Variant Hirschsprung's Disease

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Florian Friedmacher and Prem Puri

70.1 Introduction

There are a number of newborns and children who present with clinical symptoms similar to Hirschsprung's disease (HD) despite the presence of ganglion cells in rectal biopsies. Various terms, such as *chronic idiopathic intestinal pseudoobstruction*, *intestinal hypoperistalsis syndrome* or *pseudo-HD*, have been used to describe these conditions over the years. At present, there are only a few articles in the medical literature that have attempted to standardize the terminology of HD and allied intestinal disorders (Holschneider et al. 1994). In 1997, Prem Puri suggested that *variant HD* may be a more appropriate description for this heterogeneous group of functional bowel disorders (Box 70.1) in patients who suffer from chronic constipation and abdominal distension despite a ganglionic rectal biopsy (Puri 1997). Specific histological, immunohistochemical or electron microscopic investigations, combined with anorectal manometry studies, are required to distinguish between the different variants of HD (Friedmacher and Puri 2013). Although the initial diagnostic workup and subsequent management can be challenging, the majority of these patients will have a satisfactory long-term outcome.

Box 70.1 Variants of Hirschsprung's disease (HD)

Intestinal neuronal dysplasia (IND)
Intestinal ganglioneuromatosis (GNM)
Isolated hypoganglionosis (HG)
Immature ganglia (IG)
Absence of the argyrophil plexus (AP)
Internal anal sphincter achalasia (IASA)
Megacystis-microcolon-intestinal hyperperistalsis syndrome (MMIHS)

70.2 Intestinal Neuronal Dysplasia

In 1971, William A. Meier-Ruge first described intestinal neuronal dysplasia (IND) as a hyperplastic malformation of the enteric plexus (Meier-Ruge 1971). A few years later, Puri et al. (1977) reported a case of rectosigmoid aganglionosis associated with IND of the descending and transverse colon. Nowadays, IND can be classified into two clinical

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and histological distinct subtypes (Fadda et al. 1983). IND type A (IND A), occurring in less than 5% of all IND cases, is characterized by congenital aplasia or hypoplasia of the sympathetic innervation. Patients with IND A typically present in the neonatal period with episodes of abdominal distension, intestinal obstruction and diarrhea with bloody stools. IND type B (IND B) is defined by hyperplasia of the parasympathetic submucosal and myenteric plexuses, accounting for over 95% of all IND cases. Typical histological features of IND B include hyperganglionosis, giant ganglia, ectopic ganglion cells and increased activity of acetylcholinesterase (AChE) in the lamina propria and around submucosal blood vessels (Bruder and Meier-Ruge 2007a). IND occurring in association with HD is invariably IND B.

70.2.1 Epidemiology

IND occurs with an estimated incidence of 1 in 7500 newborns (Granero Cendón et al. 2007). However, the frequency of isolated IND cases seems to be highly variable with reported rates ranging between 0.3% and 40% of all rectal biopsies (Friedmacher and Puri 2013). IND immediately proximal to an aganglionic colon segment is not uncommon and has been suggested as a possible cause of persistent bowel symptoms after pull-through operation for HD (Kobayashi et al. 1995). Some authors have found IND in up to 44% of their HD patients (Montedonico et al. 2011), whereas others have rarely encountered IND in association with HD. The high variability of patient age, specimen type and applied staining methods has resulted in considerable confusion in the published literature regarding the accurate diagnostic criteria (Schäppi et al. 2013).

70.2.2 Pathogenesis

The existence of IND as a distinct histopathological entity remains controversial (Schäppi et al. 2013). Several authors have suggested that the observed changes may be either a variant of normal bowel development or a secondary acquired phenomenon caused by congenital obstruction or inflammation (Martucciello et al. 2005). An underlying autoimmune mechanism has also been proposed for IND (Friedmacher and Puri 2013). Furthermore, there may be an additional genetic component, as several familial cases of IND have been found (Martucciello et al. 2002; Moore et al. 1993). The strongest evidence that IND is a real entity actually arose from two different *Hox11L1* knockout mouse models (Puri and Gosemann 2012). In both cases, homozygous mutant mice developed megacolon at the age of 3 to 5 weeks, without any further morphological changes. Histological and immunohistochemical evaluation revealed hyperplasia of the ganglia similar to the phenotype observed in human IND. Another animal model resulting in a phenotype similar to IND was reported in rats with a heterozygous mutation of the endothelin B receptor (*Ednrb*) and showed features of hyperganglionosis, giant ganglia and hypertrophied nerve fiber strands in the submucosal plexus (Friedmacher and Puri 2013). However, mutational screening of human patients with IND has not identified any alterations in *HOX11L1* and *EDNRB* genes to date (Puri and Gosemann 2012).

70.2.3 Clinical Presentation

Most patients with IND present with chronic constipation with or without abdominal distension, thus clinically resembling HD with absence of internal anal sphincter relaxation on manometry, but with a normal contrast enema examination of the colon. It has been shown that intestinal obstruction is the most characteristic clinical feature of IND in infants and young children (Montedonico et al. 2002). Furthermore, there appears to be a high incidence of associated congenital anomalies, ranging from 25% to 30% (Puri and Gosemann 2012). The most common ones are anorectal malformations, megacystis, intestinal malrotation, congenital short bowel, hypertrophic pyloric stenosis, necrotizing enterocolitis and Down syndrome (Martucciello et al. 2002; Montedonico et al. 2002).

70.2.4 Diagnosis

The method of choice for the diagnosis of IND is rectal suction biopsy. It is essential to include a sufficient amount of submucosal tissue in the biopsy specimens. Previously, the diagnosis of IND was based on qualitative criteria, thus resulting in a high interobserver variation (Koletzko et al. 1999). Therefore, the debate about the existence of IND as a distinct histopathological entity continues, mainly due to the lack of consensus on diagnostic criteria (Martucciello et al. 2005; Schäppi et al. 2013). Initially, IND was diagnosed on the basis of AChE immunohistochemistry of nerve fibers in rectal biopsies (Kobayashi et al. 1995; Meier-Ruge et al. 1995; Montedonico et al. 2011) (Fig. 70.1a, b). However, as AChE activity in the lamina propria mucosae has been shown to be an age-dependent phenomenon that disappears at the time of submucosal plexus mat-

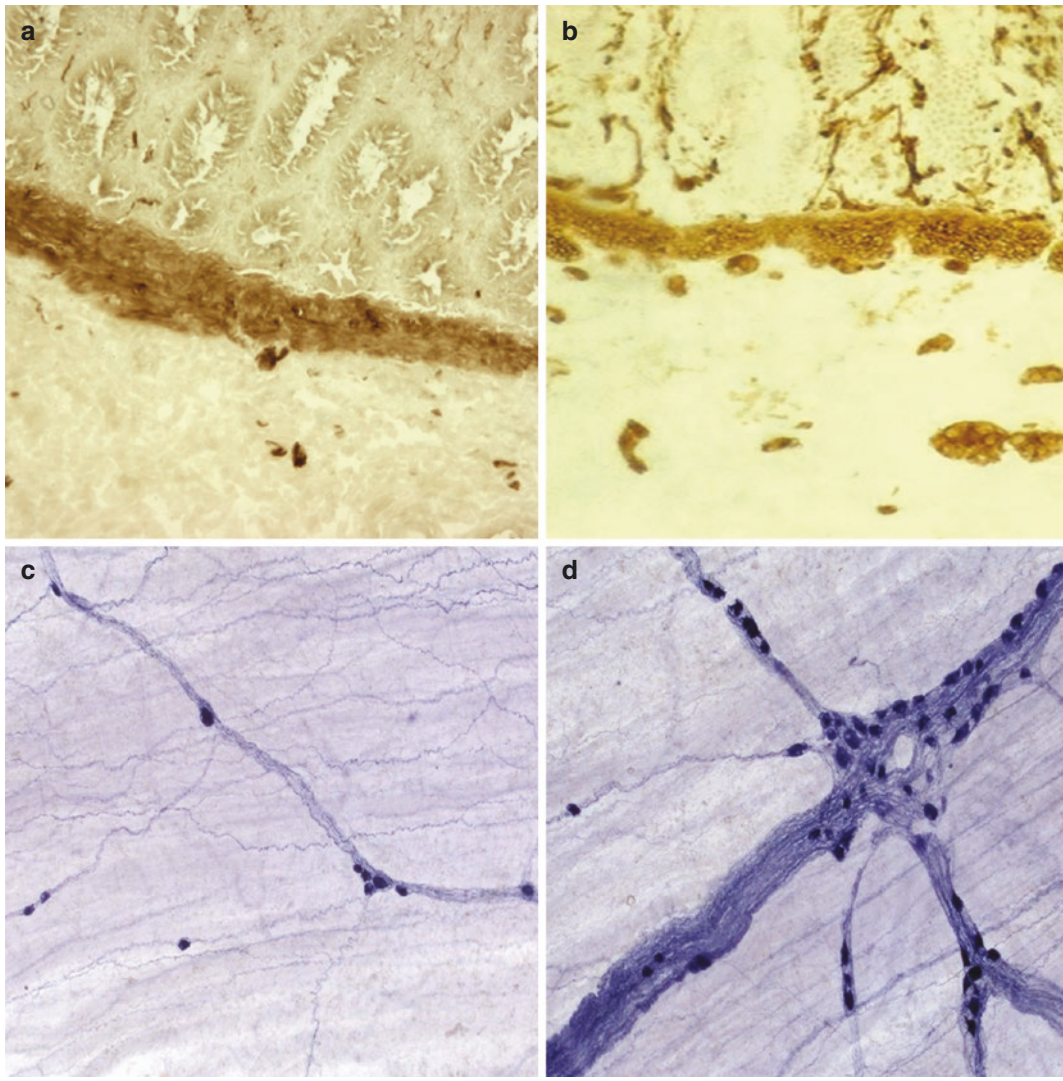

Fig. 70.1 AChE immunohistochemistry of a normal rectal suction biopsy (**a**). Rectal biopsy from a patient with IND, showing hyperganglionosis, giant ganglia and increased AChE activity in the lamina propria (**b**). NADPH-d staining of a normal submucosal plexus (**c**). Submucosal plexus of a patient with IND showing giant ganglia (**d**)

uration (Coerdt et al. 2004), more specific staining techniques were required to assess the enteric nervous system in more detail. Thus, enzyme histochemistry for lactate dehydrogenase, succinate dehydrogenase and nitric oxide synthase has been introduced to evaluate and diagnose IND B quantitatively (Meier-Ruge and Bruder 2005). Various other neuronal and glial markers, such as nicotinamide adenine dinucleotide phosphate-diaphorase (NADPH-d) (Fig. 70.1c, d), neural cell adhesion molecule (NCAM), neuron-specific enolase (NSE), cathepsin D, protein gene product 9.5 (PGP9.5), S-100 protein, peripherin, synaptophysin and cuprolinic blue, have also been used (Meier-Ruge and Bruder 2005; Meier-Ruge et al. 2004; Friedmacher and Puri 2013). Cuprolinic blue staining has been proposed as it stains the whole population of ganglion cells (Puri and Gosemann 2012), but only their cell bodies and not their axons, which makes the differentiation between the individual cell types relatively easy. In addition, defective innervation of the neuromuscular junction within the affected bowel segment of patients with IND has been identified (Friedmacher and Puri 2013). Abnormal submucosal vasculature is a further histological finding in isolated IND and IND associated with HD, which may also be a useful diagnostic feature (Friedmacher and Puri 2013). Furthermore, a reduced number of c-Kit-positive interstitial cells of Cajal (ICCs) has been demonstrated in the myenteric plexus and muscle layers of IND cases (Rolle et al. 2007). More recently, a marked reduction in the expression of phosphatase and tensin homolog (PTEN) has been discovered in the submucosal and myenteric plexuses of patients with IND, which may explain the observed motility dysfunction (Friedmacher and Puri 2013). Due to age-dependent AChE expression and the discrepancy of applied staining techniques, the most commonly used diagnostic criteria at present are as follows: (1) more than 20% of 25 submucosal ganglia must be giant ganglia containing 9 or more ganglion cells, and (2) the patient must be older than 1 year, as before that age, giant ganglia may be misinterpreted due to the fact that immature ganglia often have an incomplete differentiation in nerve cells (Bruder and Meier-Ruge 2007a; Meier-Ruge et al. 2004). The majority of patients with IND do not display any specific radiological features on contrast enema studies other than rectosigmoid distension. The rectosphincteric reflex has often been shown to be present, absent or atypical in these patients (Puri and Gosemann 2012).

70.2.5 Management

In the first instance, the management of IND B should be conservative, consisting of laxatives and enemas (Bruder and Meier-Ruge 2007a, 2007b). Most patients have been shown to respond well to this treatment strategy. However, if bowel symptoms persist longer than 6 months despite conservative bowel management, surgical or interventional treatment options should be considered (Puri and Gosemann 2012). Internal sphincter myectomy has been performed by several authors with satisfactory results, whereas others recommend the injection of botulinum toxin into the anal sphincter (Friedmacher and Puri 2013). Resection of the affected bowel segment and pull-through procedure are rarely indicated in infants and children with IND, but in adolescent or adult patients, this is often the only successful therapeutic option (Puri and Gosemann 2012). The indication for surgery should not be determined on the basis of histopathological findings alone; instead, the decision must be based on the individual patient's clinical symptoms and distress.

70.2.6 Outcome

A multidisciplinary team of experienced pediatric surgeons and gastroenterologists is crucial for the long-term follow-up of patients with IND and chronic constipation. Authors from Ireland reported functional outcome in 33 infants and children with IND (Friedmacher and Puri 2013): 64% had a good response to conservative management with normal bowel habits and did not need any surgical intervention. However, 36% of their patients underwent internal sphincter myec-

tomy after failed conservative treatment. Seven out of these 12 patients had normal bowel habits after surgery and 2 were able to stay clean with regular enemas. Three patients continued having persistent constipation after myectomy and subsequently required resection of their redundant and dilated sigmoid colon, which resulted in normal bowel habits. Other authors have achieved satisfactory results in 90% of their patients within 6 months of internal sphincter myectomy (Friedmacher and Puri 2013).

70.3 Intestinal Ganglioneuromatosis

Intestinal ganglioneuromatosis (GNM) is characterized by a diffuse proliferation of nerve fibers with marked hyperplasia of submucosal and myenteric ganglion cells causing the thickening of the bowel wall (D'Amore et al. 1991). This extremely rare but severe neoplastic condition leads to chronic bowel obstruction and is often associated with multiple endocrine neoplasia type 2B (MEN 2B), neurofibromatosis 1 or Cowden syndrome (de Krijger et al. 1998; Moline and Eng 2011).

70.3.1 Epidemiology

Although the exact incidence of intestinal GNM is unknown, it has been reported that the frequently associated MEN 2B syndrome occurs in approximately 1:4,000,000 live births (Moline and Eng 2011). Conversely, it can be estimated that intestinal GNM is present in nearly 90% of patients with MEN 2B (O'Riordain et al. 1995).

70.3.2 Pathogenesis

The pathogenesis of intestinal GNM involves complex hyperplasia of peptidergic, cholinergic and most likely adrenergic nerve fibers and neurons (D'Amore et al. 1991). Transmural GNM mainly originates from the myenteric plexus, while mucosal GNM predominantly affects the submucosal plexus and is often associated with neurofibromatosis (D'Amore et al. 1991). Furthermore, mutation analysis in patients with MEN 2B identified a de novo germline Met918Thr mutation in exon 16 of the *rearranged during transfection* (*RET*) proto-oncogene (Moline and Eng 2011), suggesting a genetic component to this condition. In addition, a recent experimental study in mice revealed that the deletion of the *Pten* gene on chromosome 10 disrupts the development of the enteric nerve system, resulting in a phenotype similar to human intestinal GNM (Friedmacher and Puri 2013).

70.3.3 Clinical Presentation

The vast majority of patients with intestinal GNM present with severe chronic constipation and abdominal distension due to intestinal obstruction (O'Riordain et al. 1995). Constipation may also fluctuate with episodes of diarrhea (de Krijger et al. 1998). The similarity of the gastrointestinal symptoms between patients with HD, IND and MEN 2B-associated intestinal GNM suggests that these three conditions could be the result of mutations actually affecting the same domain of the *RET* proto-oncogene (Friedmacher and Puri 2013). Despite the fact that gastrointestinal dysmotility is a common initial presentation of patients with MEN 2B, the rarity of this syndrome often delays the diagnosis. Further findings are mucosal neuromas of the lips and tongue, as well as medullated corneal nerve fibers, distinctive facies with enlarged lips and an asthenic "marfanoid" body habitus (Moline and Eng 2011).

70.3.4 Diagnosis

Intestinal GNM is mainly diagnosed on the basis of clinical presentation and histological analysis of full-thickness rectal biopsies, showing massive proliferation of submucosal and myenteric plexuses comprising thick nerve trunks with scattered mature neurons, giant ganglia with often 15–40 nerve cells and a high AChE activity (Torre

et al. 2002). Unlike neurofibromatosis, which occurs more commonly in the small intestine, intestinal GNM appears to be largely confined to the colon and rectum. Although AChE immunohistochemistry has often been used to show the typical submucosal and myenteric changes in intestinal GNM (i.e., increased thickness of nerve fibers), it can easily be appreciated in standard hematoxylin and eosin-stained paraffin sections (Yin et al. 2006). NSE, synaptophysin and S-100 protein immunostaining has also been applied to evaluate and diagnose intestinal GNM (D'Amore et al. 1991). It has been demonstrated that the submucosal hyperplasia can be extensive, but not as prominent as that seen in the myenteric plexus (Yin et al. 2006). As intestinal GNM is frequently associated with MEN 2B, the diagnosis should prompt additional molecular, endocrinological and oncological investigations (Feichter et al. 2009). In general, a mutational analysis of the *RET* proto-oncogene is strongly recommended in all patients with intestinal GNM and MEN 2B, as well as their first-degree relatives (de Krijger et al. 1998).

70.3.5 Management

In patients with MEN 2B-associated intestinal GNM, surgical resection of the affected bowel segment is not always necessary. In fact, it has been shown that in most cases, the gastrointestinal symptoms can be managed with daily laxatives and enemas (Cohen et al. 2002; Smith et al. 1999). However, some patients eventually require surgery for severe intestinal obstruction or stricture formation. Furthermore, all patients with intestinal GNM who carry MEN 2B mutations should undergo a prophylactic total thyroidectomy to prevent the development of medullary thyroid carcinoma (O'Riordain et al. 1995; Torre et al. 2002).

70.3.6 Outcome

Early diagnosis and treatment of patients with MEN 2B-associated intestinal GNM is essential for long-term survival. Moreover, a yearly follow-up with monitoring of basal plasma calcitonin and carcinoembryonic antigen levels for possible tumor recurrence is strongly recommended (Torre et al. 2002). Additionally, the continued surveillance of the adrenal glands with abdominal ultrasonography and urine analysis of catecholamine metabolites including metanephrine, normetanephrine, dopamine and vanillylmandelic acid is required as patients with MEN 2B have at least a 50% risk of developing pheochromocytoma (Smith et al. 1999).

70.4 Isolated Hypoganglionosis

Isolated hypoganglionosis (HG) is a rare entity that has been classified as a hypogenetic type of intestinal innervation disorder. The clinical presentation of patients with isolated HG is similar to those with classical HD, with nonspecific symptoms of severe constipation or bowel obstruction (Puri and Gosemann 2012). It has been demonstrated that congenital and acquired HG are two separate entities with different clinical features and histological findings (Taguchi et al. 2006). At present, there are only a few cases in the published literature as isolated HG is one of the rarest subtypes of intestinal innervation disorders and there remains controversy regarding its existence as a distinct isolated histopathological entity (Martucciello et al. 2005).

70.4.1 Epidemiology

Reports of finding isolated HG in rectal biopsies are rare, ranging between 0.3% and 6.4% (Friedmacher and Puri 2013; Montedonico et al. 2011). Since 1978, there have been a total of 92 cases published in the English literature and 32% of them were diagnosed in the newborn period (Dingemann and Puri 2010). However, the median age at diagnosis was 4.8 years, which was most likely due to the fact that in several patients, the diagnosis was not made until they were adolescents.

70.4.2 Pathogenesis

The pathogenesis and genetic basis of isolated HG is still largely unclear. Although various mutational analyses of the *RET* gene have been performed, neither causative missense mutations nor neutral substitutions were found (Friedmacher and Puri 2013). Some cases of isolated HG were reported to exhibit a deficient expression of c-Kit-positive ICCs within the myenteric plexus and the smooth muscle layer (Rolle et al. 2007), which may contribute to the observed motility dysfunction in the hypoganglionic bowel segment. A lack or reduced expression of NCAM-positive nerve fibers within the lamina propria and muscularis mucosae, as well as the circular and longitudinal muscle layers of patients with isolated HG, has also been described (Friedmacher and Puri 2013).

70.4.3 Clinical Presentation

The most common presenting symptoms of isolated HG are severe chronic constipation combined with intestinal obstruction and enterocolitis, thus resembling the clinical features of HD. The median age at diagnosis is considerably higher in patients with isolated HG compared to patients with HD, which is predominately diagnosed during the newborn period. Enterocolitis of the newborn has been reported to be the most serious and potentially life-threatening complication of isolated HG, similarly to HD (Dingemann and Puri 2010).

70.4.4 Diagnosis

There is an ongoing debate whether isolated HG represents an extreme form of intestinal dysganglionosis or solely a developmental abnormality of the enteric nervous system that leads to severe constipation (Martucciello et al. 2005). Hence, the diagnosis of isolated HG remains difficult and a consensus in diagnostic criteria still needs to be established. In general, a full-thickness rectal biopsy is required for the definitive diagnosis of isolated HG (Bruder and Meier-Ruge 2007b; Schäppi et al. 2013). The vast majority of reported cases have been analyzed by immunohistochemical staining showing sparse and small myenteric ganglia, absent or low AChE activity in the lamina propria as well as hypertrophy of muscularis mucosae and circular muscle (Friedmacher and Puri 2013). Further histopathological differences between resected bowel specimens from patients with isolated HG and normal bowel tissue have been discovered using AChE immunohistochemistry (Puri and Gosemann 2012). For instance, a 40% reduction in the number of nerve cells has been shown, accompanied by a doubled distance between ganglia and a three times smaller plexus area in the hypoganglionic bowel segment. These observations currently form the basis for the histopathological diagnosis of isolated HG. It has also been suggested that the size of the myenteric plexus may be an indicator of clinical severity. Therefore, various neuronal markers have been introduced to facilitate the diagnosis of isolated HG. NADPH-d staining has been used to determine the muscular nitrergic innervation and differentiation of mature from immature ganglia in patients with isolated HG, demonstrating a reduced number of positive nerve fibers in the muscularis mucosae with absent or sparse submucosal and myenteric ganglion cells (Fig. 70.2a–d) (Friedmacher and Puri 2013). Additionally, c-Kit staining has been employed to investigate the expression of ICCs and thus intestinal pacemaker activity, which is markedly decreased or even absent in patients with isolated HG (Rolle et al. 2007).

70.4.5 Management

The treatment of isolated HG is similar to that of HD. According to the literature, most patients undergo resection of the affected bowel segment with subsequent pull-through procedure (Dingemann and Puri 2010). However, the management of isolated HG should always be tailored to the individual patient's findings.

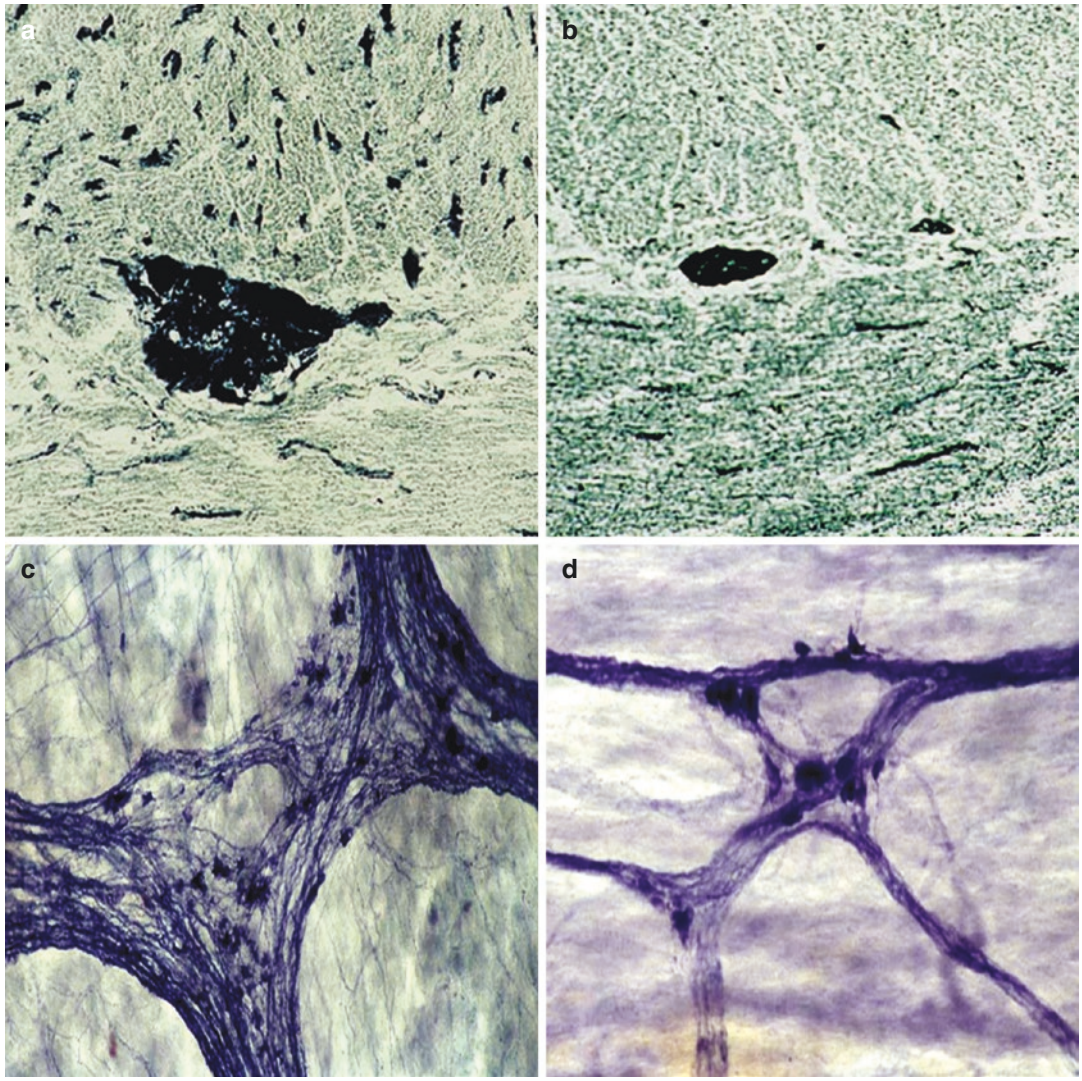

Fig. 70.2 NADPH-d staining in whole mount preparation of a normal myenteric plexus (**a**, **c**). Myenteric plexus of a patient with isolated HG showing markedly reduced number of ganglion cells (**b**, **d**)

70.4.6 Outcome

The postoperative outcome after resection of the hypoganglionic segment is usually good. Typical complications of isolated HG are enterocolitis, chronic constipation, overflow encopresis and the need for redo pull-through operation due to residual hypoganglionosis. An overall mortality rate of 8% has recently been reported with the majority of patients who died being newborns suffering from severe enterocolitis (Puri and Gosemann 2012). As the dysmotility in isolated HG may also affect the urinary system, recurrent urinary tract infections are a common problem and therefore should be monitored actively.

70.5 Immature Ganglia

Immature ganglia (IG) are normally found in rectal biopsies from premature infants presenting with functional bowel obstruction. Not surprisingly, delayed maturation of ganglion cells in the submucosal and myenteric plexuses has been

reported to be the most common cause of chronic constipation during the first year of life (Feichter et al. 2009).

70.5.1 Epidemiology

Strong epidemiological data on the incidence of IG is unfortunately lacking. In 1997, 4 (2.8%) cases of immature ganglion cells were reported in a cohort of 141 patients with intestinal neuronal malformations. More recently, 10 (5.6%) cases of IG were discovered in bowel specimens of 178 patients with variants of HD (Friedmacher and Puri 2013).

70.5.2 Pathogenesis

A combination of large (i.e., fully mature) and small (i.e., immature) ganglion cells can be found at birth (Burki et al. 2011). In the early postnatal period, ganglion cells in the submucosal plexus are generally less developed than the ones in the myenteric plexus (Smith 1968). It has further been demonstrated that this immaturity is a physiological, age-dependent phenomenon and the maturation of IG strongly correlates with the age of the patient (Holschneider et al. 1994; Smith 1968). Strong evidence supporting this theory has arisen from several animal studies showing postnatal maturation of the submucosal and myenteric plexuses (Friedmacher and Puri 2013). Hence, the finding of IG on rectal biopsy may be a reliable indicator of transient functional immaturity of the bowel (Burki et al. 2011).

70.5.3 Clinical Presentation

Patients with IG usually present with a history of chronic constipation or functional bowel obstruction resembling HD. Further clinical features may include slow transit peristalsis and insufficient defecation.

70.5.4 Diagnosis

In general, the diagnosis of IG can be made from full-thickness rectal biopsies. The ganglion cells appear very small and have a less significant nucleus with an inconspicuous nucleolus (Fig. 70.3a, b) (Smith 1968). However, with AChE immunohistochemistry, it is often not possible to distinguish between these small ganglion cells and their supporting enteric glial cells. Therefore, NADPH-d and NCAM staining have

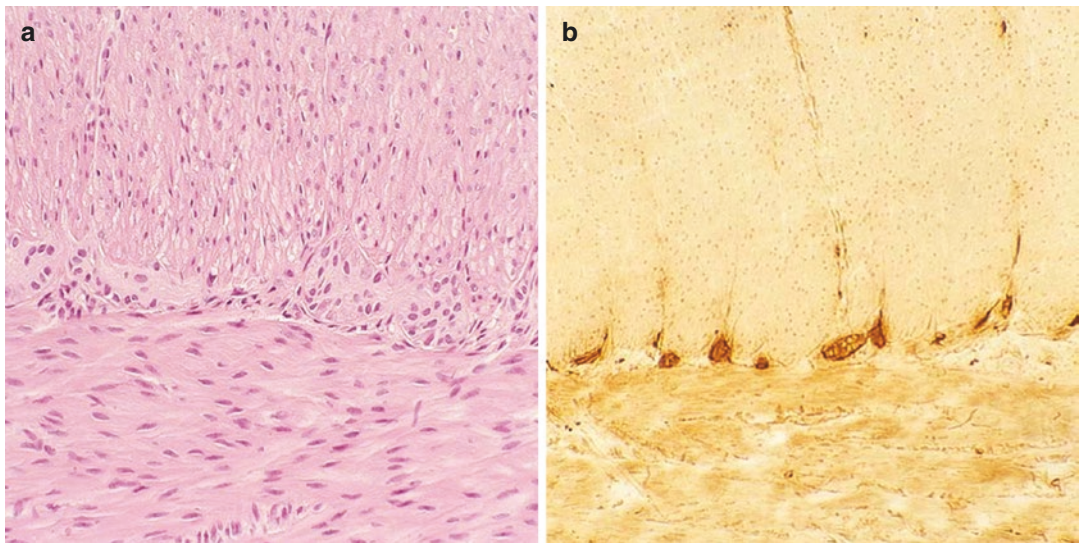

Fig. 70.3 Hematoxylin and eosin staining (**a**) and AChE immunohistochemistry (**b**) in a patient with IG, showing immature ganglion cells

been suggested as neuronal markers to show the small ganglion cells more clearly (Puri 1997). Enzyme histochemistry for succinate and lactate dehydrogenase is also commonly used to determine IG, demonstrating an absent or rather weak positive reaction (Holschneider et al. 1994; Meier-Ruge and Bruder 2005). In addition, cathepsin D has been recommended to assess the maturation of immature ganglion cells in more detail (Friedmacher and Puri 2013). Another helpful biomarker to detect IG is the apoptosis regulator B-cell lymphoma 2 (Friedmacher and Puri 2013), which clearly differentiates immature small neurons from enteric glial cells and satellite cells.

70.5.5 Management

The management of patients with IG is conservative with the use of laxatives and enemas (Friedmacher and Puri 2013).

70.5.6 Outcome

The vast majority of patients with IG can be successfully managed with conservative treatment measures until their ganglion cells are fully mature (Holschneider et al. 1994). Nevertheless, it is recommended to repeat the biopsy 12 to 18 months after initial investigation.

70.6 Absence of the Argyrophil Plexus

The lack of argyrophil cells in the myenteric plexus, which is also known as the absence of the argyrophil plexus (AP), is a rare cause of constipation and functional bowel obstruction in infants and children.

70.6.1 Epidemiology

There is a paucity of published data on the incidence of this very rare condition. In 1997, Prem Puri found three cases with an absence of the AP in their series of patients with functional bowel disorders (Puri 1997). Familial cases in the offspring of consanguineous parents and recurrence in siblings suggest that the absence of the AP may be inherited in an autosomal-recessive manner (Auricchio et al. 1996; Tanner et al. 1976).

70.6.2 Pathogenesis

There are two distinct subtypes of nerve cells in the myenteric plexus, which can be distinguished by their affinity for silver stains: (1) *argyrophil* cells and (2) *argentaffin* cells. Argyrophil cells normally comprise between 5% and 20% of the total number of myenteric neurons (Tanner et al. 1976). The processes of these cells along with extrinsic and parasympathetic fibers form a complex neuronal network within the myenteric plexus, which is involved in the regulation of gastrointestinal peristalsis and transit time. Argyrophil cells coordinate the activation of argentaffin cells, which in turn secrete specific neurotransmitters and ultimately cause contraction or relaxation of muscle fibers within the bowel wall (Tanner et al. 1976). A distinct time lag has been demonstrated between the appearance of both cell types, with argyrophil cells appearing earlier than argentaffin cells (Singh 1963). It has further been shown that there is a caudocranial gradient in the differentiation of these neuron cells in the human bowel (Singh 1963). Therefore, it is suspected that the disruption of this differentiation process may lead to an absence of the AP.

70.6.3 Clinical Presentation

The clinical symptoms of patients with the absence of the AP are highly similar to HD, thus presenting with severe constipation, moderate abdominal distension and the lack of peristalsis (Auricchio et al. 1996).

70.6.4 Diagnosis

The absence of argyrophil cells and their neuronal processes can only be demonstrated by using silver impregnation of full-thickness rectal biopsies

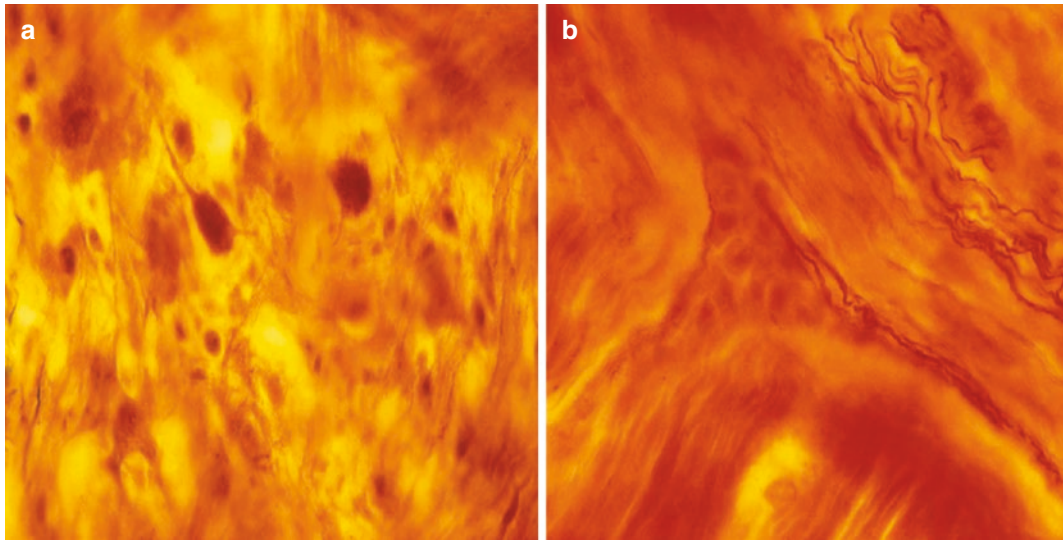

Fig. 70.4 Silver staining showing normal AP (**a**) and absence of argyrophil cells (**b**) in a patient with absence of the AP

(Fig. 70.4a, b), whereas conventional hematoxylin and eosin staining, AChE immunohistochemistry and histochemistry with other neuronal markers fail to show this abnormality (Puri 1997).

70.6.5 Management

Conservative management with laxatives and enemas is sufficient in most patients with absence of the AP. However, in some cases, internal sphincter myectomy or the formation of a colostomy may be required due to persistent constipation (Puri 1997).

70.6.6 Outcome

Conservative and surgical treatment of patients with absence of the AP usually results in a satisfactory outcome (Puri 1997).

70.7 Internal Anal Sphincter Achalasia

Internal anal sphincter achalasia (IASA) has a similar clinical presentation to HD, but with the presence of ganglion cells in rectal biopsies. Previously, IASA was referred to as ultrashort-segment HD, which is characterized by an aganglionic segment of 1 to 3 cm above the pectinate line, normal AChE activity in the lamina propria and increased AChE activity in the muscularis mucosae (Friedmacher and Puri 2012). Thus, it has been suggested that IASA is a more accurate term for this pathological entity, as many patients with the absence of the rectosphincteric reflex on anorectal manometry actually showed the presence of ganglion cells combined with normal AChE activity in rectal biopsies (Doodnath and Puri 2009).

70.7.1 Epidemiology

A total number of 395 cases with IASA have been reported in the literature since 1973 (Friedmacher and Puri 2012). However, the exact incidence of IASA is unknown.

70.7.2 Pathogenesis

Despite attempts of numerous investigators to determine the pathophysiological mechanisms of IASA in more detail, the exact pathogenesis remains unclear. Age-related changes in the

developing intramuscular innervation of the internal anal sphincter (IAS) most likely form the basis for the observed motility dysfunction (Doodnath and Puri 2009). By analyzing NADPH-d activity (Fig. 70.5a, b), it has been shown that absent to marked reduction of nitrergic innervation within the IAS of patients with IASA may be the underlying pathomechanism leading to spasm or increased tone (Friedmacher and Puri 2013). Additionally, a defective innervation of the neuromuscular junction of the IAS with decreased expression of PGP9.5 and synapsin-1 has been identified (Friedmacher and Puri 2013). Absent to markedly reduced NADPH-d and NCAM activity in the IAS of patients with IASA has also been demonstrated (Friedmacher and Puri 2013). More recently, a reduced number of c-Kit-positive ICCs have been found in the IAS of patients with IASA (Rolle et al. 2007). The deficiency in nitrergic innervation and ICCs may explain the impaired IAS relaxation in patients with IASA.

70.7.3 Clinical Presentation

In most cases, the clinical presentation of IASA is similar to that of HD. Patients with IASA generally suffer from chronic and severe constipation with or without soiling. Approximately one third of these patients have a history of abdominal distension and failure of laxative therapy (Doodnath and Puri 2009).

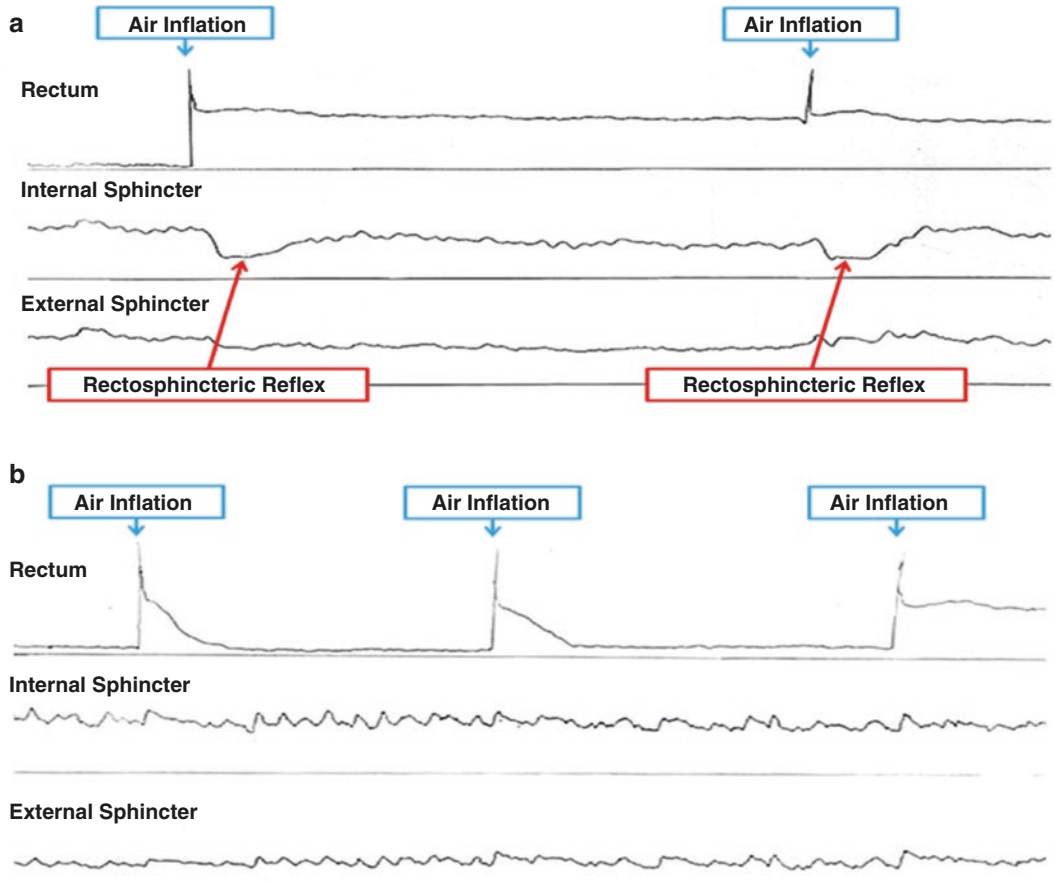

Fig. 70.5 NADPH-d staining of normal IAS (**a**). Reduced NADPH-d-positive innervations (**b**) in a patient with IASA

Fig. 70.6 Anorectal manometry showing evidence of the rectosphincteric reflex in a normal IAS (**a**). The absence of the rectosphincteric reflex on rectal balloon inflation with marked increased rhythmic activity of the IAS (**b**) in a patient with IASA

70.7.4 Diagnosis

The diagnosis of IASA is based on clinical symptoms in combination with anorectal manometry findings, showing the absence of the rectosphincteric reflex along with marked increased rhythmic activity (Fig. 70.6a, b). Furthermore, rectal biopsies demonstrate the presence of ganglion cells and normal AChE activity, as well as depletion of nitrergic nerves within the IAS (Puri and Gosemann 2012).

70.7.5 Management

Traditionally, posterior IAS myectomy has been recommended for the treatment of IASA. More recently, intrasphincteric injection of botulinum toxin has been introduced as a less invasive therapeutic alternative (Friedmacher and Puri 2013).

70.7.6 Outcome

The vast majority of patients with IASA have regular bowel movements after treatment irrespective of the therapeutic approach (Puri and Gosemann 2012). However, a recent meta-analysis has indicated that posterior IAS myectomy appears to be a more effective treatment option resulting in better functional outcome compared to intrasphincteric botulinum toxin injection (Friedmacher and Puri 2012). The rate of transient fecal incontinence, non-response and subsequent surgical procedures was significantly higher after injection of botulinum toxin, whereas long-term improvements were significantly more frequent following IAS myectomy. Interestingly, no differences were found in the postoperative use of laxatives or enemas, postoperative soiling and constipation between both procedures.

70.8 Megacystis-Microcolon-Intestinal Hypoperistalsis Syndrome

Megacystis-microcolon-intestinal hypoperistalsis syndrome (MMIHS) is an extremely rare condition and the most severe form of functional bowel obstruction in the newborn, characterized by massive abdominal distension caused by a large-dilated, non-obstructed bladder, microcolon with malrotation and decreased or absent intestinal peristalsis (Fig. 70.7a, b) (Puri and Gosemann 2012).

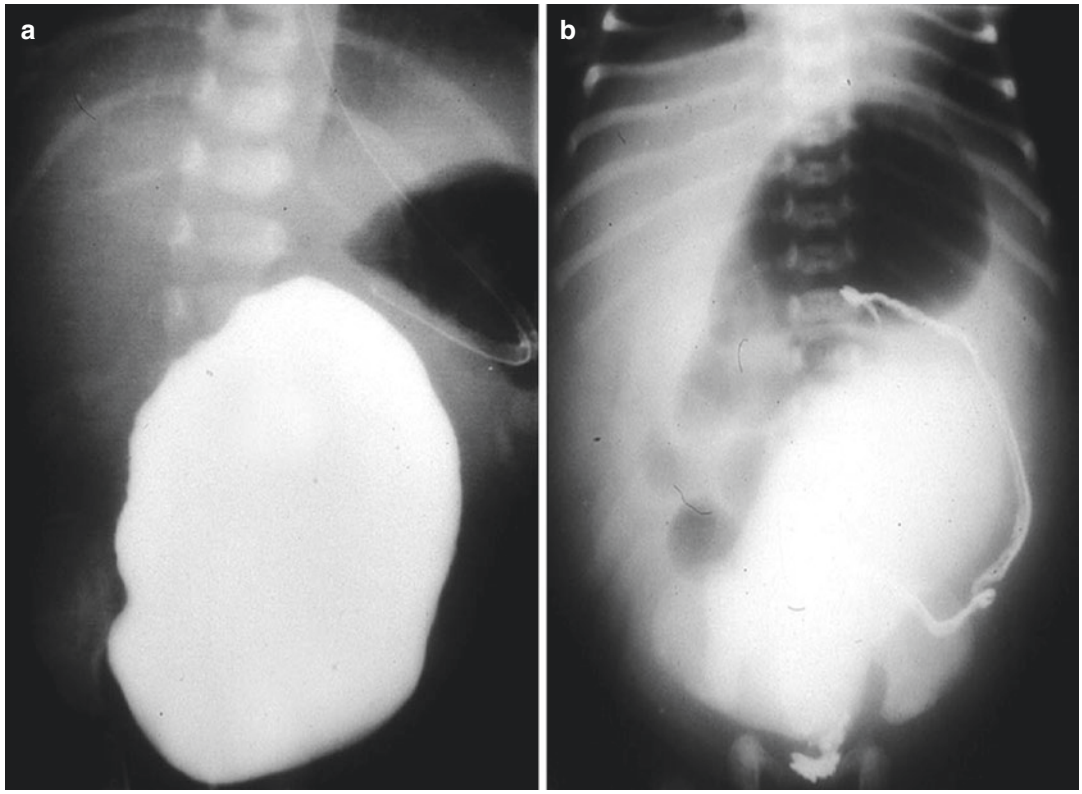

Fig. 70.7 Voiding cystourethrogram (**a**) and barium contrast enema (**b**) showing massively enlarged bladder and microcolon in a patient with MMIHS

70.8.1 Epidemiology

In the published literature, there has been a total of 450 reported MMIHS patients (Nakamura et al. 2019). A male-to-female ratio of 1:2.3 has been observed, suggesting a distinct female predominance. In comparison to affected females, male patients with MMIHS seem to have a shorter life expectancy. This is most likely due to increased severity of this condition in males compared to females (Köhler et al. 2004). Furthermore, the occurrence of familial cases in the offspring of consanguineous parents and recurrence in siblings indicates that MMIHS may be inherited in an autosomal-recessive manner (Nakamura et al. 2019).

70.8.2 Pathogenesis

MMIHS was first observed in 1976 in five newborn girls (Berdon et al. 1976), who presented with the above characteristic features. Since then, various hypotheses have been proposed to explain the pathogenesis of MMIHS. Genetic, myogenic, neurogenic and hormonal etiologies have been discussed (Puri and Gosemann 2012). However, most of these theories have been derived from case reports due to the rarity of this condition. Thus, the etiology remains poorly understood. At present, several candidate genes for MMIHS have been identified. The gene knockout of the nicotinic acetylcholine receptor (*nAChR*) in transgenic mice resulted in a phenotype similar to that of human MMIHS (Friedmacher and Puri 2013). Furthermore, it has been shown that a lack of expression of the *α3*, *β2* and *β4* subunits of *nAChR* in small bowel tissue from patients with MMIHS contributes to the pathogenesis of this rare syndrome (Puri and Gosemann 2012). *CHRNA3* and *CHRNB4* genes, which both code for the *β4* subunit of *nAChR*, are additional strong candidates (Friedmacher and Puri 2013). Published data also points to a

dysfunction within the smooth muscle layer. Vacuolar changes in the smooth muscle cells within the bowel and bladder wall have been reported in patients with MMIHS (Fig. 70.8a, b) (Puri et al. 1983). Therefore, it has been suggested that the observed smooth muscle myopathy may be the underlying cause of MMIHS (Puri et al. 1983; Rolle et al. 2002). The expression of several contractile and cytoskeleton proteins, such as αSMA, calponin, caldesmin and desmin, has also been found to be absent or decreased in the colonic smooth muscle tissue of patients with MMIHS (Puri and Gosemann 2012). Further support of this theory is derived from the findings of abnormal synaptophysin distribution in the circular muscle layer of bowel and bladder specimens, as well as increased connective tissue and atrophic smooth muscle fibers (Friedmacher and Puri 2013). Moreover, a decreased expression of ICCs in the bladder has been observed (Rolle et al. 2007). In addition to the smooth muscle dysfunction, other studies have focused on neuronal differences in MMIHS. Previously, immaturity and malfunction of autonomic nerve endings in the whole gastrointestinal tract have been demonstrated (Puri and Gosemann 2012). Axonal dystrophy and additional defects of the autonomic innervation in the intestine of patients with MMIHS have also been reported (Friedmacher and Puri 2013). In addition, an intramural inflammatory process that affects the gastrointestinal and urinary tract has been proposed in the pathogenesis of MMIHS (Puri and Gosemann 2012).

70.8.3 Clinical Presentation

On prenatal ultrasound or MRI scans, an enlarged bladder with hydronephrosis, microcolon and intestinal malrotation can often be demonstrated. The clinical presentation of MMIHS is similar to that of other severe neonatal intestinal obstruction, typically presenting with massive abdominal distension, which is a consequence of the largely dilated, non-obstructed bladder with or without upper urinary tract dilatation. The majority of patients with MMIHS require either vesicostomy or catheterization as they are unable to void spontaneously. A strikingly abnormal detrusor muscle has been identified in these patients, which is most likely the cause for the voiding dysfunction (Friedmacher and Puri 2012). Additional common findings are bile-stained vomiting, absent or decreased bowel sounds and failure to pass meconium.

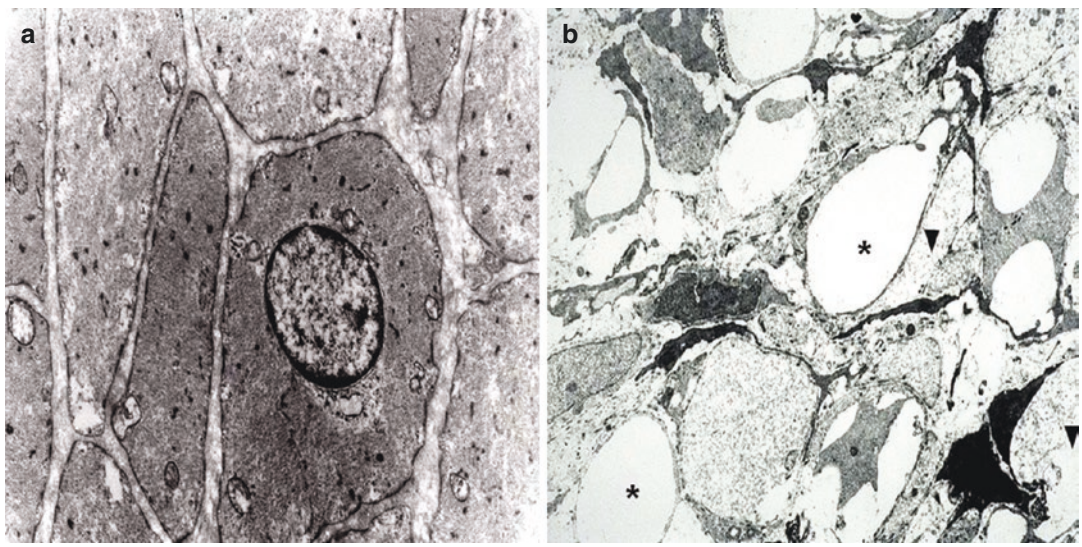

Fig. 70.8 Electron microscopy of smooth muscle cells in normal bowel (**a**). Central core degeneration resulting in empty vacuoles (*asterisks*) and increased connective tissue (*triangles*) in smooth muscle cells (**b**) of a patient with MMIHS

70.8.4 Diagnosis

There should be an emphasis on the prenatal diagnosis of MMIHS to allow for adequate prenatal counseling to enable future parents to make an informed decision regarding the continuation of the pregnancy, given the poor prognosis of this severe condition. The diagnosis of MMIHS is usually made on the basis of prenatal ultrasound findings and characteristic clinical presentation in the immediate neonatal period. An enlarged bladder and hydronephrosis can already be found on fetal ultrasound studies. An analysis of enzymatic changes in amniotic fluid in combination with MRI scans can further contribute to the prenatal diagnosis of MMIHS (Garel et al. 2006). A histological evaluation of myenteric and submucosal plexuses revealed normal ganglion cells in 77% of the investigated bowel specimens from patients with MMIHS. The remaining 23% were shown to have various neuronal abnormalities including hyper-/hypoganglionosis and immature ganglia (Friedmacher and Puri 2013). In addition, some authors found significant anomalies in smooth muscle cells from bowel and bladder specimens, such as vacuolar degeneration and thinning of the longitudinal muscle (Puri et al. 1983; Rolle et al. 2002).

70.8.5 Management

The management of patients with MMIHS is limited. A number of prokinetic drugs and gastrointestinal hormones have been tried without any success. Surgical treatment usually has to be performed for malrotation, bowel obstruction and megacystis to achieve decompression of bowel and bladder. However, in most cases, these interventions do not result in any improvement in enteral food intake, functional bowel obstruction or bladder function. Consequently, the majority of patients with MMIHS are maintained on total parenteral nutrition (TPN), which leads to further comorbidities, such as catheter sepsis, dyslipidemia, TPN-associated liver disease and eventually chronic liver failure (Gosemann and Puri 2011). Intestinal and multivisceral transplantation has recently been introduced as a valuable therapeutic alternative in patients with irreversible intestinal pathology and failure of TPN. To date, 12 multivisceral transplantations have been reported in MMIHS patients (Puri and Gosemann 2012).

70.8.6 Outcome

The survival rate of MMIHS has improved considerably in recent years from 12.6% initially to 55.6%. Between 1977 and 2011, an overall survival rate of 19.7% was reported with the oldest survivor being 24 years old. Overwhelming sepsis, multiple organ failure and malnutrition have been shown to be the most frequent causes of death in MMIHS patients (Gosemann and Puri 2011). A 3-year survival rate of 50% with all survivors tolerating enteral feeding and showing adequate gastric emptying has recently been reported (Loinaz et al. 2005). Improvements in outcome have mainly been due to more specialized care, innovations in parenteral nutrition and the introduction of multivisceral transplantation.

70.9 Conclusion

Conditions that clinically resemble HD despite the presence of ganglion cells on rectal biopsies, can be diagnosed by providing an adequate biopsy specimen and employing a variety of histological staining techniques. The two most common disorders in variant HD are IND and IASA. The majority of patients with IND can be successfully managed with conservative treatment measures or IAS myectomy if required. Thus, pull-through operation is rarely indicated in the management of IND. IASA, which is characterized by a reduction of nitrergic innervation within the IAS, can be diagnosed by anorectal manometry and successfully treated by either posterior IAS myectomy or alternatively, an intrasphincteric injection of botulinum toxin.

References

Auricchio A, Brancolini V, Casari G, Milla PJ, Smith VV, Devoto M et al (1996) The locus for a novel syndromic form of neuronal intestinal pseudoobstruction maps to Xq28. Am J Hum Genet 58:743–748

Berdon WE, Baker DH, Blanc WA, Gay B, Santulli TV, Donovan C (1976) Megacystis-microcolon-intestinal hypoperistalsis syndrome–a new cause of intestinal obstruction in the newborn: report of radiologic findings in five newborn girls. AJR Am J Roentgenol 126:957–964

Bruder E, Meier-Ruge WA (2007a) Intestinal neuronal dysplasia type B: how do we understand it today? Pathologe 28:137–142

Bruder E, Meier-Ruge WA (2007b) Hypoganglionosis as a cause of chronic constipation. Pathologe 28:131–136

Burki T, Kiho L, Scheimberg I, Phelps S, Misra D, Ward H et al (2011) Neonatal functional intestinal obstruction and the presence of severely immature ganglion cells on rectal biopsy: 6 year experience. Pediatr Surg Int 27:487–490

Coerdt W, Michel JS, Rippin G, Kletzki S, Gerein V, Müntefering H et al (2004) Quantitative morphometric analysis of the submucous plexus in age-related control groups. Virchows Arch 444:239–246

Cohen MS, Phay JE, Albinson C, DeBenedetti MK, Skinner MA, Lairmore TC et al (2002) Gastrointestinal manifestations of multiple endocrine neoplasia type 2. Ann Surg 235:648–654

D'Amore ESG, Manivel JC, Pettinato G, Niehans GA, Snover DC (1991) Intestinal ganglioneuromatosis: mucosal and transmural types. A clinicopathological and immunohistochemical study of 6 cases. Hum Pathol 22:276–286

de Krijger RR, Brooks A, van der Harst E, Hofstra RM, Bruining HA, Molenaar JC et al (1998) Constipation as the presenting symptom in de novo multiple endocrine neoplasia type 2B. Pediatrics 102:405–408

Dingemann J, Puri P (2010) Isolated hypoganglionosis: systematic review of a rare intestinal innervation defect. Pediatr Surg Int 26:1111–1115

Doodnath R, Puri P (2009) Internal anal sphincter achalasia. Semin Pediatr Surg 18:246–248

Fadda B, Maier WA, Meier-Ruge W, Schärli A, Daum R (1983) Neuronal intestinal dysplasia. Critical 10-years' analysis of clinical and biopsy diagnosis. Z Kinderchir 38:305–311

Feichter S, Meier-Ruge WA, Bruder E (2009) The histopathology of gastrointestinal motility disorders in children. Semin Pediatr Surg 18:206–211

Friedmacher F, Puri P (2012) Comparison of posterior internal anal sphincter myectomy and intrasphincteric botulinum toxin injection for treatment of internal anal sphincter achalasia: a meta-analysis. Pediatr Surg Int 28:765–771

Friedmacher F, Puri P (2013) Classification and diagnostic criteria of variants of Hirschsprung's disease. Pediatr Surg Int 29:855–872

Garel C, Dreux S, Philippe-Chomette P, Vuillard E, Oury J-F, Muller F (2006) Contribution of fetal magnetic resonance imaging and amniotic fluid digestive enzyme assays to the evaluation of gastrointestinal tract abnormalities. Ultrasound Obstet Gynecol 28:282–291

Gosemann JH, Puri P (2011) Megacystis microcolon intestinal hypoperistalsis syndrome: systematic review of outcome. Pediatr Surg Int 27:1041–1046

Granero Cendón R, Millán López A, Moya Jiménez MJ, López Alonso M, De Agustín Asensio JC (2007) Intestinal neuronal dysplasia: association with digestive malformations. Cir Pediatr 20:166–168

Holschneider AM, Meier-Ruge W, Ure BM (1994) Hirschsprung's disease and allied disorders–a review. Eur J Pediatr Surg 4:260–266

Kobayashi H, Hirakawa H, Puri P (1995) What are the diagnostic criteria for intestinal neuronal dysplasia? Pediatr Surg Int 10:459–464

Kobayashi H, Hirakawa H, Surana R, O'Briain DS, Puri P (1995) Intestinal neuronal dysplasia is a possible cause of persistent bowel symptoms after pull-through operation for Hirschsprung's disease. J Pediatr Surg 30:253–257

Köhler M, Pease PW, Upadhyay V (2004) Megacystis-microcolon-intestinal hypoperistalsis syndrome (MMIHS) in siblings: case report and review of the literature. Eur J Pediatr Surg 14:362–367

Koletzko S, Jesch I, Faus-Kebetaler T, Briner J, Meier-Ruge W, Müntefering H et al (1999) Rectal biopsy for diagnosis of intestinal neuronal dysplasia in children: a prospective multicentre study on interobserver variation and clinical outcome. Gut 44:853–861

Loinaz C, Rodriguez MM, Kato T, Mittal N, Romaguera RL, Bruce JH et al (2005) Intestinal and multivisceral transplantation in children with severe gastrointestinal dysmotility. J Pediatr Surg 40:1598–1604

Martucciello G, Pini Prato A, Puri P, Holschneider AM, Meier-Ruge W, Jasonni V et al (2005) Controversies concerning diagnostic guidelines for anomalies of the enteric nervous system: a report from the fourth international symposium on Hirschsprung's disease and related neurocristopathies. J Pediatr Surg 40:1527–1531

Martucciello G, Torre M, Pini Prato A, Lerone M, Campus R, Leggio S et al (2002) Associated anomalies in intestinal neuronal dysplasia. J Pediatr Surg 37:219–223

Meier-Ruge W (1971) Casuistic of colon disorder with symptoms of Hirschsprung's disease. Verh Dtsch Ges Pathol 55:506–510

Meier-Ruge WA, Ammann K, Bruder E, Holschneider AM, Schärli AF, Schmittenbecher PP et al (2004) Updated results on intestinal neuronal dysplasia (IND B). Eur J Pediatr Surg 14:384–391

Meier-Ruge WA, Brönnimann PB, Gambazzi F, Schmid PC, Schmidt CP, Stoss F (1995) Histopathological criteria for intestinal neuronal dysplasia of the submucosal plexus (type B). Virchows Arch 426:549–556

Meier-Ruge WA, Bruder E (2005) Pathology of chronic constipation in pediatric and adult coloproctology. Pathobiology 72:1–102

Moline J, Eng C (2011) Multiple endocrine neoplasia type 2: an overview. Genet Med 13:755–764

Montedonico S, Acevedo S, Fadda B (2002) Clinical aspects of intestinal neuronal dysplasia. J Pediatr Surg 37:1772–1774

Montedonico S, Cáceres P, Muñoz N, Yáñez H, Ramírez R, Fadda B (2011) Histochemical staining for intestinal dysganglionosis: over 30 years experience with more than 1,500 biopsies. Pediatr Surg Int 27:479–486

Moore SW, Kaschula ROC, Cywes S (1993) Familial and genetic-aspects of neuronal intestinal dysplasia and Hirschsprung's disease. Pediatr Surg Int 8:406–409

Nakamura H, O'Donnell AM, Puri P (2019) Consanguinity and its relevance for the incidence of megacystis microcolon intestinal hypoperistalsis syndrome (MMIHS): systematic review. Pediatr Surg Int 35:175–180

O'Riordain DS, O'Brien T, Crotty TB, Gharib H, Grant CS, van Heerden JA (1995) Multiple endocrine neoplasia type 2B: more than an endocrine disorder. Surgery 118:936–942

Puri P (1997) Variant Hirschsprung's disease. J Pediatr Surg 32:149–157

Puri P, Gosemann JH (2012) Variants of Hirschsprung disease. Semin Pediatr Surg 21:310–318

Puri P, Lake BD, Gorman F, O'Donnell B, Nixon HH (1983) Megacystis-microcolon-intestinal hypoperistalsis syndrome: a visceral myopathy. J Pediatr Surg 18:64–69

Puri P, Lake BD, Nixon HH, Mishalany H, Claireaux AE (1977) Neuronal colonic dysplasia: an unusual association of Hirschsprung's disease. J Pediatr Surg 12:681–685

Rolle U, O'Briain S, Pearl RH, Puri P (2002) Megacystis-microcolon-intestinal hypoperistalsis syndrome: evidence of intestinal myopathy. Pediatr Surg Int 18:2–5

Rolle U, Piaseczna-Piotrowska A, Puri P (2007) Interstitial cells of Cajal in the normal gut and in intestinal motility disorders of childhood. Pediatr Surg Int 23:1139–1152

Schäppi MG, Staiano A, Milla PJ, Smith VV, Dias JA, Heuschkel R et al (2013) A practical guide for the diagnosis of primary enteric nervous system disorders. J Pediatr Gastroenterol Nutr 57:677–686

Singh I (1963) The prenatal development of enterochromaffin cells in the human gastro-intestinal tract. J Anat 97:377–387

Smith B (1968) Pre- and postnatal development of the ganglion cells of the rectum and its surgical implications. J Pediatr Surg 3:386–391

Smith VV, Eng C, Milla PJ (1999) Intestinal ganglioneuromatosis and multiple endocrine neoplasia type 2B: implications for treatment. Gut 45:143–146

Taguchi T, Masumoto K, Ieiri S, Nakatsuji T, Akiyoshi J (2006) New classification of hypoganglionosis: congenital and acquired hypoganglionosis. J Pediatr Surg 41:2046–2051

Tanner MS, Smith B, Lloyd JK (1976) Functional intestinal obstruction due to deficiency of argyrophil neurones in the myenteric plexus: familial syndrome presenting with short small bowel, malrotation, and pyloric hypertrophy. Arch Dis Child 51:837–841

Torre M, Martucciello G, Ceccherini I, Lerone M, Aicardi M, Gambini C et al (2002) Diagnostic and therapeutic approach to multiple endocrine neoplasia type 2B in pediatric patients. Pediatr Surg Int 18:378–383

Yin M, King SK, Hutson JM, Chow CW (2006) Multiple endocrine neoplasia type 2B diagnosed on suction rectal biopsy in infancy: a report of 2 cases. Pediatr Dev Pathol 9:56–60