Neurocristopathies and Particular Associations with Hirschsprung's Disease

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18.1 Introduction

Primitive neural cells migrate from the neural crest during embryogenesis to reach their target organs. They then undergo differentiation into melanocytes, adrenal medulla cells, C cells of the thyroid, sympathetic ganglia and the enteric nervous system (ENS) of visceral ganglia, sensory tracts of cranial and spinal nerves, as well as the membranous bones of the face and palate.

Neurocristopathies (a unifying concept of conditions which arise from a common site of neural crest development [13]) arise from disturbance of cellular development and tissue of neural crest origin and result in a number of clinical phenotypes, which include a variety of tumors. These tumors occur largely from disturbances in the oncogenes and tumor suppressor genes involved in cellular development. The combination of Hirschsprung's disease (HSCR) with a neurocristopathy strongly indicates the need to investigate the sympathetic amine precursor uptake decarboxylase system for associated lesions.

18.2 Neurocristopathies Associated with HSCR

Because the etiology of HSCR is thought to be largely genetic in nature, the observed genetic variation in HSCR has become an emerging resource for studying the complex pathophysiology of this multifaceted condition as well as understanding reported clinical associations. At a molecular level, HSCR appears to arise as the result of a disruption of normal developmental molecular signaling. Major susceptibility genes known to be involved include the RET (REarranged during Transfection) protooncogene, at 10q11.2, the recessive EDNRB gene, located at 13q22 and its ligand endothelin 3 (EDN3), and the glial cell line-derived neurotrophic factor (GDNF) [1, 129].

The resulting neurocristopathies include the following:

- Neurocristopathies associated with the RET protooncogene
- Neurocristopathies associated with the endothelin system (EDNRB, EDN3) and SOX10
- Congenital central hypoventilation syndrome (CCHS)
- Other rarer neurocristopathies

18.2.1 Neurocristopathies Associated with the RET Protooncogene

The relationship to the major susceptibility gene RET protooncogene, at 10q11.2 and multiple endocrine neoplasia (MEN) syndromes appears to be highly significant. The association is now clearly understood to be genetic in nature and early identification may have implications for preventative and early intervention strategies.

18.2.1.1 The Role of the RET Protooncogene in HSCR and MEN Syndromes

The RET protooncogene appears to be the most significant susceptibility gene in HSCR where it appears to result from loss of function. Although major mutations appear to account for up to 50% of familial and 30% of nonfamilial cases [145], a number of lesser genetic variations have been identified in RET which also appear to play a significant role. These include specific alleles at RET-1VS, certain single nucleotide polymorphisms (SNPs; e.g. A45A) as well as specific haplotypes (haplotype 0) [1, 17, 18, 98, 131]. It is not yet clear whether these variations can give rise to HSCR by haploinsufficiency per se or whether lesser mutations require the multiplicative effects of other disturbed signaling pathways.

The pathophysiology involved in the MEN and related syndromes appears to be reliant upon completely different sites on the RET protooncogene and results in a RET-activating rather than a RET loss of function action. RET protooncogene mutations have now been associated with MEN 2A and 2B syndromes, familial medullary thyroid carcinoma (MTC), and (partly) papillary thyroid carcinoma. The position of mutations seems to be important in terms of the phenotypic expression. For example, those RET variations associated with the six cysteine positions in the extracellular region of the RET protooncogene [20, 66] plus exon 14 SNP S826S [126] and the 918 mutation in exon 16 [79, 132] have been strongly associated with MTC.

18.2.1.2 The Multiple Endocrine Neoplasia Syndromes

The association between HSCR and MEN-related syndromes concerns mainly the MEN2 syndromes (A and B) and HSCR. MEN1 is a clinical syndrome consisting of pituitary, parathyroid, pancreatic neuroendocrine tumors not genetically related to RET mutations, its current association being with chromosome 11q13, and it is usually not associated with HSCR.

MEN2 Syndromes

MEN2A is an autosomal dominant genetic condition characterized by the development of a number of tumors including pheochromocytoma, MTC, thyroid C cell hyperplasia and parathyroid tumors. There are distinct genotype–phenotype correlations in MEN2. The most common subtype is MEN2A (Sipple syndrome), which includes two known variants: associations with HSCR, and associations with cutaneous lichen amyloidosis.

The association of aganglionic megacolon with megaloureter, pheochromocytoma and neuromatosis [139] actually preceded the landmark report by Sipple of a 14 times higher association between pheochromocytoma and thyroid tumors [86, 142]. A diffuse ganglioneuromatosis (GN) within the wall of the stomach, and small and large intestine was then identified at autopsy in two patients with MTC and pheochromocytoma [166], thus expanding the concept of a neurocristopathy. Steiner et al. [147] introduced the term multiple endocrine neoplasia syndrome which is transmitted in an autosomal dominant manner [69]. The association of MEN2 syndromes with the RET protooncogene subsequently appeared in the literature and is discussed later in this chapter.

MEN2B, on the other hand, is an association of pheochromocytoma, MTC, C cell hyperplasia, and ocular and gastrointestinal ganglioneuromata in patients with marfanoid features. Isolated cases of GN probably represent incomplete gene penetrance.

Familial MTC represents the familial transmission of MTC without the full features of MEN 2, and is sometimes referred to as MEN3 (Froboese syndrome)

Clinical Features of the MEN Syndromes

MEN2 syndromes are defined by the presence or absence of pheochromocytomas, hyperparathyroidism, MTC and other characteristic clinical features. It has not yet been possible to differentiate between the thyroid carcinomas or pheochromocytomas of the MEN2A and MEN2B syndromes on histological grounds, and patients with MEN2A are generally completely asymptomatic in the preclinical phase. Similar to other genetically determined premalignant conditions (e.g. familial polyposis coli and the APC gene), the prevention of the resulting neoplasms depends entirely on familial pedigree, genetic analysis and timely removal of the target organ. It is important to identify patients with MEN2 early as 52–75% of MTC have lymph node metastases at the time of clinical diagnosis. The high morbidity and associated mortality in these radio- and chemoresistant tumors makes surgical preventative removal of the target organ an essential goal of treatment.

The clinical presentation of pheochromocytoma is well described. It may be asymptomatic or missed where patients present early. Because of the association with MEN2, patients with pheochromocytoma should be screened for blood chemistry and calcitonin levels and, if necessary, referred for genetic evaluation

MEN2B on the other hand presents with marfanoid features as well as the classical ganglioneuromas of the oral cavity and gastrointestinal system. The gastrointestinal involvement associated with the MEN2B syndrome means that patients not uncommonly present with intractable chronic constipation and megacolon. Intestinal obstruction resulting from a colonic mucosal neuroma has also been described [119].

Genetic Aspects of the MEN2 Syndromes

The MEN2 syndromes result from gene upregulation as a result of germline activating mutations in the RET protooncogene. In general, HSCR and MTC affect different parts of the RET gene but a certain amount of genetic overlap leads to therapeutic dilemmas in apportioning risk (see 18.2.1.4 HSCR and MEN-related RET Mutations).

Activating mutations of RET appear to be of the order of 1:500,000 in the general population [127]. Many are de novo genetic variations which involve germline mutations in exons 10, 11, 13, 14, 15, and 16 of the RET protooncogene in at least 92% of patients with MEN2 presenting with MTC [21]. MEN2B is a less common subtype, but is mostly associated with exon 16 (M918T) RET mutations [24].

Recent advances have resulted in a clearer understanding of RET function and the effect of RET mutations on RET signaling and activation (e.g. MTC) or inactivation (e.g. HSCR) by means of a number of different mechanisms. As the resulting mutant proteins appear to determine the phenotypic expression, the higher the penetrance of the MEN2 phenotype, the earlier and more aggressive the cancer. The method by which RET mutations produce cancer is less clear, as mutations are mostly de novo and the cause unknown. Radiation exposure is the only clear factor associated with thyroid carcinoma and can actually be capable of inducing RET mutations [32], but is usually absent from the patient's history.

MEN is caused by "gain of function" variations in the cysteine-rich extracellular domain of RET and is associated with variations at one of the six cysteine residues (viz. 609, 611, 618, 620, 630 and 634 positions). The MEN2A mutations probably activate RET by inducing disulfide-linked homodimerization [8, 133]. In addition, RET extracellular domain mutations may result in the unfolding of RET by affecting polarity (e.g. C620S). The RET2B mutation (significantly more than the RET2A mutation) results in an increase in Ret-MEN specific potentiated phosphorylation of tyro 1062 (Y1062; a RET multiple effector docking site that mediates the recruitment of the Shc adaptor and of phosphatidinylinositol-3 kinase, P13K, at the Y1062 docking site) [27, 130]. RET MEN2B has been shown to be more active in associating Shc and in causing constitutive activation of the Ras/mitogen-activated protein and P13K/Akt cascades [27].

In the light of the apparent genotype–phenotype correlation between RET and MEN2 [23, 168] and the identification of specific sites on chromosome 10q11.2 associated with MEN2A [106] and MEN2B [76, 104], predictive DNA testing for MEN2 is now possible. Genetic screening for RET has been shown to be an extremely sensitive marker in MEN2 syndromes [106] with the majority of mutations relating to the cysteine radicals in exons 10, 11 and 16. As a result, the diagnosis of MEN2 is currently mostly confirmed on the basis of the genetic features, although the clinical phenotype remains important. Effective management therefore depends on early diagnosis and the gene carriers can now be identified before any clinical or biochemical abnormalities are present. These children can therefore be offered a prophylactic thyroidectomy which is successful in preventing the development of MTC with its associated high rate of metastatic disease (Fig. 18.1).

It is therefore clear that genetic screening should occur prior to the onset of any clinical symptoms to allow adequate early risk assessment and prophylactic management. It has been established that RET testing is vastly superior to calcitonin in identifying preclinical cases with specificity approaching 95–100% [106]. Mutations of codons 634 and 618 have been found in the youngest patients (3 and 7 years, respectively) making this a high-risk age group [91]. On the other hand, codons 790, 620 and 611 appear to be associated with an intermediate risk, and codons 768 and 804 with a relatively low risk of developing MTC. Nevertheless, a 12-year-old patient in an intermediate risk group has been reported with MTC, stressing its relevance in the prepubertal age group [91].

In most patients with MEN2B a methionine to threonine substitution occurs at position 918 (M918T) of the

Fig. 18.1 Familial MEN2A and C634S RET. Mother had pheochromocytoma plus MTC. Two affected children were treated by total thyroidectomy

RET-kinase domain. This currently appears to be the most significant alteration in oncogenesis, and may be of prognostic significance. The tumors display aggressive behavior and distant metastatic spread [10, 58–60, 76, 104].

In addition to the known sites, there is also over-representation of the variant S836S in patients with MTC [71, 126]. Associations with RET polymorphisms L769L, V804M and S904S have also been reported [93], although not consistently [165]. The role of these other RET variations is unclear as many authors of the various studies fail to state whether all 21 exons of the RET gene were investigated in a systematic manner or whether only the specific exons known to be associated were probed.

Patients with phenotypic features resembling MEN2B require genetic testing in spite of a negative family history because of the high incidence of spontaneous mutations (approximately 50%) [29, 153].

Treatment of MEN Syndromes

The multiple neoplasias encountered in MEN are treated on their own merits. Prophylactic total thyroidectomy is performed on gene carriers in accordance with their risk stratification. Screening should at least include the cysteine-containing codons 10, 11 and 16, but should also include exons 13 and 14. It is now established that the risk groups are determined by the genotype and should be used to dictate timing of prophylactic surgery [92]. In MEN2B it is recommended that testing should be done before 1 year of age (particularly in 883/918 codon mutations) and before 5 years in MEN2A (especially in the presence of mutations of codons 611, 618, 620 and 634). The assessment of risk in patients with isolated intestinal GN with the same genetic background without other features of MEN2B then remains problematic, and is addressed in section 18.2.1.4 Intestinal Ganglioneuromatosis.

As it is difficult to entirely predict tumor risk in affected individuals, it has been recommended that children with HSCR plus RET abnormalities undergo prophylactic thyroidectomy in accordance with their risk profile [141]. A high incidence of early aggressive tumors in MEN2B warrants an aggressive surgical approach with early prophylactic thyroidectomy in gene carriers (less than 1 year of age). Colonic disease in MEN2B is generally managed conservatively where possible. A localized segment of affected colon may be resected, but more commonly, especially where the small bowel is affected, there is little therapeutic benefit to be gained from such surgery.

18.2.1.3 HSCR and MEN-Related RET Mutations

The uncommon association between HSCR and MEN2 in the same patient is extremely interesting, as opposite effects have to occur in the RET protooncogene for this to take place. "Gain of function" variations result in MEN syndromes, and "loss of function" mutations result in HSCR [151], and these would have to take place simultaneously. Mulligan et al. [105] suggested that mutations at RET codons 618 and 620 not only give rise to MEN2A and familial MTC but also may predispose to a low penetrance way to HSCR.

Although cosegregation of these two conditions is uncommon, there are reports in at least 24 families of documented RET mutations associated with HSCR and MEN2A [12, 19, 22, 31, 47, 78, 105, 114, 115, 125, 134, 141, 159]. Recorded RET mutations in patients with cosegregation of HSCR with MEN include C609Y (n=2) [9, 109], C611S (n=1) [109], C618R (n=5), C618S (n=3) [22, 31, 115], C620R (n=8), C620S (n=4) [16, 78, 109, 125, 134] and C620W (n=1). We have reported a further case of a C620W mutation occurring in a patient with long-segment HSCR but without yet developing MTC.

The 620 mutation has been named the Janus mutation and is of interest as it accounts for approximately half of the reported cases of cosegregating MTC and HSCR, although it makes up only 12% of genetic variations associated with MTC itself [65]. The importance of this mutation is demonstrated by the reported case of familial MTC occurring in a patient with a C620S mutation 12 years after surgical correction of HSCR [134], the mother having developed MTC 7 years after the child's birth. On the other hand, Fernandes et al. [63] reported a kindred with a C620S mutation MTC but without HSCR . They suggested that the observed RET mutation had little to do with the development of HSCR in these patients and hypothesized that another area of RET is responsible for the HSCR phenotype. Our patient had total colonic aganglionosis and other genetic variations apart from the C620W in exon 10, namely a further RET SNP in exon 13 plus an exon 4 (831 G/A) SNP in EDNRB (which was probably neutral).

The hypothesis that the 620 mutation has a dual function is supported by the report of Arighi et al. [7] who have provided an theoretical explanation for the dual phenotypic Janus mutation at cys 620 of RET. Working with Madin-Darby canine kidney cells (MDCK) with a transfected C620S mutation, they demonstrated that although the mutation impairs the GDNF-induced effects on cell migration, differentiation and cell survival, it also simultaneously results in increased rapid cell proliferation. This dual action may also be true of certain other RET genetic variations. Borst et al. [22] suggested that the 618 RET codon could also predispose patients with MEN to HSCR in a similar manner. More information is required before this picture becomes clear, but based on current knowledge it does appear as if the 620 codon mutation has a dual or Janus potential.

18.2.1.4 Intestinal Ganglioneuromatosis

GN is an uncommon condition affecting peripheral nerves in the intestinal wall. It is important to note the transmural nature of the hypertrophied nerves (Fig. 18.2) to distinguish it from the thickened peripheral nerves seen in association with HSCR and the thickened nerves sometimes visible on low rectal biopsy. Although it displays similarities to the circumscribed or diffuse neuromatosis encountered in certain patients with neurofibromatosis, GN usually presents as an isolated condition with pseudoobstruction (presumably related to incomplete penetrance of the genetic defect [61].

GN is known to occur in association with MEN2B where there are also GN of the lips and tongue. This association with the MEN syndromes links it to the RET protooncogene and as a result, it potentially carries the risk of MTC and pheochromocytoma. Further ganglioneuromas (GN) of the ENS are also a possibility. In addition, GN may also be associated with abnormal neuropeptide secretion (e.g. VIP) [49, 123, 140] and diarrhea especially when it involves the small bowel and pancreas [140]. Although it has been described in animals [39], as part of intestinal neuronal dysplasia [43, 62] or part of intermuscular plexus hyperplasia [123], it must be seen as a separate entity, preferably with its identity being confirmed genetically.

Although often asymptomatic, initially patients may present with constipation or diarrhea [30] or in a similar manner to those with HSCR [94]. Other reported clinical features include failure to thrive, chronic diarrhea and abdominal distension. Radiological features include abnormal haustral patterns of the colon with thick mucosal folds, defective peristaltic movements and possible colonic diverticulae [5, 52]. In addition, areas of spasm and dilatation of the colon are often present [94], and it may even mimic Crohn's disease on radiological assessment [35]. Esophageal dysmotility has also been reported [48].

Rectal biopsy may show the massive transmural hypertrophy of nerve fibers among autonomic ganglia of the ENS. Ganglion cells are usually present in normal numbers and in our own studies PGP9.5 staining was within normal limits [83]. On the other hand, neural markers neurofilament protein and S100 protein demonstrated some variation with a marked increase in S100 staining being observed in the muscularis propria (but not in the lamina propria) as well as a mild reduction in neurofilament protein staining in both layers.

Patients with MEN2B often present with symptoms related to the ganglioneuromas of the intestinal wall [30]. Gastrointestinal symptoms may precede the clinical presentation and may lead to the diagnosis. There is a clear association with diarrhea (possibly on the basis of excessive VIP secretion). The relationship to constipation and recurrent episodes of pseudoobstruction and a Hirschsprung-like clinical picture is a definite mode of presentation. Verdy et al. [159] reported a connection with MEN syndromes in 9 out of 92 patients in their series which is the highest on record.

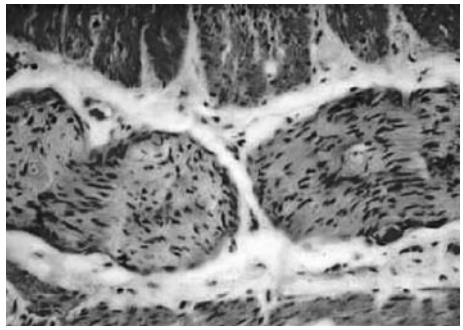

Fig. 18.2 Section of bowel wall demonstrating the massive transmural hypertrophy of nerve fibers typical of ganglioneuromatosis (H&E, ×25) (Photo P Beale, used with permission)

When associated with the MEN syndromes, the risk of thyroid carcinoma is increased but there is little available information as to the risk of developing MEN-related tumors in patients presenting with an isolated intestinal GN during childhood. The answer to this conundrum must surely lie in the genetic abnormalities associated with the condition. There is at least one reported patient, a 27-year-old man, who developed the phenotypic expression afterwards and diagnosis and prevention could have been obtained from earlier genetic investigation [11]. There is also a report of another patient with typical ganglioneuromas in whom the diagnosis was not made until tumors were present [111]. Shekitka et al. [138] concluded that the solitary polypoid ganglioneuroma of the gastrointestinal tract did not carry the same risk of neurofibromatosis or RET as the diffuse type.

The histological features of ganglioneuromatosis and its place in the neurocristopathies are of interest. Ganglioneuromas of the ENS are rare tumors, which consist of ganglion cells, nerve fibers and supporting cells. There are at least two morphological patterns of GN [43], the polypoid and diffuse types. Transmural GN affects all layers of the bowel wall which show neural hyperplasia with predominantly the myenteric plexus being involved, and is the form generally associated with MEN syndromes. The other form, mucosal GN (often as polyps [34, 100]), is more associated with von Recklinghausen's disease, adenocarcinoma of the colon and multiple adenomas with megacolon. The significance of GN is that it may be an indicator of the genetic background which may carry the risk of eventual cancer [111].

A germline RET codon 918 mutation has been reported in apparently isolated GN of the intestine [144]. Many series on intestinal dysplastic conditions contain

similar patients without the phenotypic features of a MEN syndrome, but with GN of the bowel. The risk of MTC is unclear (and hence its prevention by prophylactic thyroidectomy). The question as to whether the risk to patients with GN but without the features of a MEN syndrome can be predicted genetically has not yet been answered. Little is known about the way in which the exon 16 (M918T) germline mutation relates to GN, but it was present in all three patients reported by Smith et al. [144]. What is known is that pheochromocytoma cells transfected with RET M918T mutation are resistant to nerve growth factor inhibition [26], which may well explain the overgrowth of nerve elements. This study supports earlier findings of increased nerve growth stimulating activity acting preferentially on sympathetic nerve terminals [49].

It is well documented that the RET/GFR-alpha-1/GDNF complex is responsible for a signal which is essential for the survival of early crest derived neural precursors which in turn colonize the gut giving rise to the ENS [70, 151]. The RET 2B mutation (significantly more than the RET 2A mutation) results in an increase in Ret-MEN-specific potentiated phosphorylation of tyro 1062 (Y1062). Y1062 is a RET multiple effector docking site that mediates the recruitment of the Shc adaptor and of P13K at the Y1062 docking site. The MEN mutations convert RET into a dominant mutant protein which results in activation of its tyrosine kinase activity and tumor formation via the downstream mediator Shp-2 [42].

18.2.2 Neurocristopathies Associated with Endothelin System (EDNRB, EDN3) and SOX10

18.2.2.1 Waardenburg Syndrome

Waardenburg syndrome (WS) is a human genetic condition characterized by defective melanocyte function (with pigmentation anomalies of the skin, hair and iris; Fig. 18.3), cochlear sensorineural deafness and craniofacial abnormalities [160]. It occurs in association with intestinal aganglionosis as the uncommon Shah-Waardenburg subtype (WS4) [137]. EDNRB-deficient cells have been shown not to develop into differentiated pigmented melanocytes [77] and mutations of the EDN3 gene also appear to be important in WS [33, 64].

The Waardenburg-HSCR association is uncommon in most series and we have encountered only 1 patient out of more than 500 patients with HSCR. It does appear in kindreds, however [120, 121], where no increasing penetrance of aganglionosis was observed between generations in 25 unrelated kindreds (i.e. almost identical aganglionic length) [15, 85, 113].

This Waardenburg-HSCR association is transmitted in an autosomal recessive manner and appears to be related to genes at 13q22 (EDNRB) [121] and other related genes required for the normal development of the neural crest cells migrating to the eye, inner ear and colon. Experiments with Sp (Splotch) mutation on chromosome 1 in mouse models have provided a link to a PAX3 deletion (a transcription factor with two highly conserved DNA motifs) [54]. It has since been shown that PAX3 functions with SOX10 to activate c-ret transcription and that interruption of these pathways at various stages will result in intestinal aganglionosis [87]. To emphasize the role of EDNRB, a mouse model with exon 2 and 3 mutations has been reported to demonstrate the features of WS4 [97].

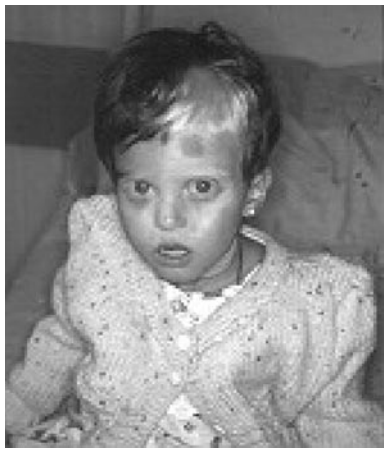

Fig. 18.3 Waardenburg-Shah association of HSCR with WS4. Note the white forelock

Thus, at least four genetic links are currently associated with the Waardenburg-Shah phenotype (viz. a transcription factor from SOX10, EDN3, the EDNRB gene [98], and a possible link to the MITF gene) [161].

18.2.2.2 The Role of SOX10

It is now understood that SOX10 activity (corresponding to the animal dominant megacolon DOM trait) modulates a number of critical signaling pathways controlling the differentiation of neural crest-derived nerve cells and melanocytes [101]. In addition to the known PAX3–SOX10–c-ret functions, interaction between SOX10 and the severity of aganglionosis has been shown in an animal model [28]. The presence of certain EDNRB mutations was shown to increase penetrance. In addition to EDNRB, further links have been demonstrated between SOX10 and RET (MOLa) binding sites in the RET promoter region where SOX10 has been shown to regulate transcription from the RET M promoter [161]. Lack of the normal SOX10-mediated RET activation may therefore also lead to intestinal aganglionosis. In addition,

overexpression of other genes coding for myelin proteins may result in some of the syndromic neurological associations of HSCR. A report of a patient with pseudoobstruction and SOX10 (without EBNRB and EDN3) mutations, and no pigmentation disorder [118], demonstrates its importance in intestinal neuronal development.

It is clear therefore that dosage-sensitive heterozygosity with incomplete penetrance of SOX10 could predispose to HSCR, whereas homozygosity would result in more complex neurocristopathies associated features of HSCR and WS [2]. WS has also been associated with mutations of the MITF (microphthalmia-associated transcription factor) gene [88, 161] which encodes a transcription factor with the basic helix-loop-helix leucine zipper (bHLH-zip) motif, which has been shown to be involved in melanocyte differentiation [110].

18.2.3 Congenital Central Hypoventilation Syndrome

Congenital central hypoventilation (CCHS, Ondine's curse) is an uncommon syndrome occasionally associated with HSCR (14–20% of cases), as well as with tumors of neural origin and autonomic dysfunction HSCR-CCHS (Haddad's syndrome). It is mostly associated with long-segment aganglionosis. CCHS involves a loss of autonomic control and is often associated with other autonomic nervous system abnormalities such as tonic pupil and other ophthalmic anomalies, especially when it occurs in association with HSCR [40]. It is a life-threatening condition as it results in an impaired ventilatory response to hypercarbia and hypoxemia, and patients often spend long periods on mechanical ventilatory support.

It has been reported to occur in 1 in every 200,000 live births in France [157]. It affects boys and girls equally and may be familial [73], the recurrence risk to sibs being 4%. These sib pairs together with identified genetic links with HSCR and associated tumors suggest a genetic basis for this syndrome. The pathogenesis of CCHS is most likely multigenic, although novel mutations of the RET and EDN3 genes have been reported [14]. A novel RET mutation (R114H) has been described [81, 128] as well as a corresponding GDNF variation [2]. Variations in brain-derived neurotrophic factor gene have been reported [162]. The CCHS-like picture resulting from a disrupted RNX gene (HOX11) in an animal model, in embryonic stem cells [96], has not been replicated in humans. Other workers have reported PHOX2B as a candidate [3], and more recently, heterozygous mutations of the paired-like homeobox gene PHOX2B have been identified in 91% of patients. It is not infrequently associated with tumors such as neuroblastomas [124], ganglioneuromas and ganglioneuroblastomas. Because of the known genetic associations, it is reasonable to speculate that the latter two arise in situations of lower gene penetrance.

18.2.4 Other Rarer Neurocristopathies

18.2.4.1 Extended Plasticity of the Enteric Nervous System

This group of conditions incorporates those variants of HSCR in which plasticity of the ENS appears to not follow the usual course and the plasticity of the ENS is prolonged. These conditions include prolonged or delayed maturity of ganglion cells, segmental aganglionosis and acquired postoperative aganglionosis.

Immaturity of Ganglion Cells

A wide spectrum of dysplastic features occur in the bowel in HSCR, one of which is immaturity of cells. This has been seen mostly in neonates and premature infants, the so-called "immaturity of prematurity". The ENS function in these patients appears to improve with maturation and is mostly managed conservatively. It may, however, persist giving rise to clinical problems.

It has been observed that, although differentiation of ENS neurons occurs early, a significant pool of precursor cells persists in the ENS, and the numbers of enteric neurons continue to increase until well after birth or hatching [67]. Immaturity of ganglion cells has been reported to influence the function of the intestine [25, 57]. Immaturity must be interpreted in the light of the gestational age, postnatal age and knowledge of the variations in normal postnatal development. In addition, the recognition of immature cells is not always easy as other cells such as hypertrophied glial cells and fibroblasts may lead to misinterpretation [6]. These immature ganglion cells have a smaller, darker nucleus without a recognizable nucleolus [6]. Special staining methods may be necessary to clarify the ganglion cell morphology and identify immature cells [108, 135].

To a certain extent, ENS immaturity may also explain the relatively low levels of acetylcholinesterase (AChE) not infrequently observed in neonatal ganglion cells [45], and the increase in staining patterns over time. The immature or developing cells would express AChE as they attempt to differentiate, and the timing of this would depend on the proportion of immature cells present.

18.2.4.2 Segmental Aganglionosis (Zonal Aganglionosis or Skip Lesions)

HSCR is normally defined as a functional obstruction resulting from congenital absence of ganglion cells in the myenteric plexuses of the distal segment of the gastrointestinal tract. A single distal aganglionic region therefore extends from the anal margin to the level of the proxi-

mal ganglionated bowel. Segmental aganglionosis, on the other hand, involves only a limited segment of bowel interposed between segments of normally innervated bowel. Understanding this phenomenon poses considerable theoretical and practical challenges.

Despite it being reported very early on in HSCR [75, 85, 146, 152], the existence of zonal aganglionosis is often questioned on theoretical grounds [170]. It has. however, been described in both children [4, 46, 72, 74, 89, 95, 116, 136, 149, 170] and adults [68], as well as in a number of animals [148]. It has been reported as including both the small bowel and the large bowel, and occasionally the appendix [4].

Munakata and Holschneider [107] classified the reported cases into:

- Single zonal aganglionosis or hypoganglionosis with distal normal innervation (ten patients)
- Double zonal analysis with distal normal innervation (four patients)
- Zonal normoganglionic or hypoganglionic colon within aganglionic intestine (eight patients)

The generally held view that all enteric neuroblasts arise from the vagal crest [117] and populate the bowel in a craniocaudal wave gives rise to certain theoretical difficulties in understanding how zonal aganglionosis could come about. Possible etiologic causes include the following:

- Anoxic damage to the myenteric plexus
- Migratory theory: a meeting point of the craniocaudal neuroblast migration as well as the neuroblasts arising from the sacral outflow
- Unfavorable microenvironment hypothesis
- Intrauterine inflammation or viral infection
- A primary abnormality of the developing gastrointestinal anlage

The hypoxic theory is discussed in the next section (18.2.4.3 Acquired Aganglionosis). The migratory hypothesis lacks support and there is little evidence that the sacral outflow produces a significant contribution to the ganglionation of the terminal bowel. In fact, the contrary appears to be the case [117]. In contrast to the migratory theory, a localized defect in the microenvironment of the specific segment of bowel resulting in a failure of enteric neurons to differentiate and undergo normal development and undergo apoptosis appears a distinct possibility. The pathogenesis of this condition would then depend upon developing and migrating neural crest cells confronting a segmental abnormal and hostile and microenvironment as a result of deranged intracellular signaling systems relating to the specific genes and gene protein.

The plasticity of the ENS after birth has long been the subject of debate. Current concepts include the idea that average neuronal activity levels are maintained by a set of homeostatic plasticity mechanisms, which adjust levels to achieve stability [158]. Recent findings demonstrate the important role of Hox genes (e.g. SOX10) in promoting the survival of neural crest precursors prior to differentiation [101]. Mutations may lead to apoptosis, thus offering a further explanation of ENS plasticity.

A primary abnormality of the developing gastrointestinal anlage appears to be a real possibility. It is currently supported by recent animal experiments on embryos of ls/ls minus mice (a model of classic short-segment aganglionosis) [82] in which a transient phase in the migratory pattern has been demonstrated. It would seem that ganglion cells appear in the middle colon of these mice as a result of an extramural phase of neuroblast migration at a stage when they are still absent from the ascending colon and distal large intestine. This unique observation suggests some sort of theoretical understanding of zonal aganglionosis. Should ENS development be arrested and persist after birth, it would give rise to the same clinical picture as reported by Martin et al. [95], where the ascending and descending colon were aganglionic with ganglion cells present in the middle colon. There are also similarities to one of the cases reported by Yunis et al. [170] and Taguchi et al. [149], and the zonal hypoganglionosis reported by Kadair et al. [80] could probably also be explained in this way.

18.2.4.3 Acquired Aganglionosis

Secondary aganglionosis following pull-through procedures for HSCR is a rare event. We previously reported an incidence of 1.5% in our series (5 patients out of 324 HSCR patients with pull-through operations) [38]. All the patients had a satisfactory initial postoperative course, but developed recurrent symptoms such as abdominal distension, pain and constipation, and in some cases soiling, several months later. Carefully controlled rectal biopsies above the level of the original anastomosis in these patients indicated that the previously histologically proven ganglionic pulled-through segment had become aganglionic.

Previous studies have been criticized because of possible sampling errors whereby the biopsy may have been taken from the level of residual aganglionic bowel inadvertently or deliberately retained at the original procedure (e.g. Rehbein's procedure or the anterior rectal wall following a Duhamel procedure). Nevertheless, acquired aganglionosis has been reported following the Swenson [44, 55, 56], Duhamel [90, 122, 164] and Soave procedures [37, 41]. As in our patients, the aganglionosis in all these patients seems to have been acquired postoperatively, the pulled-through bowel being ganglionated at the time of surgery. The pathogenetic mechanism by which aganglionosis may be acquired following pull-through procedures remains uncertain, but a number of possibilities exist. These include vascular insufficiency as well as a number of other possible mechanisms.

Since the first description of this condition by Ehrenpreis in 1965 [55], vascular impairment of the pulled-through segment with consequent neuronal hypoxia has been postulated. The evidence attributing a vascular cause to HSCR still seems to be largely based on circumstantial evidence, however [103]. The fact that hyaline fibrosis was observed in certain vascular walls together with an increase in fibrous tissue in the submucosa in two of our patients [38] and in one reported by Ehrenpreis in 1965 [55, 56] would appear to support this hypothesis. On the other hand, fibrosis has not been a feature of other studies [37]. There is some experimental data supporting a vascular accident in the pathogenesis of HSCR [53], and abnormal arteries have been found in aganglionic areas and in the transitional zone of resected bowel [90, 150]. Although it is a stated view that marked regional differences in the sensitivity of the neuromuscular system to hypoxia in experiments on the large intestine of piebald mice [167] could possibly account for the divergent experimental results [37, 51, 53, 55, 164], the possibility still exists that hypoxia of the pulled-through segment could lead to degeneration or a failure of differentiation of developing or immature ganglion cells. On the other hand, in other animal experiments [50, 99], selective ischemia failed to cause aganglionosis and ganglion cells were still clearly identifiable after the hypoxic event in spite of other features of hypoxia in the mucosa and muscle. Meijers et al. [99] concluded that the induction of such ischemia at an early stage of development results in stenosis or intestinal atresia without selective loss of enteric neurons. It is also possible that other pathogenetic mechanisms such as environmental toxins play a part in acquired aganglionosis. Degeneration and destruction of colonic ganglia have been experimentally produced in animals by injection or administration of various toxins [55, 112, 169], but the hypothesis appears to lack clinical support. Acquired intestinal aganglionosis has also been reported in association with circulating immunoglobulin G class enteric neuronal antibodies in high titer [143]. This is of particular interest due to other observations of increased immunoglobulins in congenital aganglionosis [102], and raises the question as to the role played by the immune system in the pathophysiology of aganglionosis.

In addition to acquired aganglionosis following pull-through procedures, there are a number of reports of acquired aganglionosis occurring without surgery [155, 156, 163]. In all these patients the diagnosis of HSCR, although clinically suspected, was eliminated by the presence of distinctive ganglion cells on rectal biopsy. Following several months of clinical intestinal obstruction, repeat rectal biopsies revealed hypertrophic nerves and an absence of ganglion cells typical of HSCR. Touloukian and Duncan [154], reporting acquired aganglionosis in a stressed premature baby with enterocolitis, attributed it to ischemia generated by the redistribution of the capillary circulation away from the gut during a state of shock. Chow et al., reporting a patient with degenerated ganglion cells and a mononuclear infiltrate in the submucosa of the rectum at the age of 5 days and subsequent aganglionosis at 7 months [36], speculated that a viral infection, probably acquired in utero, could be the cause of HSCR in some patients. Smith et al. [143] reported two patients with enteric ganglionitis with a loss of neurons together with vacuolated nerve cells surrounded by $CD3^+$ and $CD4^+$ T lymphocytes.

We emphasize the need for repeated sequential biopsies in patients with recurrent symptoms and features of HSCR following pull-through procedures. The specific etiology and pathogenesis of this entity needs to be elucidated.

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