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Clinical aspects of neurointestinal disease: Pathophysiology, diagnosis, and treatment

Allan M. Goldstein [a,*], Nikhil Thapar [b], Tennekoon Buddhika Karunaratne [c,d], Roberto De Giorgio [c,d]

[a] Department of Pediatric Surgery, Center for Neurointestinal Health, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA
[b] Division of Neurogastroenterology and Motility, Department of Gastroenterology, UCL Institute of Child Health and Great Ormond Street Hospital, London, UK
[c] Department of Medical and Surgical Sciences and Gastrointestinal System, University of Bologna and St. Orsola-Malpighi Hospital, Bologna, Italy
[d] Centro di Ricerca BioMedica Applicata (C.R.B.A.), University of Bologna and St. Orsola-Malpighi Hospital, Bologna, Italy

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ABSTRACT

The enteric nervous system (ENS) is involved in the regulation of virtually all gut functions. Conditions referred to as enteric neuropathies are the result of various mechanisms including abnormal development, degeneration or loss of enteric neurons that affect the structure and functional integrity of the ENS. In the past decade, clinical and molecular research has led to important conceptual advances in our knowledge of the pathogenetic mechanisms of these disorders. In this review we consider ENS disorders from a clinical perspective and highlight the advancing knowledge regarding their pathophysiology. We also review current therapies for these diseases and present potential novel reparative approaches for their treatment.

1. Introduction

The principal function of the gastrointestinal (GI) tract is to ensure the proper digestion and absorption of nutrients and the expulsion of undigested residue and unwanted waste. This complex process requires the coordinated propulsion of endoluminal contents along the length of the GI tract, which in turn relies on the activity of specialized cells, including smooth muscle cells (SMC; final effectors of contraction and relaxation), interstitial cells of Cajal (ICC; gut pacemakers and regulators of neuronal input to the SMC), and a hierarchy of intrinsic and extrinsic neurons to regulate the motor programs (Knowles et al., 2013). While the propagation, absorption, and excretion of food and waste have long been appreciated as key aspects of gut function, the GI tract also has a myriad of other critical roles necessary for maintaining health and homeostasis. These include its capacity to sense and respond to its luminal environment, its role in controlling immune activation (i.e. distinguishing nutrients and commensal bacteria from toxins, allergens, and pathogens) and initiating inflammation, as well as its ability to monitor and control microbiota composition. All of these aspects of GI function share a common feature: they rely on the intestinal tract's intrinsic network of neuronal and glial cells, referred to as the enteric nervous system (ENS).

The ENS is a morphologically and functionally complex system that controls, largely independent of central and peripheral nervous system input, virtually all gut functions (Furness, 2005). It is comprised of a very large number of neuronal and glial cells (about the same number as are in the mammalian spinal cord) that arise from the embryonic neural crest and become organized into two major plexuses: the myenteric (Auerbach's plexus), which extends along the entire length of the GI tract, and the submucosal (Meissner's plexus), which is present from the stomach to the rectum. Enteric neurons can be classified into functionally distinct subpopulations (e.g. intrinsic primary afferent neurons, motor neurons, and interneurons) synaptically linked in reflex circuitries (Furness, 2005). The central nervous system (CNS) can modulate some intrinsic reflexes, particularly in the esophagus, stomach and rectum, via sympathetic and parasympathetic pathways (Furness, 2012). However, the ENS has the ability to control most gut functions independent of CNS input, including secretion, absorption, vascular tone, and motility. Any injury to the ENS, whether congenital or acquired, thus results in intestinal neuropathies that can cause clinical symptoms and lead to significant morbidity.

This review focuses on neurointestinal diseases, which are those conditions associated with abnormalities of enteric innervation. From a biologic perspective, neuronal defects in the GI tract can arise from various etiologies. Developmental disorders

* Corresponding author.
E-mail address: agoldstein@partners.org (A.M. Goldstein).

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represent one cause, in which any aspect of enteric neural crest cell development might be perturbed, including cell migration, proliferation, survival, differentiation, or patterning. Disordered ENS development typically gives rise to congenital enteric neuropathies and manifests in early childhood. Hirschsprung disease is the best characterized of these developmental conditions and therefore the first disease discussed in this review. Many other neurointestinal diseases are acquired, and these are generally believed to be caused by neuronal degeneration, immune-mediated inflammation, or infection. These factors, however, are often intertwined. Neurodegeneration can be associated with prominent inflammation in the enteric ganglia, whether as the cause of the inflammation or as its consequence. Infection can lead to autoantibodies against enteric neuronal antigens and subsequent loss of neurons. Understanding the normal biology of ENS development and maintenance, and defining the pathophysiology that underlies the various neurointestinal diseases, are essential in order to develop improved therapies for these conditions.

It is important to note that the emphasis on the ENS in this review is not intended to undervalue the critical contributions of the SMC, ICC, or extrinsic innervation (autonomic nervous system, spinal cord and brain) to GI health and disease. Rather, it is only intended to focus the review on this specific area of interest. The reader is referred to excellent reviews on those topics (Sanders et al., 2014; Huizinga et al., 2009). We have also taken a neurocentric approach, which is not meant to ignore the importance of the enteric glia, but rather reflects our limited current understanding of their normal function and their role in intestinal disease. Thus, the broad objective of this review is to provide an update on the molecular and clinical features of neurointestinal diseases, to give an overview of therapeutic options for their management, and to present the potential future use of neuroregenerative approaches for their treatment. We hope that by presenting the basic biology of these diseases in a clinical context, the reader will appreciate the significant unmet needs faced by clinicians caring for patients with any form of neurointestinal disease and consequently identify areas where further fundamental research might contribute to improving clinical care.

2. Hirschsprung disease

Hirschsprung disease (HSCR) is a congenital enteric neuropathy that causes functional bowel obstruction as a result of distal intestinal aganglionosis (Panza et al., 2012). First described by Harald Hirschsprung in 1888, HSCR occurs in approximately 1 in 5000 births with a male predominance (4:1 male to female ratio in short-segment disease) (Hirschsprung, 1888). HSCR is characterized by the absence of enteric ganglion cells in the submucosal and myenteric plexuses along variable lengths of the distal bowel. In most cases, children present in the neonatal period with delayed passage of meconium beyond the first 24–48 h of life. Other symptoms and signs are those of complete or partial intestinal obstruction, including feeding difficulties, abdominal distension, vomiting (which may be bilious), and constipation (or obstipation). The most common form is short-segment (S-HSCR) affecting approximately 80% of cases and involving no more than the rectosigmoid colon. Less commonly, the aganglionic segment is longer (L-HSCR), extending proximal to the rectosigmoid. The aganglionosis can involve the entire colon in 8–10% of patients, or, rarely, the entire intestine (Kapur, 1999).

HSCR is a classic example of a neurocristopathy, a disease arising from abnormalities of neural crest-derived cells. In HSCR, enteric neural crest-derived cells fail to complete their rostrocaudal migration along the length of the intestine, leaving variable lengths of distal gut without ganglion cells (Goldstein et al., 2013). The failure to complete migration can be caused by defects in neural crest cell migration, proliferation, survival, or differentiation. HSCR is most commonly an isolated finding, but can occur along with other disorders, most commonly Down syndrome, which is present in about 10% of cases (Heanue and Pachnis, 2007). Since neural crest cells also give rise to melanocytes, abnormalities of pigmentation occasionally co-exist with HSCR. Shah-Waardenburg syndrome (WS4), caused by mutations in *SOX10*, *EDNRB*, or *ET3*, is a neurocristopathy characterized by abnormal pigmentation of the hair, skin, and eyes, intestinal aganglionosis, and congenital hearing loss due to absence of neural crest-derived cells in the cochlea of the inner ear (Pingault et al., 1998). Congenital central hypoventilation syndrome (CCHS), in which autonomic control of spontaneous breathing is impaired, can co-exist with HSCR in a condition called Haddad syndrome (Amiel et al., 2003). This neurocristopathy is associated with mutations in *PHOX2B*, a transcription factor expressed by neural crest cells, which give rise not only to the ENS but also to parts of the autonomic nervous system, accounting for the respiratory defects in this disease. Mowat-Wilson is a syndrome characterized by mental retardation, epilepsy, delayed motor development, and HSCR and has been associated with mutations in *ZFHX1B*, a zinc finger homeobox gene that encodes Smad-interacting protein 1 (Cacheux et al., 2001).

Several observations initially indicated the genetic origin of the disease, including: (i) average risk of recurrence in siblings of 3–4%, about 200-fold higher than in the normal population; (ii) increased prevalence in males; (iii) association with other genetic diseases and chromosomal abnormalities; and (iv) presence of genetic models of aganglionosis with a specific mode of inheritance. The high proportion of sporadic cases (~80%), variable clinical expression (i.e. variable lengths of aganglionosis among relatives), and incomplete penetrance (i.e. some mutation carriers do not have aganglionosis) support a multigenic model to explain the predominantly non-Mendelian inheritance pattern of non-syndromic cases. Linkage analyses in large HSCR families led to the identification of the *RET* gene (10q11.2) as the first gene shown to be involved in HSCR (Gabriel et al., 2002). Coding sequence mutations in *RET* account for 15–35% of patients with sporadic HSCR and 50% of familial cases (Goldstein et al., 2013). Recent data suggest that non-coding mutations in *RET* within a conserved enhancer-like sequence in intron 1 confer susceptibility to HSCR, whereas a variant in the 3′-untranslated region confers a "protective" haplotype that is underrepresented in HSCR (Emison et al., 2005; Griseri et al., 2007). In addition to *RET*, mutations have been identified in over a dozen other genes, but these account for a minority of cases (Goldstein et al., 2013; Wallace and Anderson, 2011). While RET mutations are the major risk factor in this disease, evidence suggests that they may not be sufficient on their own to result in aganglionosis (Goldstein et al., 2013). The majority of cases appear to be multigenic, comprising a combination of RET mutations with genetic abnormalities at other loci, with these interactions impacting the incidence and severity of the aganglionosis. These types of interactions have been observed for RET and EDNRB signaling and for EDNRB and SOX10 (Carrasquillo et al., 2002; Cantrell et al., 2004). An important role has also been described for modifier genes. These are genes that, when mutated, do not result in a phenotype but, when present with a mutation in another gene, they worsen the effect. Examples of modifier genes include neuregulin 1 (NRG1) and L1CAM (Wallace and Anderson, 2011). These modifier genes may help to explain the phenotypic variability and incomplete penetrance characteristic of HSCR.

The diagnosis of HSCR should be considered in neonates presenting with the delayed passage of meconium, or in older infants and children with severe constipation. The gold standard for the diagnosis of HSCR is histological assessment of tissue obtained by

deep rectal biopsy (suction biopsy or full-thickness biopsy) showing the complete absence of submucosal (and myenteric) ganglion cells. The presence of hypertrophied acetylcholinesterase-positive nerve trunks, representing projections of extrinsic nerve fibers, in the muscularis mucosae and lamina propria provides additional confirmation (Qualman et al., 1997), as does the absence of calretinin-positive nerve fibers in the lamina propria and submucosa (Qualman et al., 1997; Knowles et al., 2010; Kapur et al., 2009). It is important to note that a physiologic zone of hypoganglionosis is normally present in the distal 1–3 cm of the rectum and may lead to false-positive results if that distal segment is biopsied (Weinberg, 1975). In contrast, if taken too proximally, rectal biopsy may miss a very short segment of clinically important aganglionosis (Ballard, 1996).

Anorectal manometry (ARM) should not be used as a sole diagnostic tool for HSCR in neonates and infants, but in older children may provide a useful screening test in patients presenting with symptoms suggestive of HSCR. Whereas the presence of a rectoanal inhibitory reflex on ARM would reasonably exclude HSCR in older children, its absence should lead to a rectal biopsy to definitively make the diagnosis. Contrast enemas are useful radiologic studies that often suggest the diagnosis in the first place by demonstrating a tonically contracted distal colon, reflecting the aganglionic segment, and possibly also giving a rough indication of the length of aganglionosis prior to surgery.

The standard treatment for HSCR involves surgical removal of the aganglionic bowel and transition zone, which refers to the relatively hypoganglionic bowel proximal to the aganglionosis. The normoganglionic intestine is then anastomosed to the rectum or anus, depending on the specific procedure performed. A number of techniques have been described to fashion the distal anastomosis, and these are named for their surgical pioneers, most notably Soave, Swenson, and Duhamel. For short-segment aganglionosis, a single-stage transanal operation is commonly performed (Langer et al., 2003).

Surgical treatment of HSCR is undoubtedly life-saving and allows many children to live normal lives. For many patients, however, the long-term outcomes following surgery are far from perfect, with the main problem being fecal incontinence and the significant psychosocial impact that this can have (Laughlin et al., 2012; Catto-Smith et al., 2007; Jarvi et al., 2010; Niramis et al., 2008). Severe constipation can also occur after surgery, possibly related to residual abnormalities in the remaining, presumably “normoganglionic” bowel, or to the failure of normal internal anal sphincter relaxation, which is associated with HSCR. Children with HSCR are also at risk for a potentially life-threatening inflammatory process termed Hirschsprung-associated enterocolitis (HAEC), which can occur before or after surgical removal of the aganglionic segment (Gosain and Brinkman, 2015). HAEC, which usually manifests with abdominal distension and diarrhea, often accompanied by fever and vomiting, can progress to sepsis. HAEC is usually treated with antibiotics and rectal irrigations to evacuate stool from the distal bowel. Since RET signaling is essential for formation of Peyer's patches (aggregates of lymphoid tissue in the gut wall), patients with HSCR and a *RET* mutation may have defects in intestinal immunity, which might contribute to the risk for HAEC (Veiga-Fernandes et al., 2007; Gosain et al., 2015). Several studies implicate alterations in the fecal microbiome as contributing to the pathogenesis of HAEC (Yan et al., 2014; Frykman et al., 2015). This notion is supported by a recent prospective, multicenter, randomized controlled trial showing that administration of probiotics reduces both the frequency and severity of HAEC (Wang et al., 2015).

3. Esophageal achalasia

Achalasia is a disorder of the esophagus characterized by the absence of peristalsis and impaired swallow-induced relaxation of the lower esophageal sphincter (LES), resulting from loss of intrinsic inhibitory neurons in the myenteric plexus. The clinical manifestation is characterized by dysphagia for both solid food and liquids, regurgitation of undigested food, respiratory complications, chest pain, and weight loss. The condition is characterized by a defect of intrinsic inhibitory neurons, which release nitric oxide (NO) and vasoactive intestinal polypeptide (VIP), both in the esophageal body and LES (Hoshino et al., 2013). The resulting imbalance between defective inhibitory innervation and apparently spared excitatory (cholinergic/tachykininergic) neuronal components likely underlies the pathogenesis. It remains unclear why esophageal myenteric neurons are preferentially targeted and irreversibly damaged by the immune system, although several lines of evidence indicate that infectious and immune factors could underlie the etiopathogenesis of this neuropathy (Gyawali, 2016). These factors likely act independently and their influence may be different in different subsets of patients. The myenteric plexus in patients with end-stage achalasia has been found to have an extensive reduction in ganglion cells in association with lymphocytic and eosinophilic infiltrates (Goldblum et al., 1996a). A follow-up study examined the histopathological features of esophageal specimens from patients with early-stage achalasia undergoing surgical myotomy. Inflammation was found in both stages, but fibrosis was only seen in end-stage disease, suggesting a spectrum of histopathological changes during the course of the disease (Goldblum et al., 1996b). These results are consistent with other reports that have shown myenteric inflammatory infiltrates (hence the term “myenteric ganglionitis”) represented by CD3+ and CD8+T cells in both early- and end-stage achalasia, as well as a normal number of myenteric ganglion cells in the early stage of the disease (Clark et al., 2000; Raymond et al., 1999).

Infectious agents, possibly viruses, might trigger an immune response to esophageal myenteric neurons. This was demonstrated by previous work showing herpes zoster virus in patients with the sporadic form of achalasia, although this has been debated (Robertson et al., 1993; Birgisson et al., 1997). A causal link between viral infections and neuropathological alterations has not been definitively demonstrated yet, although an increase in lymphocytic proliferation has been noted in esophageal biopsies of achalasic patients after exposure to herpes simplex 1, supporting a role for an immune response against viral agents infecting esophageal neurons in predisposed patients (Castagliuolo et al., 2004).

Other studies have suggested that circulating autoantibodies might cause immune-mediated neuronal damage in patients with achalasia (Eaker, 1998; Verne et al., 1998), although the autoantibodies could certainly be the consequence of neuronal injury and the expression of normally occult neuronal antigens. Serum of achalasic patients has been shown to contain anti-neuronal antibodies that react against rodent enteric neurons (Latiano et al., 2006; Moses et al., 2003). In both studies, the immunohistochemical pattern was characterized by immunoreactivity toward the nucleus and cytoplasm of myenteric and submucosal neurons, although targets of this immune response are unknown. Intriguingly, the immunolabelling was specific for enteric neurons and was not detected in neurons of the spinal cord, sensory ganglia, or superior cervical ganglia (Moses et al., 2003). Another study of sera from 18 achalasic patients showed that applying the sera to gastric fundus muscle strips of healthy individuals led to an abnormal neurochemical code with reduced nitrergic/VIPergic neurons, increased cholinergic neurons and altered relaxation response when electrical stimuli were

applied (Bruley des Varannes et al., 2006). These observations suggest that other factors, other than antineuronal antibodies, are present in the sera of achalasic patients and may contribute to the ENS dysfunction (De Giorgio et al., 1999).

Although achalasia is mainly sporadic in origin, a genetic predisposition has been suggested. Achalasia can be found in association with rare *syn*dromic disorders with an autosomal recessive inheritance, including familial esophageal achalasia (OMIM 200400) and the achalasia microcephaly syndrome (OMIM 200450). Causative genes for these two conditions have not been identified as yet, but for achalasia-addison-alacrima, also referred to as Allgrove syndrome (AAAS, OMIM 231550), the genetic basis has been elucidated. AAAS was originally reported in two pairs of siblings with achalasia of the cardia and glucocorticoid deficiency. Other clinical signs included defective tear formation (alacrima) and other signs of dysautonomia. The causative *AAAS* gene has been mapped to chromosome 12q13, encoding the protein ALADIN. Several *AAAS* mutations have been identified in patients with a broad spectrum of clinical presentations, while patients with sporadic achalasia do not carry *AAAS* mutations, indicating that different mechanisms can contribute to the pathogenesis of idiopathic achalasia (Di Nardo et al., 2005). Based on the evidence collected to date, the current view suggests that idiopathic achalasia can be defined as an inflammatory/autoimmune disease of unknown etiology with loss of inhibitory neurons in the esophageal myenteric plexus.

Patients presenting with dysphagia and regurgitation, classic hallmarks of achalasia, should be investigated with high-resolution esophageal manometry, which will show a lack of peristalsis and incomplete LES relaxation. According to the Chicago classification for esophageal motility disorders, three manometrically distinct types of achalasia exist: type I, the “classic form” (usually with a markedly dilated esophagus), is characterized by an integrated relaxation pressure (IRP) above the upper limit of normal and complete lack of peristalsis throughout the esophagus; type II is characterized by esophageal compression (mean IRP > upper normal limit), abnormal peristalsis and pan-esophageal pressurization with $\geq$ 20% of swallows; and type III, formerly referred to as “vigorous achalasia”, also exhibits mean IRP > upper limit of normal and abnormal peristalsis, with preserved fragments of distal peristalsis or premature (spastic) contractions with $\geq$ 20% of swallows (Bredenoord et al., 2012). Although quite technical, this classification carries clinically meaningful implications, including certainty of diagnosis, treatment response with either dilatation or surgery, and the natural evolution of the disease (Scherer et al., 2009; Pandolfino et al., 2008). In clinical practice, fluoroscopic imaging during ingestion of a contrast agent will show the classic “bird beak” narrowing at the LES and also identify whether esophageal dilatation exists and to what extent. Because manometry and imaging allow for safe and accurate diagnosis in virtually all cases, histopathological examination of biopsies, other than for research protocols, is not indicated. An exception to this may occur in the small subset of patients with a suspected underlying paraneoplastic syndrome in whom myenteric ganglionitis might be identified at the tissue level. In those rare cases, a search for circulating antineuronal autoantibody should be undertaken, as well as imaging to search for a possible occult malignancy (Clark et al., 2000).

In adults and children, medical treatments for patients with achalasia have limited efficacy and are used only as a temporizing strategy while the patient awaits either dilatation or surgery. Medical therapy is, however, appropriate for subjects deemed unsuitable for an interventional procedure. Pharmacological options include nitrates (nitroglycerin and isosorbide dinitrate), phospho-diesterase-5 inhibitors (e.g., sildenafil), calcium-channel blockers (e.g., nifedipine), and direct injection of botulinum toxin into the LES (Di Nardo et al., 2008). Surgical myotomy, which involves longitudinally dividing the LES, or pneumatic dilatation to physically stretch the LES, remain the definitive treatments. A recent double-blind controlled trial showed that surgery (laparoscopic Heller myotomy) was not superior to pneumatic dilatation at a mean follow-up of 43 months in an international cohort of 210 patients with achalasia (Boeckxstaens et al., 2011). In children, in whom achalasia is much less common than in adults, a recent systematic review concluded that available data could not determine whether surgical myotomy or pneumatic dilatations were superior (Sharp and Peter, 2015). It is important to note that while both interventions effectively reduce LES pressure in most patients, they do not restore esophageal peristalsis, which can continue to cause significant symptomatic dysphagia.

4. Chagas disease

Chagas disease is caused by the parasite *Trypanosoma cruzi* a member of the Trypanosomatidae family transmitted to humans by triatomine insects called Reduviidae beetles or “kissing bug” (Meneghelli, 1985). The disease is endemic in South and Central America and causes > 15,000 deaths annually (Clayton, 2010). Acute symptoms go largely unattended and the infection subsides without treatment. However, some patients develop chronic infection, leading to cardiomyopathy, urinary system involvement (megaloureter) and/or motor dysfunction of the gastrointestinal tract. Gastrointestinal involvement occurs less frequently than cardiomyopathy and is more common in the Southern part of South America (Argentina, Bolivia, Chile, Paraguay, Uruguay, and parts of Brazil) than in northern South America, Central America, and Mexico. This variation is thought to result from differences in the predominant *T. cruzi* genotypes (Miles et al., 1981). The esophagus (megaesophagus) and colon (megacolon) are mainly affected, although the disease may progress to any segment of the bowel and extrahepatic biliary tract (Meneghelli, 1985; Clayton, 2010).

A progressive degeneration and loss of the intrinsic innervation of the digestive system represent the neuropathological hall mark of Chagas resulting from an immune cross-reactivity between the *T. cruzi* flagellar antigen Fl-160 (a surface protein of 160-kDa) and a structurally similar 48-kDa protein expressed by mammalian axons and myenteric neurons (Andersson et al., 2003). The histopathologic analysis shows an immune infiltrate in the myenteric plexus leads to enteric neuron degeneration and loss in a similar fashion to that observed in idiopathic cases of enteric ganglionitis (see below). In addition to an immune cell-mediated response, patients with chronic Chagas’ disease show high titers of circulating antibodies directed against the type 2 muscarinic acetylcholine receptor, which is widely expressed on smooth muscle cells, but not on neurons. These autoantibodies play a pathogenic role in Chagasic achalasia as they bind to muscarinic receptors and evoke muscle contraction (Goin et al., 1999).

Treatment options for chronic Chagas disease are limited and have variable efficacy. Both nifurtimox and *o*-benznidazole yielded eradication of the infection only in about 10% of treated patients and are not thought to influence the progression of gastrointestinal disease (Bern et al., Moore). The management of gastrointestinal Chagas disease is similar to that of idiopathic achalasia or constipation related to megacolon, with the use of drugs and myotomy for the former and laxatives for the latter (de Oliveira et al., 1998). Surgical resection remains the mainstay of therapy in patients with end-stage disease, i.e. those with severe megaesophagus or megacolon (Teixeira et al., 2006).

4.1. Gastroparesis

Gastroparesis, meaning "paralyzed stomach," results in a significant delay in the emptying of solids and liquids from the stomach. Symptomatic gastroparesis is characterized by nausea, vomiting, bloating, early satiety, and epigastric pain. In adults, gastroparesis can be divided into two common forms: idiopathic gastroparesis, accounting for 50% of cases, and diabetic gastroparesis (Horowitz and Fraser, 1995; Patrick and Epstein, 2008). In children, 70% of cases appear to be idiopathic, with the remainder accounted for by drugs, viral infections, or post-surgical (Waseem et al., 2012; Sigurdsson et al., 1997).

Nearly any pathological condition that disrupts the neuromuscular function of the stomach can result in gastroparesis. Alterations in the extrinsic neural supply to the stomach are known to contribute to its pathophysiology. While the pathogenesis of idiopathic gastroparesis is unknown, it has been proposed that abnormalities of intrinsic gastric innervation can also be causative (You et al., 1981; Shellito and Warshaw, 1984). Recent data based on histopathological characterization of laparoscopic biopsies showed ICC depletion or abnormalities in 39% and 48% of idiopathic and secondary (diabetic) gastroparesis cases, respectively, with neuropathic changes observed in 14% and 17% of cases (Grover et al., 2012). Tissue analysis of a patient who underwent total gastrectomy showed hypoganglionosis with features suggestive of neuronal dysplasia. In this report, abnormalities of myenteric and intramuscular ICC, along with neuronal alterations, were described, suggesting a more generalized process. Neuronal inflammation, manifested as myenteric ganglionitis, has also been described in gastroparesis. Several conditions, including paraneoplastic syndromes, are associated with inflammatory neurodegenerative alterations and gastroparesis (Chinn and Schuffler, 1988). Cases of idiopathic inflammatory neuropathies with gastroparesis have also been described. For example, a patient with intractable vomiting had a dense lympho-plasmacellular infiltrate in the gastric myenteric plexus (De Giorgio et al., 2000). Treatment with steroids improved the symptoms, suggesting an important role for immunity and supporting the use of anti-inflammatory therapy in patients with proven myenteric ganglionitis (De Giorgio et al., 2000). Additional causes include post-viral gastroparesis and mitochondrial disease, which were identified in 18% and 8%, respectively, of children with gastroparesis in a recent review (Rodriguez et al., 2012).

A diagnosis of gastroparesis can be made based on clinical assessment that includes (1) symptom evaluation (postprandial fullness, early satiety, nausea, vomiting, upper abdominal bloating), (2) exclusion of mechanical causes for delayed gastric emptying, such as hypertrophic pyloric stenosis in children, and (3) objective evidence from a gastric emptying assay performed using either ^{99}Tc-scintigraphy or ^{13}C octanoic acid breath test. Additional tests can be used to determine possible causes for the gastroparesis, such as electrolyte abnormalities or diabetes mellitus, which are among the most common secondary forms of gastroparesis (Pasricha and Parkman, 2015). Gastric biopsies are not included in the diagnostic work-up for gastroparesis.

The most common therapeutic strategies include dietary modification, nutritional supplements, and prokinetic drugs. For feeding strategies in children with gastroparesis some consideration may need to be given to the type of protein delivered as well as its degree of hydrolysis as there is evidence suggesting that gastric emptying is more rapid with breast milk and predominant whey-based feeds (Meyer et al., 2015). The two most commonly used drugs, metoclopramide and domperidone (the latter not commercially available in the U.S.), have been shown to be effective in ameliorating symptoms in patients with gastroparesis (Acosta and Camilleri, 2015). Both compounds, however, should be used with caution because of their extrapyramidal side effects (metoclopramide) and increased risk of sudden cardiac death (domperidone). Promising results have been obtained using a ghrelin agonist (relamorelin), newly developed motilin agonist (camicinal), 5-HT$_4$ receptor agonists (velusetrag), and neurokinin-1 receptor antagonists (aprepitant). These medications all deserve further investigation in clinical trials (Stein et al., 2015). In medically refractory cases, operations, including pyloroplasty or gastrojejunostomy, can be performed to accelerate gastric emptying, but have been met with variable results (Di Nardo et al., 2008). The evidence of a predominant reduction of ICC, rather than a neuropathy, in a considerable number of patients may provide a cellular basis for the efficacy of gastric pacing, which is generally reserved for the most severe and refractory cases (Maranki and Parkman, 2007). Interestingly, gastric pacing results in symptom control, but without improvement in gastric emptying time.

4.2. Idiopathic hypertrophic pyloric stenosis

Failure of the stomach to empty properly also occurs in a condition referred to as idiopathic hypertrophic pyloric stenosis (IHPS). This disease presents at around one month of age and affects approximately 1 in 500 children. The hallmark of the disease is the lack of nitric oxide synthase (nNOS) in myenteric ganglia of the pyloric region (Vanderwinden et al., 1992). The absence of muscle relaxation due to the inhibitory denervation results in a continuous tonic contraction of the pylorus followed by muscle hypertrophy, thereby impeding gastric emptying. The pathogenic role of deficient nitrergic innervation is supported by the Nos1$^{-/-}$ mouse model, which exhibits gastric obstruction similar to that occurring in the human disease (Mashimo et al., 2000). IHPS can be either sporadic or associated with several conditions, including Turner syndrome, phenylketonuria, and trisomy of chromosome 18 (Goyal and Hirano, 1996). Although the hypertrophic muscle forms a palpable epigastric mass, the force exerted by antral contractions may be sufficient to overcome the malfunctioning pylorus and propel some gastric contents into the duodenum, at least in the early phase of the disease. Surgery is the only therapeutic option currently available.

4.3. Chronic intestinal pseudo-obstruction

The term chronic intestinal pseudo-obstruction (CIPO) refers to a relatively rare, disabling, and potentially life-threatening condition characterized by severe intestinal dysmotility, which results in a clinical presentation of bowel obstruction, often with radiological signs of air-fluid levels, but in the absence of any mechanical blockage. CIPO is an important cause of chronic intestinal failure in 15% of pediatric and about 20% of adult CIPO patients (Stanghellini et al., 2007; De Giorgio et al., 2011). While some cases of CIPO are due to a wide array of recognized pathological conditions, including myxedema, Duchenne muscular dystrophy, hypothyroidism, hypoparathyroidism, celiac disease, Chagas disease and mitochondrial disorders, most cases have an unknown etiopathogenesis (Stanghellini et al., 2007; De Giorgio et al., 2011; Sekino et al., 2012). The ability to collect full-thickness intestinal samples using minimally invasive approaches, combined with technical advances for biopsy assessment, is reducing the uncertainty associated with CIPO cases otherwise labeled as "idiopathic" (Lindberg et al., 2009). A better appraisal of the underlying neuro-ICC-muscular changes that occur in CIPO and other gastrointestinal motility disorders was recently proposed by the Gastro 2009 International Working Group on Gastrointestinal Neuromuscular Disorders (GINMD) (Knowles et al., 2009). Based on this classification, CIPO can be due to underlying neuropathies, myopathies and, albeit with lesser supportive evidence,

Table 1
Main treatment options in patients with CIPO.

Treatment options	Comments	References
Nutritional support:	Mainstay of therapy;	Messing and Joly (2006)
• Enteral nutrition	Preferred if possible, based on residual intestinal peristalsis;	Joly et al. (2011)
• Total parenteral nutrition	TPN is required in severe cases when other supportive methods fail.	Pironi et al. (2012) Billiauws et al. (2014)
Non-opioid analgesics	Manage intractable pain with analgesic drugs other than opioids (the latter can cause narcotic	Lauro et al. (2015)
• Opioid withdrawal	bowel syndrome and related life-threatening sequelae).	
Antibiotics	Abnormal motility leads to small intestine bacterial overgrowth. No controlled studies prove which antibiotics work best; largely empirical based on individual experience.	De Giorgio et al. (2011)
Prokinetics	Prokinetics showed disappointing results; prucalopride, however, has good rationale with some promising initial results.	Emmanuel et al. (2012)
Immunotherapy	For patients with underlying inflammatory neuropathy.	De Giorgio and Camilleri (2004)
Surgery	Occasionally indicated, mainly for intestinal decompression by ostomy creation, for feeding tube placement, or for bowel transplantation in well-selected, TPN-dependent, end-stage patients.	Stanghellini et al. (2005) and Abu-El-magd et al. (2012)

mesenchymopathies, depending on whether neuronal, muscular, or ICC cells, respectively, are primarily involved (Farrugia, 2008). Notably, combined abnormalities (e.g. neuro-myopathy, neuro-mesenchymopathy, etc.) are increasingly recognized (De Giorgio et al., 2004a).

Two main histopathologic findings are seen in CIPO associated with enteric neuropathies: inflammatory and degenerative. Inflammatory (or immune-mediated) neuropathies are characterized by CD3+ T lymphocytes (and, to a lower extent, plasma cells) infiltrating enteric neurons in the two ganglionated plexuses of the ENS. Most commonly it is the myenteric plexus that is targeted by the inflammatory infiltrate, hence the clinico-pathologic term "myenteric ganglionitis" (De Giorgio et al., 2004b). Evidence of myenteric lymphocytic ganglionitis in small bowel full thickness biopsies has been reported in 29% and 34% of patients with CIPO (Lindberg et al., 2009; Knowles et al., 2004). The diagnosis of lymphocytic ganglionitis is easily made when an overt infiltration of lymphocytes is seen in the myenteric ganglia (Knowles et al., 2009). A low-grade myenteric ganglionitis, with a less abundant inflammatory infiltrate, can also occur in CIPO and in other non-CIPO forms of enteric dysmotility (Lindberg et al., 2009). A quantitative analysis of lymphocytes (or other immune cells) may be required to establish a correct diagnosis of inflammatory neuropathy underlying cases of severe gut dysmotility. Currently, the lack of normative data on the number of lymphocytes or other immune cells within myenteric ganglia in healthy controls hampers a clear definition of myenteric ganglionitis. Based on the International Working Group on GINMD, more than 5 lymphocytes per ganglion is indicative of myenteric ganglionitis (Knowles et al., 2009). A low-grade ganglionitis was described in 59 patients with enteric dysmotility, one-third of whom had CIPO, and the mean number of lymphocytes per ganglion was 5.1 (Lindberg et al., 2009). Left untreated, a lymphocytic myenteric ganglionitis can progress and evoke neuronal degeneration and loss, even leading to complete ganglion cell depletion (De Giorgio et al., 2002). Further research on lymphocytic ganglionitis is needed to establish clear criteria for diagnosing this entity and to determine its pathogenic relevance in CIPO.

Patients with lymphocytic myenteric ganglionitis may develop a humoral response characterized by anti-neuronal nuclear antibodies, such as to the ANNA-1 protein, also referred to as anti-Hu (De Giorgio et al., 2003). Notably, these autoantibodies have been demonstrated to compromise the ascending reflex pathway of peristalsis in in vitro preparations. Studies using primary neuronal cell culture have shown that anti-neuronal antibodies can elicit neuronal hyperexcitability and evoke apoptotic and autophagic mechanisms (De Giorgio et al., 2003, 2008). In conclusion, both immune-mediated mechanisms involving lymphocytes infiltrating myenteric ganglia and anti-neuronal autoantibodies can lead to an inflammatory neuropathy and CIPO.

Non-lymphocytic inflammatory neuropathies, such as eosinophilic or mast cell ganglionitis, have been identified in pediatric and adult patients with CIPO (Schäppi et al., 2003; Accarino et al., 2007). These non-lymphocytic forms of inflammatory neuropathy are extremely uncommon and therefore their clinico-pathological features remain poorly defined. Mesenchymopathies, due to abnormalities in the ICC network, have been documented in various gastrointestinal motor abnormalities, including patients with CIPO (Farrugia, 2008). We have observed decreased ICC density, loss of processes, and damaged intracellular cytoskeleton and organelles as revealed by c-Kit immunohistochemistry in 5 of 11 CIPO patients (Stanghellini et al., 2005). Given the significant physiological role of ICCs in gut motility, injury of these cells could certainly contribute to severe enteric dysmotility. However, the International Working Group on GINMD considered it premature to attribute an etiological role to ICC changes in gut motility disorders, with the exception of diabetic gastroparesis (Knowles et al., 2009).

The main therapies used in patients with CIPO are listed in Table 1. Nutritional support (either enteral or, more commonly, parenteral), along with non-opioid analgesics for severe abdominal pain, antibiotics for bacterial overgrowth, and prokinetic drugs represent the major therapies in this condition. In CIPO, minimally invasive techniques are increasingly being used to obtain full-thickness biopsies for a histopathological search for neuro-ICC-muscular abnormalities to aid in the clinical diagnosis, guide therapy, and predict prognosis. In pediatric and adult CIPO, the presence of a myopathy is a predictor of poor outcome, as compared to those with a neuropathy (Mann et al., 1997). Furthermore, from a therapeutic standpoint, the identification of an inflammatory or an immune-mediated neuropathy or myopathy may prompt immunosuppressive therapy (Knowles et al., 2013; Schäppi et al., 2003; Ruuska et al., 2002; De Giorgio and Camilleri, 2004). Possible non-pharmacological options include plasmapheresis and immunoglobulin treatment in carefully selected patients. Special consideration should be given to elderly patients with CIPO and evidence of an inflammatory infiltrate on biopsy or the presence of circulating anti-neuronal antibodies. In these cases, the clinician needs to consider a paraneoplastic process with involvement of the gut and a thorough work-up should be undertaken to identify an occult underlying malignancy (Knowles et al., 2013).

4.4. Neuronal intranuclear inclusion disease (NIID)

NIID is a progressive neurodegenerative disorder affecting the three main neuronal systems, including central, peripheral and enteric neurons. The neuropathological hallmark, represented by eosinophilic inclusions typically detectable within neuronal nuclei, is associated with neuronal loss of variable degree. Molecular

studies reveal that these eosinophilic inclusions contain expanded polyglutamine tracts and are positive for ubiquitin and small ubiquitin-like modifier-1 (SUMO-1) (Kimber et al., 1998). These factors post-translationally modify numerous proteins via processes called ubiquitination and sumoylation, respectively, altering protein localization, metabolism, and function. Abnormalities in these processes may account for the accumulation of intraneuronal protein aggregates (i.e. inclusion bodies) seen in this disease. Neurological manifestations usually start in childhood, although adult onset has also been reported. The clinical expression depends on the predominant central, peripheral, or enteric neuronal loss (Zannolli et al., 2002). When autonomic dysfunction characterizes the clinical picture, then the whole gastrointestinal tract can be affected with severe impairment of gut motility, including dysphagia, gastroparesis, and CIPO in combination with urinary abnormalities, such as neurogenic bladder (Sone et al., 2005). NIID is usually sporadic, although familial cases have been reported. Rectal or colonic biopsies with histopathological identification of eosinophilic inclusions in the submucosal ganglion cell bodies may help in the diagnostic work-up. Currently, there are no specific treatment options available other than general supportive measures.

4.5. Ganglioneuromatosis in MEN2B

MEN2B is a rare autosomal dominant syndrome that affects 1 in 30,000 individuals and is characterized by an early-onset of an aggressive form of medullary thyroid carcinoma. Pheochromocytomas (50% of cases) and a marfanoid (tall and thin) body build are also seen in MEN2B. Mucosal neuromas involving lips ("blubbery lips") and eyelids (leading to thickening and eversion) represent other features in these patients (Raue and Frank-Raue, 2010). Gastrointestinal symptoms, including constipation, megacolon, or CIPO, beginning in infancy or early childhood occur in up to 80% of patients, with diffuse ganglioneuromatosis of the gastrointestinal tract occurring in about 40% of cases (Wray et al., 2008). A specific germ-line point mutation (methionine to threonine) in exon 16 of the *RET* gene (M918T) occurs in 95% of patients (Eng et al., 1996). Other rarer mutations involve exon 15 (A833F) (Smith et al., 1997) or codon 691 (G691S) leading to isolated ganglioneuromatosis (Nguyen et al., 2006). Patients with intestinal ganglioneuromatosis involving both myenteric and submucosal plexus should be tested for *RET* mutation. In these positive cases, prophylactic thyroidectomy is indicated. Adrenal gland surveillance with ultrasound scanning and urinary fractionated catecholamines is recommended to identify the presence of a pheochromocytoma (Smith et al., 1999).

4.6. Intestinal Neuronal Dysplasia (IND)

IND represents a hotly debated entity, largely due to disagreement regarding its existence as a pathologic condition (Kapur, 2003) and disagreement regarding its histopathology (Koletzko et al., 1999). IND type B, which comprises about 98% of described cases, presents in infants or older children as severe constipation. It can occur in isolation or proximal to the aganglionic segment in a patient with HSCR (Kobayashi et al., 1995). Diagnostic criteria vary regarding the details, but broadly speaking are based on the presence of neuronal hyperplasia specifically in the submucosal plexus. One set of criteria includes the presence of giant submucosal ganglia, defined as containing >8 ganglion cells per ganglion, in at least 20% of submucosal ganglia examined (Meier-Ruge et al., 2006), and often also the presence of ectopic ganglia in the lamina propria (Meier-Ruge et al., 1995; Kapur, 2001). Interestingly, later descriptions of IND require that the child be >1 year of age for this diagnosis because of the recognition that the submucosal ganglia of infants contain a larger number of cells per ganglion due to their immaturity (Meier-Ruge et al., 2006). Importantly, the diagnosis of IND applies only to patients with these histologic features in the large intestine, not the small intestine, where the importance of these findings is unknown. Most children with IND are treated conservatively for their constipation, with surgical removal of the abnormal colon reserved for the most severe cases.

No definitive animal model of IND exists. Rats with a heterozygous mutation of *EDNRB* have histologic features reminiscent of IND, with larger and denser submucosal ganglia present in the colon (von Boyen et al., 2002). However, while the histology is consistent with IND, the rats have no intestinal dysmotility and develop normally. A recent study in avian embryos showed that inhibition of Shh signaling led to the development of large and ectopic submucosal ganglia (Nagy et al., 2016), suggesting the possibility that Shh may be associated with this condition. Since IND has been identified proximal to the aganglionic segment in HSCR (Kobayashi et al., 1995), a search for mutations in HSCR-related genes (*RET*, *GDNF*, *EDNRB*, and *ET3*) was performed in 20 patients with IND, but failed to uncover any abnormalities (Gath et al., 2001).

5. Chronic constipation

Chronic constipation is a functional disorder of the gastrointestinal tract characterized by difficult and infrequent bowel movements. Depending on the definition used, estimates indicate that about 10–15% of adults and up to 30% of children in the general population suffer from constipation (Ford et al., 2013; van den Berg et al., 2006). Although in children no specific gender, age or socioeconomic associations are evident, in adults the condition most commonly affects women (female:male ratio of 2–3:1), elderly, non-whites, and those with a lower socio-economic status (van den Berg et al., 2006; Brandt et al., 2005; Longstreth et al., 2006). From a pathophysiologic standpoint, three main subtypes of constipation exist: (1) normal transit (also referred to as functional constipation), (2) slow transit, and (3) obstructed defecation (also called dyssynergic defecation when no mechanical obstruction is present) (Wald, 2015).

Data regarding neuromuscular abnormalities in chronic constipation have been obtained in the minority of patients who undergo surgery for the most severe forms of the disease, predominantly slow transit constipation (STC) with colonic inertia (CI), which mainly affects young women and has also been described in children without an obvious sex bias (Giorgio et al., 2013). Recent studies have shown that tissue analysis in STC/CI shows features indicative of an underlying enteric neuropathy, a finding previously predicted using gastrointestinal manometry to assess bowel motility in these patients (Bassotti and Villanacci, 2006; Bassotti et al., 1996). Alterations of colonic innervation in STC/CI were initially characterized by silver staining, showing a reduction in the total number of argyrophilic neurons and structural alterations in both neuronal perikarya and axons (Smith, 1967; Krishnamurthy et al., 1985). Other reports described a reduction of enteric neural elements (cell bodies and/or processes) as a common feature in patients with STC/CI who underwent surgery (Porter et al., 1998; Faussone-Pellegrini et al., 1999; Wedel et al., 2002a). Furthermore, these neuronal abnormalities are often associated with reduced ICC and enteric ganglion cells (He et al., 2000; Lyford et al., 2002; Wedel et al., 2002b). In a cohort of patients with a severe and intractable form of STC, it has been found that: 1) ICCs were significantly decreased; 2) enteric neuronal loss was present, partly ascribed to apoptosis; and 3) enteric glial cell number was markedly reduced in both the submucosal and

myenteric plexuses (Bassotti et al., 2006a). However, in the same study, the examination of the terminal ileum of patients with intractable STC/CI revealed only a reduction of enteric glial cells, while neuronal and ICC alterations were not detected at this level (Bassotti et al., 2006b). In a study of children with severe STC, abnormal contractile patterns present on high resolution colonic manometry were significantly associated with histopathological abnormalities primarily involving enteric neurons, but also included changes in ICCs and smooth muscle (Giorgio et al., 2013). Giorgio et al. (2013) showed that a failure of motor quiescence between bisacodyl-induced high amplitude propagating sequences reliably predicted neuromuscular pathology. Other results suggest that colonic dysmotility in STC/CI patients can be attributable to more subtle imbalances of enteric neurotransmitters and neuropeptides. An excessive production of NO in the colonic myenteric plexus of patients with STC has been suggested as a pathophysiological mechanism eliciting a constant inhibition of propulsive contractile activity (Tomita et al., 2002). However, this possibility is still debated, since other studies investigating mediators like VIP, substance P, neuropeptide Y, or serotonin gave contradictory results in tissue specimens of patients with STC (Koch et al., 1988; Lincoln et al., 1990; Tzavella et al., 1996; Sjolund et al., 1997). A major limitation to finding a consistent histopathological diagnosis and likely contributing to the wide spectrum of neuromuscular abnormalities reported is the large variability in disease phenotype, anatomic source of the sample, morphological methods for tissue evaluation, and the lack of normative data to detect subtle changes in enteric neuronal, glial, or muscular structure (Knowles and Farrugia, 2011). The field would benefit from improved diagnostic methods and the development of a clear and comprehensive nosology to more accurately define subtypes of constipation.

Current management of severe constipation primarily consists of laxatives, given orally or as suppositories. Many types of laxatives are used, based on their mechanism of action, including the following classes of agents (with examples): bulk-formers (fiber), stool softeners (docusate), lubricants (mineral oil), hyperosmotic agents (polyethylene glycol or lactulose), and stimulants (bisacodyl or senna). Other agents include serotonin agonists (prucalopride), which stimulate motility by activating 5-HT_4 receptors on enteric neurons, or chloride channel activators (lubiprostone) or guanylatecyclase-C agonists (linaclotide), which soften stool by increasing fluid secretion by the gut epithelium. Other agents, including newer 5-HT_4 agonists (naronapride and velusetrag), the ileal bile acid transporter A3309 (elobixibat), and ghrelin agonists (relamorelin), represent the forefront of treatment (Camilleri, 2014). In children there is limited experience with many of these newer medications. A recent multicentre double-blind placebo-controlled trial of prucalopride in children with functional constipation failed to show any benefit, although it may be of value in some cases of severe constipation (Mugie et al., 2014). Although lubiprostone showed some efficacy in pediatric constipation in an open-label study, this remains to be shown in a larger multicentre trial (Hyman et al., 2014). When medical management fails, surgery can be considered in carefully selected patients. Subtotal colectomy has long been the standard approach for intractable constipation (Gladman et al., 2005). In children, and possibly in adults, antegrade enemas via a catheterizable appendicostomy or a cecostomy tube have proven to be effective (Randall et al., 2014; Patel et al., 2015). In the most severe cases, total proctocolectomy with ileoanal anastomosis or diverting ileostomy have been used (Christison-Lagay et al., 2010). A better understanding of the underlying causes of severe constipation is needed in order to develop improved pharmacotherapies that can target specific abnormalities in a given individual.

5.1. Future therapies

One of the most promising areas of research in neurointestinal disease is the development of novel cell-based therapies. Recent advances in cell biology and molecular genetics have enhanced the identification and isolation of stem cells from a variety of adult tissues, including the gut. In the human gastrointestinal tract, multipotent stem cells have been found to be present, not only during development, but also postnatally, well into late adult life (Metzger et al., 2009a). The identification of enteric neural stem cells represents an exciting opportunity to test new therapies for the ENS (Kapur, 2000). Human enteric neural stem cells can be collected by relatively noninvasive techniques, such as routine endoscopy, and have been transplanted *ex vivo* into strips of aganglionic intestinal muscle, where they differentiate into enteric neurons and glial cells (Metzger et al., 2009b).

Autologous cell transplantation offers the possibility of using a patient's own cells to replace missing or injured enteric neurons in a variety of neurointestinal diseases. Autologous cells would eliminate issues around immunologic rejection and ethical concerns raised by other sources of stem cells. Hotta et al. (2015) recently successfully isolated isogenic enteric neuronal progenitors from the ganglionated bowel of Ednrb-deficient mice and transplanted them into the postnatal aganglionic rectum of Ednrb-deficient hosts. In considering autologous transplantation as a potential therapy, one needs to consider that ENS stem cells obtained from HSCR patients might be impaired in their biological function (Hotta et al., 2009). However, in HSCR the “abnormal” enteric neural crest-derived cells are able to migrate a significant distance along the bowel, from foregut to distal hindgut in the majority of cases, failing to colonize only the very distal end. In fact, in the recent study of isogenic cell transplantation into Ednrb-deficient mice, transplanted cells demonstrated a normal capacity for self-renewal and neuronal differentiation (Hotta et al., 2015). However, this was an issue for mice carrying the monoisoformic Ret51 (miRet51) mutation, in which only the Ret51 isoform is expressed, resulting in a HSCR-like phenotype with distal colonic aganglionosis and intestinal obstruction (Heanue and Pachnis, 2007). Enteric progenitors harvested from these mice show delayed differentiation compared to wild-type controls (Heanue and Pachnis, 2007). These cells could be genetically modified to restore a normal phenotype, for example by restoring the Ret9 isoform within the miRet51 ENS progenitors, which has been shown to rescue the differentiation anomalies (Heanue and Pachnis, 2007).

An alternative source of autologous cells for transplantation is induced pluripotent stem cells (iPSC). Transfection of key transcription factors into a patient's somatic cells (e.g. fibroblasts or peripheral blood leukocytes) allows these cells to reprogram into a multipotent-like state, and these cells are referred to as iPSC (Takahashi and Yamanaka, 2006). For cell therapy this technology offers the possibility of overcoming the need for harvesting ENS stem cells from the gut and instead using an easily accessible source of autologous cells, such as the skin. iPSC can be expanded in vitro and genetically engineered, either by introduction of a normal gene to replace a mutant allele or by gene editing to correct a known mutation (Urnov et al., 2010). iPSC were recently successfully induced to enteric neural crest progenitors, and subsequently differentiated into enteric neurons (Fattahi et al., 2016). These cells were able to migrate and differentiate appropriately in embryonic aneural chick intestine and to colonize adult mouse colon (Fattahi et al., 2016). In patients with a known mutation, a variety of techniques can be used to correct the mutation using homologous recombination or a genome editing tool, such as CRISPR, TALENs, or ZFNs (Xue et al., 2016). Recent publications have demonstrated successful genome modification of iPSC harboring known disease-causing mutations in several genetic

diseases, including sickle cell disease (Zou et al., 2011), beta-thalassemia (Song et al., 2015), and spinal muscular atrophy (Corti et al., 2012).

While it appears plausible that cell-based approaches could be used to treat enteric neuropathies, many challenges remain, including identification of the most appropriate diseases to treat, optimal cell sources, strategies for maximizing cell expansion, methods for efficient and targeted cell delivery, and issues with immune response. Addressing these challenges will usher in a new era in the treatment of enteric neuropathies and offer hope to patients with these difficult conditions.

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