSCR patients | | \( \text{miR-124} \) in aganglionic colon | \( \text{miR-124 and SOX9 target gene is overexpressed in the aganglionic colon} \) |
| 5  | Zhu et al. [150] | 2015 | 254 HSCR cases and 265 controls | SNP genotyping (rs2910164, rs11614913), qRT-PCR | The expression of miR-146a is higher for GG than CC genotype; G allele of rs2910164 is associated with HSCR (p < 0.005 OR, 1.54; 95% CI, 1.06-2.23) | G allele of rs2910164 is a risk factor for HSCR, increasing miR-146a expression and decreasing the expression of ROB O 1 which might affect cell proliferation and migration of NCCs |
| 6  | Li et al. [151] | 2014 | 88 HSCR cases and 75 controls | qRT-PCR, western blot | \( \text{miR-200a} \) & \( \text{miR141} \) associated with PTEN miRNA and protein (p < 0.05) | Family of miR200 might play important roles in the pathogenesis of HSCR with coregulator PTEN. |
| 7  | Lei et al. [152] | 2014 | 78 HSCR colon samples and 66 controls | Cell counting kit 8(CCK-8) | \( \text{miR-195} \) in HSCR (p < 0.05) | Abrupt expression of miR-195 might be involved in the pathogenesis of HSCR by DIEXF expression level. |
| 8  | Sharan et al. [147] | 2015 | 80 stenotic HSCR colon samples, 80 dilated HSCR colon samples, and 80 controls | qRT-PCR, western blot, transwell assay, flow cytometry, dual-luciferase reporter assays | \( \text{miR-206} \) in HSCR compared to control (p < 0.05), \( \text{SDPR} \) | \( \text{miR-206 inhibitor suppresses cell migration and proliferation without affecting cell cycle and apoptosis; silencing of SDPR can reduce the extent of the suppressive effect of the miR-206 inhibitor.} \) |
| 9  | Gao et al. [143] | 2017 | 6 HSCR colon samples and 3 controls | Data set screening GSE77296, target gene prediction by miRWalk, Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathway, and gene ontology (GO) term analyses, DEM-target gene interaction network analysis | \( \text{miR-141-3p} \) \( \text{miR-30a-3p} \) | Two miRNAs (miR-141-3p and miR-30a-3p), MAPK signaling pathway, and two major genes (FZI33 and DOK6) involved in the pathogenesis of HSCR |
| 10 | Tang et al. [153] | 2015 | 69 HSCR (42 S-HSCR, 27 L-HSCR) 49 controls | qRT-PCR, Western blot, cell proliferation, cell cycle, and apoptosis, transwell assay, dual luciferase reporter assays | \( \text{miR-218-1} \) \( \text{SLIT2, RET, PLAG1} \) (p < 0.05) | Overexpression of SLIT2 inhibits cell migration by binding to ROB O 1 receptor |
| 11 | Zhu et al. [154] | 2015 | 80 HSCR colon samples and 77 controls | qRT-PCR, western blot, a dual-luciferase reporter assay, transwell assay, CCK8 assay, flow cytometry | \( \text{miR-192/215 in HSCR} \) (p = 0.0001) \( \text{Nidogen1 (NID1)} \) | Reduced mir-192/215 inhibits cell migration and proliferation; silencing of NID1 can reduce the extent of the suppressive effect of \( \text{miR-192/215 inhibitor} \) |
| 12 | Li et al. [140] | 2016 | 76 HSCR colon samples and 3 control | miRNA target prediction by miRWalk software, qRT-PCR | Significant (p < 0.05) Upregulated \( \text{SDPR} \); \( \text{miR-142-3p, miR-142-5p, miR-146b-5p, miR-306-3p, dan miR-429} \); Downregulated \( \text{miR-885-3p, miR-141-3p, miR-192/215-SIGLEC-8} \) (p < 0.05) | miRNA play important roles in the complex and multifactorial pathophysiology of HSCR |
| 13 | Lei et al. [155] | 2016 | 70 HSCR colon samples and 62 controls | qRT-PCR, western blot, dual-luciferase reporter assay, transwell assay, CCK8 assay, flow cytometry | \( \text{miR-215, } \text{Sialic acid binding like lectin 8 (SIGLEC-8)} \) (p < 0.05) | \( \text{miR-215 causes SIGLEC-8 inhibition by binding directly to the 3'-UTR region of SIGLEC-8} \); Silencing of SIGLEC-8 can reduce the extent of the effect of suppressing cell migration and proliferation due to the decline of \( \text{miR-215} \) | JARS2-miR-215-SIGLEC-8 pathway might play role in the pathogenesis of HSCR |

(continued on next page)
Table 2

| No. | Researcher | Years | Sample | Method | Result | Conclusion |
|-----|------------|-------|--------|--------|--------|------------|
| 14  | Pan et al. [145] | 2017 | 60 HSCR colon samples and 47 controls | qRT-PCR, dual-luciferase reporter assay, transwell assay, CCK8 assay, flow cytometry | \( \text{miR-369-3p, } \text{miR-508} \) (p < 0.05) | Aberrant expression of miR-369-3p may be involved in the pathogenesis of HSCR by regulating the expression of SOX4; dysregulation of miR-369-3p and SOX4 suppresses cell migration and proliferation |
| 15  | Wang et al. [156] | 2017 | 20 HSCR colon samples and 20 controls | qRT-PCR, western blot, dual-luciferase reporter assay, transwell assay, CCK8 assay, flow cytometry | \( \text{miR-483-5p, } \text{miR-668-3p} \) (p < 0.05) | miR-483-5p may play a role in the pathogenesis of HSCR by inhibiting cell migration and proliferation |
| 16  | Wu et al. [144] | 2018 | 24 HSCR colon samples and 20 controls | qRT-PCR, western blot, dual-luciferase reporter assay, transwell assay, CCK8 assay, flow cytometry | \( \text{miR-214, } \text{miR-23a} \) (p < 0.001) | miR-214 plays a role in the pathogenesis of HSCR |
| 17  | Hu et al. [157] | 2018 | 8 HSCR colon samples and 8 controls | qRT-PCR, western blotting, CCK-8 assay, luciferase reporter assay | \( \text{miR-431-5p, } \text{miR-369-3p} \) | Reduced miR-431-5p expression in ENCCs proliferation by targeting LRSAM1 |
| 18  | Gunadi, et al. [146] | 2019 | 21 HSCR ganglionic (G), aganglionic (A), and 13 controls | qRT-PCR | \( \text{miRNA-206} \) (2-fold) in HSCR-G and \( \text{miR-9, miR-124, miR-141, miR-181b, miR-188, miR-214 and miR-302a} \) [11]. | To date, most miRNAs have been analyzed as segregated or individual components for specific target genes. However, these genes can interact with each other and they are activated by several specific signaling pathways, mainly RET/GDNF, EDNRB/EDN3, and Semaphorin pathways. The mechanism of action of miRNA can also be "one on many" or "many on one", therefore, a more thorough study of miRNAs and their effect, either as a single molecule or a group of molecules on gene(s) activation pathways would provide better insight into their role in the pathogenesis of HSCR. |

Another study attempted to identify the diagnostic marker for HSCR by analyzing miRNA that was expressed differently in the serum of HSCR patients and control subjects. However, the molecular mechanisms of those miRNAs involved in HSCR etiology have not been fully elucidated. Li et al performed an analysis of the most relevant regulatory pathways associated with target genes from miRNA that were identified previously, revealing that many key pathways of cellular signaling can be disrupted by changes in miRNA expression. Fifty miRNA targets were experimentally validated for HSCR through identification via miRWalk software, GoGene database, and NCBI PubMed [140]. The study also identified signaling pathways that are related to cell proliferation and migration (RET, MAPK, phosphatase and tensin homolog [PTEN], PI3K/AKT, Hedgehog, p53, p21, and ZEB2), apoptosis (BCL-2, PTEN, PI3K/AKT, p53, p21, and c-Jun), and inflammation (TNFa, IL1β, IL-6, MAPK, and PI3K/AKT) [140].

The signaling pathway associated with RET (MAPK and PI3/AKT) contributes greatly to the pathogenesis of HSCR. [141] In this study, transcripts of 14 genes involved in those pathways (RET, FGF, MAPK3, IL1B, JUN, MAPK1, MAP2K1, TGFβ, TNF, TP53, AKT, FOS, p21, and PTEN) are targets of miRNA that are expressed differently in HSCR. This study found a decreased expression of RET and molecules involved in RET-related signaling pathways. Six miRNAs (miR-142-3p, miR-142-5p, miR-146b-5p, miR-369-3p, and miR-429) were significantly upregulated in the aganglionic segments (p < 0.05), and one miRNA, namely miR-885-3p, was significantly downregulated (p < 0.05) [140].

The identification of miRNA profiles can identify RET dysregulation and RET-related signaling pathways, and miRNA can be individually or collectively influenced synergistically. Changes in the molecules related to the regulation of target genes and signaling pathways are involved in the pathogenesis of HSCR [140].

The regulation of Semaphorin expression by miRNA is found to vary, both in the physiological system and in pathological conditions such as immunity, cardiovascular system, nervous system, and cancer. However, the existing research data is still elementary, so further research is necessary. Semaphorin, Neuropilin 1 and 2, and Plexin are molecules affected by miRNA. Several miRNAs modulate Semaphorin signalling, namely miR-9, miR-27a/b, miR-124, miR-141, miR-181b, miR-188, miR-214 and miR-302a [11].

To date, most miRNAs have been analyzed as segregated or individual components for specific target genes. However, these genes can interact with each other and they are activated by several specific signaling pathways, mainly RET/GDNF, EDNRB/EDN3, and Semaphorin pathways. The mechanism of action of miRNA can also be "one on many" or "many on one", therefore, a more thorough study of miRNAs and their effect, either as a single molecule or a group of molecules on gene(s) activation pathways would provide better insight into their role in the pathogenesis of HSCR.

4. Conclusion

HSCR is a developmental disorder of the ENS due to an abnormality in migration, proliferation, differentiation, and preservation of ENCCs. A comprehensive understanding of the complex pathogenesis of HSCR, involving genetic, epigenetic, cellular, and molecular events during the development of the ENS is essential. Epigenetics is known to be involved in the occurrence of HSCR and this could be a way of prevention and treatment in the future.

This review showed that there are many mechanisms involved in the development of NCCs and the ENS, which confirms that aberrant genetic and epigenetic patterns can contribute to the development of neurocrystalpathy. Some miRNA regulation appears to directly affect the expression of target genes and/or their allied receptors, also exerting an indirect effect by modulating the molecules that regulate the expression of target genes and signaling molecules, including transcription factors, influencing the exchange of receptors with certain cell subcompartments, or regulating the release and exposure of allied receptors at the right time and location. Apart from identifying the direct target of miRNA, it will be interesting to investigate how miRNAs participate in the molecular network and see how that network can ultimately modulate protein levels. Several studies show that the knockout of multiple single miRNAs
does not cause obvious phenotypic abnormalities or phenotypes with incomplete penetration. Hence, it is important to thoroughly analyze not only each miRNA separately but also groups of multiple miRNAs that target a single mRNA or are involved in the same signaling pathway.

Declarations

Author contribution statement

All authors listed have significantly contributed to the development and the writing of this article.

Funding statement

R. Diposarosa, Edhyana Sahiratmadja, Y. Sribudiani was supported by the Ministry of Research and Technology/National Research and Innovation Agency (KEMENRISTEK/BRIN), the Republic of Indonesia (1827/UN6.3.1/LT/2020).

Data availability statement

No data was used for the research described in the article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

References

[1] A. Torrogllosa, M.M. Alves, R.M. Fernández, G. Antínolo, R.M. Hofstra, Epigenetics in ENS development and Hirschsprung disease, Dev. Biol. 417 (2016) 209–216.
[2] M.M. Alves, et al., Contribution of rare and common variants determine complex diseases–Hirschsprung disease as a model, Dev. Biol. 382 (2013) 320–329.
[3] D. Schriemer, et al., Regulators of gene expression in Enteric Neural Crest Cells are putative Hirschsprung disease genes, Dev. Biol. (2016) 1–11.
[4] C. Tomuschat, P. Purk, RET gene is a major risk factor for Hirschsprung’s disease: a meta-analysis, Pediatr. Surg. Int. 31 (2015) 701–710.
[5] J. Amiel, S. Lyonnet, Hirschsprung disease, associated syndromes, and genetics: a review, J. Med. Genet. 38 (2001) 729–739.
[6] Y. Sribudiani, et al., Identification of variants in RET and IHH pathway members in a large family with history of hirschsprung disease, Gastroenterology 155 (2018).
[7] A.K. Yadav, G. Chopra, Clinics in surgery the evolving genetic landscape of Hirschsprung’s disease: advances in genetic and stem cell studies, Nat. Rev. Neurosci. 8 (2007) 151–157.
[8] P.H. Strobl-Mazzulla, M. Marini, A. Buzzi, Epigenetic landscape and miRNA regulation of variants in RET and IHH pathway members in a large family with history of hirschsprung disease, Gastroenterology 155 (2018).
[9] N. Tjaden Butler, P. Trainor, The developmental etiology and pathogenesis of Hirschsprung disease, J. Pediatr. Surg. (2000) 35 1017–1025.
[10] J. Wilkinson, D.H. Edgar, S.E. Kenny, Future therapies for Hirschsprung’s disease, Semin. Pediatr. Surg. 21 (2012) 364–370.
[11] A.J. Burns, N.M. Le Douarin, 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 (1998) 4335–4347.
[12] R.P. Kapur, Colonization of the murine hindgut by sacral crest-derived neural precursors: experimental support for an evolutionarily conserved model, Dev. Biol. 227 (2000) 146–155.
[13] A.J. Burns, D. Champion, N.M. Le Douarin, Sacral neural crest cells colonise aganglionic hindgut in vivo but fail to compensate for lack of enteric ganglia, Dev. Biol. 219 (2000) 30–43.
[14] I.L. Lake, R.G. Heuckeroth, Enteric nervous system development: migration, differentiation, and disease, Am. J. Physiol. Gastrointest. Liver Physiol. 305 (2013).
[15] F. Obermayr, R. Hotta, H. Enomoto, H.M. Young, Development and developmental disorders of the enteric nervous system, Nat. Rev. Gastroenterol. Hepatol. 10 (2013) 43–57.
[16] H.M. Young, et al., Dynamics of neural crest-derived cell migration in the embryonic gut musculature, Dev. Biol. 270 (2004) 455–473.
[17] A.J. Barlow, A.S. Wallace, N. Thapar, A.J. Burns, Critical numbers of neural crest cells are required in the pathways from the neural tube to the foregut to ensure complete enteric nervous system formation, Development 135 (2008) 1681–1691.
[18] D.F. Newgreen, B. Southwell, L. Hartley, I.J. Allan, Migration of enteric neural crest cells in relation to growth of the gut in avian embryos, Cells Tissues Organs 127 (1996) 105–115.
[19] M.J. Simpson, D.C. Zhang, M. Mariani, K.A. Landman, D.F. Newgreen, Cell proliferation drives neural crest cell invasion of the intestine, Dev. Biol. 302 (2007) 552–568.
[20] D.F. Newgreen, R. Mitterman, E.A. Peters, Morphology and behaviour of neural crest cells of chick embryo in vitro, Cell Tissue Res. 203 (1979) 115–140.
[21] K.A. Landman, M.J. Simpson, D.F. Newgreen, Mathematical and experimental insights into the development of the enteric nervous system and Hirschsprung’s Disease, Dev. Growth Differ. 49 (2007) 277–286.
[22] J.B. Furness, B.P. Callaghan, L.R. Rivera, H.J. Cho, The enteric nervous system and gastrointestinal innervation: integrated local and central control, Adv. Exp. Med. Biol. 817 (2014). Springer New York.
[23] H.M. Young, A.J. Bergner, T. Müller, Acquisition of neuronal and glial markers by neural crest-derived cells in the mouse intestine, J. Comp. Neurol. 456 (2003) 1–11.
[24] V. Pavlovska, M.H.H. Schmidt, Neuron-glia interaction in the developing and adult enteric nervous system, Cells 10 (2021) 1–20.
[25] N. Bondurand, D. Natarajan, A. Barlow, N. Thapar, V. Pachnis, Maintenance of mammalian enteric nervous system progenitors by Sox10 and endothelin 3 signalling, Development 133 (2006) 2075–2086.
[26] J. Nishino, T.L. Saunders, K. Sagane, S.J. Morrison, Lgi4 promotes the proliferation and differentiation of glial lineage cells throughout the developing peripheral nervous system, J. Neurosci. 30 (2010) 15282–15290.
[27] A. Chalazonitis, P. D’Aoutremaux, T.D. Pham, A.J. Kesler, M.D. Gershon, Bone morphogenetic proteins regulate enteric gliogenesis by modulating ErbB3 signalling, Dev. Biol. 350 (2011) 64–79.
[28] N. Bondurand, E.M. Southard-Smith, Mouse models of Hirschsprung disease and other enteric nervous system disorders: new and old players, Dev. Biol. 417 (2016) 139–157.
[29] T. Widowati, et al., RET and EDNRB mutation screening in patients with Hirschsprung disease: functional studies and its implications for genetic counseling, Eur. J. Hum. Genet. 24 (2016).
[30] J.H. Kim, et al., New variations of the EDNRB gene and its association with sporadic Hirschsprung’s disease in Korea, J. Pediatr. Surg. 41 (2006) 1708–1712.
[31] J.M. Tilghman, et al., Molecular genetic anatomy and risk profile of hirschsprung’s disease, N. Engl. J. Med. 380 (2019) 1432–1432.
[32] G. Gnudi, et al., Aberrant expressions and variant screening of sema3a in Indonesian Hirschsprung patients, Front. Pediatr. 8 (2020) 1–7.
[33] C.M. Sergi, O. Caluaseriu, H. McColl, B.D. Eisenstat, Hirschsprung’s disease: clinical dysmorphology, genes, micro-RNAs, and future perspectives, Pediatr. Res. 81 (2017) 177–191.
[34] J. Amiel, et al., Hirschsprung disease, associated syndromes and genetics: a review, J. Med. Genet. 45 (2008) 1–14.
[35] A. Durbece, et al., GDNF signalling through the Ret receptor tyrosine kinase, Nature 381 (1996) 789–793.
P. K. Potkhozheva, et al., Neutrin, a relative of glial-cell-line-derived neurotrophic factor, Nature (1996) 384 467–470.

R.H. Baloh, et al., Arternin, a novel member of the GDNF ligand family, supports peripheral and central neurons and signals through the GFRoα3-RET receptor complex, Neuron 21 (1999) 1291–1302.

J. Milbrandt, et al., Persephin, a novel neurotrophic factor related to GDNF and neurturin, Neuron 28 (2000) 245–253.

C.F. Iba, Structure and physiology of the RET receptor tyrosine kinase, Cold Spring Harb. Perspet. Biol. 5 (2015) a009134.

H. Gui, et al., RET and NRG1 interplay in Hirschsprung disease, Hum. Genet. 132 (2013) 229–242.

M. Ruiz-Ferrer, et al., Novel mutations at RET ligand genes preventing receptor activation as a candidate gene for Hirschsprung disease susceptibility, Genomics 203 (2017) 216–221.

J. Rossi, et al., Alimentary tract innervation deficits and dysfunction in mice lacking GDNF family receptor-z2, J. Clin. Invest. 112 (2003) 707–716.

P.T. Darbec, L.B. Blaabjerg, A. Schuchardt, F. Costantini, V. Pachnis, Common origin and developmental dependence on a c-r of subsets of enteric and sympathetic neuroblasts, Development 122 (1996) 349–358.

T.A. Heaney, V. Pachnis, Expression profiling the developing mammalian enteric nervous system identifies candidate and candidate Hirschsprung disease genes, Proc. Natl. Acad. Sci. U.S.A. 103 (2006) 6919–6924.

N. Nagy, A.M. Goldstein, Endothelin-3 regulates neural crest cell proliferation and differentiation in the hindgut enteric nervous system, Dev. Biol. 293 (2006) 203–217.

S. Eketj, M. Garcia-Barceló, et al., TTF-1 and RET promoter SNPs: regulation of RET signaling, Endocr. Relat. Canc. 20 (2013) 595–608.

R.M. Hofstra, et al., RET and GDNF gene scanning in Hirschsprung patients with hydrocephalus, vesico-ureteral reflux and a balanced translocation t (3; 12) in a patient with hydrocephalus, vesico-ureteral reflux and a balanced translocation t (3; 12), Eur. J. Hum. Genet. 5 (1997) 247–251.

P. Edery, et al., Mutation of the endothelin-3 gene in the Waardenburg-Hirschsprung disease (Shah-Waardenburg syndrome), Nat. Genet. (1996) 12 442–444.

R.M. Hofstra, et al., A homoygous mutation in the endothelin-3 gene associated with a combined Waardenburg type 2 and Hirschsprung phenotype (Shah-Waardenburg syndrome), Nat. Genet. (1996) 12 445–447.

R.M. Hofstra, et al., A loss-of-function mutation in the endothelin-converting enzyme 1 (ECE-1) associated with hirschsprung disease, cardiac defects, and autonomic dysfunction, Am. J. Hum. Genet. 64 (1999) 304–307.

H. Yamanagawa, et al., Dual genetic pathways of endothelin-mediated intercellular signaling revealed by targeted disruption of endothelin converting enzyme-1 gene, Rev. Physiol. Biochem. Pharmacol. 125 (1997) 1–39.

E.M. Southard-Smith, L. Kos, W.J. Pavan, Sox10 mutation disrupts neural crest development in Dom Hirschh ruin mouse model, Nat. Genet. (1998) 60–64.

C. Paratore, C. Eichenberger, U. Suter, L. Sommer, Sox10 haploinsufficiency affects maintenance of axons in a mouse model of Hirschsprung disease, Hum. Mol. Genet. (2011) 3075–3085.

J. Kim, L. Lo, E. Dormand, D.J. Anderson, Sox10 maintains multipotency and inhibits neuronal, Neuron 30 (2003) 17–31.

D. Lang, et al., Pnoc3 is required for enteric ganglia formation and functions with Sox10 to modulate expression of c-ret, J. Clin. Invest. 106 (2000) 963–971.

G. Buchanan, et al., Structural and functional consequences of glialteme kinase variation in the androgen receptor, 2004, pp. 1677–1692, 13.

M. Nagahimada, H. Ohta, A. Auton, L. Suresh, Sox10 haploinsufficiency-associated neurturin mutations in PHOX2B dysregulate Sox10 expression, J. Clin. Invest. 122 (2012) 3145–3158.

J. Amiel, et al., Polyalanine expansion and frameshift mutations of the paired-like homeobox gene PHOX2B in congenital central hypventilation syndrome, Nat. Genet. (2003) 459–461.

D. Trochet, et al., PHOX2B genotype Allows for prediction of tumor risk in congenital central hypventilation syndrome, Am. J. Hum. Genet. 76 (2005) 421–426.

S. Elworthy, J.P. Pinto, A. Petitter, M.L. Cancela, R.N. Kelsh, Phox2b function in the enteric nervous system is conserved in zebrafish and is sox10-dependent, Dev. Biol. 306 (2007) 253–264.

J. Amiel, et al., Large-scale deletions and SMAD1P1 truncating mutations in syndromic hirschsprung disease with involvement of midline structures, Am. J. Hum. Genet. 69 (2001) 1370–1377.

L. Garaveli, et al., Hirschsprung disease, mental retardation, characteristic facial features, and mutation in the geneZEHKB1 (SIP1): confirmation of the Mowat-Wilson syndrome, Am. J. Med. Genet. 116A (2003) 385–388.

L. Stanchina, T. Putte Van De, M. Goossens, D. Huybrekeck, N. Bondurand, Genetic interaction between Sox10 and Zfhb1 during enteric nervous system development, Dev. Biol. 341 (2010) 416–428.

T. Van de Putte, et al., Mice lacking Zfhb1, the gene that codes for Smad-600, providing insight into the cellular basis of Goldberg-Shprintzen syndrome, Development 135 (2008) 599–608.

N. Okamoto, et al., Hydrocephalus and Hirschsprung’s disease with a mutation of LICAM, J. Hum. Genet. 49 (2004) 334–337.

S.A. Vign, S.G. Alley, L.M. Randolph, A.H. Ford, A.C.E. Shin, LICAM mutation in association with X-linked hydrocephalus and Hirschsprung’s disease, Pediatr. Surg. Int. 25 (2009) 823–825.

P. Gineri, et al., Complex pathogenesis of Hirschsprung’s disease in a patient with hydrocephalus, vesico-ureteral reflux and a balanced translocation t (3; 17)(p12; q11), Eur. J. Hum. Genet. 17 (2009) 1482–1490.

R.B. Anderson, et al., The cell adhesion molecule Li is required for chain migration, Gastroenterology 130 (2006) 1221–1232.
[120] K.N. Turner, M. Schachner, R.B. Anderson, Cell adhesion molecule Li affects the rate of differentiation of enteric neurons in the developing gut, Dev. Dynam. 238 (2009) 708–715.

[121] B. Luzon-Toro, et al., Mutational spectrum of semaphorin 3A and semaphorin 3D genes in Spanish hirschsprung patients, PloS One 8 (2013).

[122] J. Gonzales, et al., Semaphorin 3A controls enteric neuron connectivity and is inversely associated with synaptin 1 expression in Hirschsprung disease, Sci. Rep. (2020) 1–13.

[123] R.B. Anderson, A. Berger, M. Taniguchi, H. Fujisawa, A. Forrai, Effects of different regions of the developing gut on the migration of enteric neural crest-derived cells: a role for Sema3A, but not Sema3F, Dev. Biol. 305 (2007) 287–299.

[124] I.T. Shepherd, J.A. Raper, Collapsin-1/semaphorin D is a repellent for chick ganglion of Remak axons, Dev. Biol. 212 (1999) 42–53.

[125] A. Sukegawa, et al., The concentric structure of the developing gut is regulated by Semaphorin 3A and 3D, Dev. Biol. 212 (1999) 42.

[126] E.S.W. Ngan, et al., Hedgehog/notch-induced premature gliogenesis represents a new disease mechanism for Hirschsprung disease, Orphanet J. Rare Dis. 14 (2019) 1–6.

[127] A. Sharan, et al., Down-regulation of miR-206 is and Suppresses Cell Migration and Proliferation in Cell Models, 2015, pp. 12105–2105.

[128] G. Egger, G. Liang, A. Aparicio, P.A. Jones, Epigenetics in human disease and future therapies of Hirschsprung disease - a review, Clin. Epigenet. 11 (2019).

[129] E.G. Jaroy, et al., An operational definition of epigenetics, Genes Dev. 23 (2009) 781–783.

[130] W. Pan, et al., Upregulation of MiR-369-3p suppresses cell migration and proliferation by targeting SOX4 in Hirschsprung’s disease, J. Pediatr. Surg. 52 (2017) 1363–1370.

[131] H. Lei, et al., Decreased MiR-200a/141 suppress cell migration and proliferation by targeting PTEN in Hirschsprung’s disease, Cell. Physiol. Biochem. 48 (2018) 1398–1409.

[132] H. Zhu, et al., A common polymorphism in pre-miR-146a underlies Hirschsprung disease risk in Han Chinese, Exp. Mol. Pathol. 97 (2014) 511–514.

[133] B. H. Hu, L. Cao, X. ye Wang, L. Li, Downregulation of microRNA-431-5p promotes cell proliferation by targeting LRSAM1 in Hirschsprung disease, J. Neurochem. 134 (2015) 39–46.

[134] K. Mi, D. Chen, W. Wang, H. Gao, Study of the effect of miR-124 and the SOX9 target gene in Hirschsprung’s disease, Mol. Med. Rep. 9 (2014) 1839–1843.

[135] B. Hu, L. Cao, X. ye Wang, L. Li, Downregulation of microRNA-431-5p promotes cell proliferation by targeting LRSAM1 in Hirschsprung disease, Orphanet J. Rare Dis. 14 (2019) 1–6.

[136] A. Sharan, et al., Down-regulation of miR-206 is and Suppresses Cell Migration and Proliferation in Cell Models, 2015, pp. 1–6.

[137] E. R. Gibney, C. M. Nolan, Epigenetics and gene expression, Heredity 105 (2010) 42–53.

[138] D. Zhu, et al., Nidogen-1 is a common target of microRNAs MiR-192/215 in the pathogenesis of Hirschsprung’s disease, J. Cell Mol. Med. 19 (2015) 1197–1207.

[139] J. Gonzales, et al., Semaphorin 3A controls enteric neuron connectivity and is inversely associated with synaptin 1 expression in Hirschsprung disease, Dev. Dynam. 238 (2009) 1967–1966.

[140] P. Brodersen, O. Voinnet, Revisiting the principles of microRNA target recognition and mode of action, Nat. Rev. Mol. Cell Biol. 10 (2009) 141–148.

[141] S. Li, et al., MiRNA Profiling reveals dysregulation of RET and RET-regulating pathways in hirschsprung’s disease, PloS One 11 (2016) 1–14.

[142] W. Tang, et al., Specific serum microRNA profile in the molecular diagnosis of Hirschsprung’s disease, J. Cell Mol. Med. 18 (2014) 1580–1587.

[143] J. Mi, D. Chen, W. Wang, H. Gao, Study of the effect of miR-124 and the SOX9 target gene in Hirschsprung’s disease, Mol. Med. Rep. 9 (2014) 1839–1843.

[144] H. Zhu, et al., A common polymorphism in pre-miR-146a underlies Hirschsprung disease risk in Han Chinese, Exp. Mol. Pathol. 97 (2014) 511–514.

[145] H. Li, et al., Decreased MiR-200a/141 suppress cell migration and proliferation by targeting PTEN in Hirschsprung’s disease, Cell. Physiol. Biochem. 34 (2014) 543–555.

[146] W. Tang, et al., SLIT2/ROBO1-miR-218-1-RET/PLAG1: a new disease pathway involved in Hirschsprung’s disease, J. Cell Mol. Med. 19 (2015) 1197–1207.

[147] D. Zhe, et al., Nidogen-1 is a common target of microRNAs MiR-192/215 in the pathogenesis of Hirschsprung’s disease, J. Neurochem. 134 (2015) 39–46.

[148] C. Physiology, Erratum: aberrant reduction of mir-141 increasedCD47/CUL3 in hirschsprung’s disease (Cellular Physiology and Biochemistry (2015) 32 (1655–1667), Cell. Physiol. Biochem. 48 (2018) 1398–1399.

[149] H. Lei, et al., Decreased MiR-200a/141 suppress cell migration and proliferation by targeting PTEN in Hirschsprung’s disease, Cell. Physiol. Biochem. 34 (2014) 543–555.

[150] H. Li, et al., MiR-195 affects cell migration and cell proliferation by down-regulating DIELXP in Hirschsprung’s Disease, BMC Gastroenterol. 14 (2014) 1–7.

[151] W. Tang, et al., SLIT2/ROBO1-miR-218-1-RET/PLAG1: a new disease pathway involved in Hirschsprung’s disease, J. Cell Mol. Med. 19 (2015) 1197–1207.

[152] D. Zhe, et al., Nidogen-1 is a common target of microRNAs MiR-192/215 in the pathogenesis of Hirschsprung’s disease, J. Neurochem. 134 (2015) 39–46.

[153] C. Physiology, Role of MiR-215 in Hirschsprung ’S Disease Pathogenesis by Targeting SIGLEC-8, 2016, pp. 1646–1655.

[154] G. Wang, et al., Downregulation of microRNA-483-5p promotes cell proliferation and invasion by targeting GFRA4 in hirschsprung’s disease, DNA Cell Biol. 36 (2017) 930–937.

[155] B. Hu, L. Cao, X. ye Wang, L. Li, Downregulation of microRNA-431-5p promotes enteric neural crest cell proliferation via targeting LRSAM1 in Hirschsprung’s disease, Dev. Growth Differ. (2019) 294–302.

[156] X. Lv, et al., Molecular function predictions and diagnostic value analysis of plasma exosomal miRNAs in Hirschsprung’s disease, Epigenomics 12 (2020) 499–522.