Intestinal Neuronal Dysplasia Type B

An Updated Review of a Problematic Diagnosis

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Intestinal neuronal dysplasia type B (IND B) is a controversial entity that is diagnosed regularly in some parts of the world and largely disregarded in others. Problematic issues that contribute to this controversy have been cited for decades, but unfortunately they remain inadequately addressed and often ignored by pathologists who render the diagnosis, clinicians who manage these patients, and investigators who publish research on this subject. In this update, we consider how the diagnosis of IND B originated and how the diagnostic criteria have evolved since their inception. We reflect upon the significant problems that prevent universal acceptance of IND B as cause of intestinal dysmotility, and we illustrate how some contemporary publications perpetuate unjustified misconceptions about IND B based on evidence that lacks sufficient scientific rigor. The focus of this review is on the histopathology of IND B, recognizing that evidence-based clinical studies are impossible without a reliable pathologic diagnosis. Finally, we discuss research findings, some relatively recent, that may be relevant to the pathogenesis of IND B, and suggest what needs to be done to establish whether IND B is a neurodevelopmental defect and, if so, how it should be managed.

HISTORY OF IND B

William Meier-Ruge is credited with providing the first description of IND B in 1971. At the time, he had pioneered acetylcholinesterase (AChE) histochemical staining of mucosal nerve fibers as a diagnostic adjunct for Hirschsprung disease (HSCR), and he reported on patients with HSCR-like clinical findings and HSCR-like patterns of AChE histochemical staining in their rectal (mucosal + submucosal) biopsies, but in whom the presence of enteric ganglion cells excluded HSCR. This observation and associated histopathologic findings in rectal biopsy specimens became the diagnostic criteria for IND B, 1 of 2 types of intestinal neuronal dysplasia. Little has been written about type A, perhaps because it is claimed to be rare and diagnosis requires glyoxylic acid–induced catecholamine fluorescence studies of frozen sections. In contrast, a 2017 PubMed search identified more than 100 papers related to type B, the subject of this update.

Intestinal neuronal dysplasia type B was described originally as a malformation of the parasympathetic submucosal plexus characterized by an HSCR-like pattern of AChE-positive innervation in the lamina propria...
increased density and size of submucosal ganglia and nerve fibers. Submucosal neural hyperplasia was defined subjectively with reference to “giant ganglia” but without formal morphometric analyses or comparative control data. In 1990, a conference was held in Frankfurt, from which Borchard, Meier-Ruge, and colleagues published a brief consensus paper in German that listed formal diagnostic histopathologic criteria for IND B (Table 1). It is important to note that the 1990 Frankfurt Consensus criteria rely entirely on frozen sections, AChE-enzymatic and lactate dehydrogenase (LDH)-enzymatic histochemistry, and a combination of subjective findings and quantitative assessments of submucosal ganglion cells. The consensus publication does not specify how many ganglia need to be assessed, how ganglion cells should be counted (eg, with or without visible nuclei), how many ganglion cells per ganglion constitute a giant ganglion, what percentage of ganglia must be giant ganglia, what source of normative data were used to develop these criteria, or whether the criteria are valid for any age, intestinal site, or procedure other than suction rectal biopsy.

To this day, many studies purport to use the 1990 Frankfurt Consensus criteria, despite the fact that Meier-Ruge has (co)authored multiple modifications since then. Modified criteria evolved gradually, primarily because of a series of papers coauthored by Meier-Ruge in the mid-1990s that clarified in detail how enzyme histochemistry should be performed and ganglion cells counted. During this period, Meier-Ruge and colleagues reported that abnormal AChE-positive innervation is an unreliable diagnostic feature after age 9 months, emphasized that submucosal ganglion cell hyperplasia was the key diagnostic feature of IND B, and commented that:

in IND B, the most characteristic finding is the occurrence of giant ganglia, containing on average seven-to-ten nerve cells, but occasionally showing up to 16 LDH-positive nerve cells. These giant ganglia comprise only 3-5% of all ganglia seen in a given case and are usually not observed in the distal rectum (within 6-7 cm of the pectinate line).

Because of the latter observation, Meier-Ruge insists that diagnostic biopsies be obtained 8 to 10 cm proximal to the dentate line, which is much farther from the anus than the typical biopsy used to rule out HSCR.

Inherent in the original description and all modified criteria is the concept of ganglion size, which refers to the number of ganglion cells in a histologic cross section of an individual submucosal ganglion. The most recent Meier-Ruge modified criteria (Table 2) are based solely on the proportion of “giant submucosal ganglia,” defined as more than 8 ganglion cells per ganglion in a 15-μm-thick frozen section that has been stained histochemically for LDH to highlight mature and immature ganglion cells. A ganglion cell is considered “any part of a ganglion cell body (with or without visible nucleus),” and if the same ganglion is sampled in multiple adjacent sections, only the section with the greatest number of ganglion cells per ganglion is included. Finally, the new criteria stipulate that IND B should not be diagnosed in a patient younger than 1 year. These modified criteria will be referred to as the 2006 Meier-Ruge criteria for the remainder of this review.

At the same time as Meier-Ruge and colleagues developed modified diagnostic criteria based solely on the percentage of giant submucosal ganglia in frozen sections stained with LDH enzyme histochemistry, Kobayashi and colleagues championed the diagnosis of IND B using hematoxylin–eosin (H&E)–stained sections and AChE histochemistry, without LDH histochemistry. In addition, they touted various other ancillary methods (eg, NADPH diaphorase histochemistry, immunohistochemistry for a host of neural antigens) as satisfactory or superior alternatives. The latter studies were confounded by the absence of a gold standard for diagnosis, in part because LDH enzyme histochemistry was not employed. Nonetheless, in a 1995 summary of their own experience, they concluded the following:

Our data show that the exclusive use of suction rectal biopsies stained by AChE is not sufficient for demonstrating heterotopic ganglia and giant ganglia in IND. In neonatal cases, it is essential to use other markers such as neural cell adhesion molecule, nerve growth factor receptor, and NADPH-diaphorase to make the diagnosis of IND.

They also recommend that patients suspected to have IND on suction rectal biopsies should have a full-thickness biopsy for detailed examination of the submucosal and myenteric plexuses. Neither the authors’ specific alternative methodologies nor their recommendation for follow-up

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**Table 1. Diagnostic Criteria for Intestinal Neuronal Dysplasia Type B**

<table>
<thead>
<tr>
<th>1990 Frankfurt Consensus&lt;sup&gt;a&lt;/sup&gt;</th>
<th>2006 Meier-Ruge&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Obligate</strong></td>
<td>A minimum of 25 submucosal ganglia must be analyzed</td>
</tr>
<tr>
<td>Hyperplastic submucosal ganglia (“giant ganglia”)</td>
<td>More than 20% of submucosal ganglia must be giant ganglia</td>
</tr>
<tr>
<td>Increased AChE-positive fibers in the adventitia around submucosal blood vessels</td>
<td>A giant ganglion contains more than 8 nerve cell cross sections</td>
</tr>
<tr>
<td>Facultative</td>
<td>Patient must be older than 1 year</td>
</tr>
<tr>
<td>Increased AChE-positive fibers in the muscularis mucosae and/or lamina propria</td>
<td>Biopsy should be at least 8 cm proximal to pectinate line</td>
</tr>
<tr>
<td>Ectopic ganglion cells in the lamina propria and/or muscularis mucosae</td>
<td>(recommendation given is 9 cm)</td>
</tr>
<tr>
<td>Biopsy should be &gt;5 cm proximal to pectinate line</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Requires LDH and AChE enzyme histochemistry, and 15-μm frozen sections.

<sup>b</sup> Requires only LDH histochemistry and 15-μm frozen sections.
full-thickness biopsy appears to have been adopted by other groups.17 However, the concept that IND B can be diagnosed without specifically using the Meier–Ruge method insinuated its way into the surgical and pathologic literature, led to ambiguity in the definition of IND B, and further confused an already controversial diagnosis.

Intestinal neuronal dysplasia type B was first described as a finding in rectal biopsies from patients who underwent biopsy to exclude HSCR. These patients were typically younger than 1 year and had clinical features suggestive of HSCR (eg, constipation, abdominal distension, bilious emesis). Intestinal neuronal dysplasia type B was conceived as a neuropathologic etiology for dysmotility1,2,4 and managed by partial colonic resection in some patients.2

Soon after the initial description, IND B was observed proximal to the aganglionic segment in some patients with HSCR and has been regarded by some physicians as a marker of functionally abnormal bowel that should be resected along with the aganglionic segment and transition zone.18 Unless otherwise specified, topics covered in this review are considered equally relevant to both IND and HSCR-associated IND B.

**ISSUES THAT HAVE HAMPERED DELINEATION OF IND B AND ITS ACCEPTANCE AS A CLINICALLY SIGNIFICANT ENTERIC NEUROPATHY**

Multiple factors stand in the way of universal acceptance of IND B as a clinically important neuropathic phenotype with functional implications for the affected portion of bowel. These issues can be grouped as in the following sections.

**Inappropriate Extrapolation of 1990 Frankfurt Consensus or 2006 Meier-Ruge Diagnostic Criteria to Sections Prepared by a Different Method**

As mentioned previously, the original and modified Meier–Ruge criteria used to diagnose IND B are based on 15-μm–thick frozen sections stained histochemically for LDH activity (+/− succinate dehydrogenase +/− AChE).20 Section thickness and staining technique are extremely important because the most consistent criterion for the diagnosis is the relative abundance of giant submucosal ganglia, which are recognized by counting ganglion cells in individual tissue sections. Given the sizes of ganglion cells and the fact that even a cross-sectioned ganglion cell cytoplasm without visible nucleus is included in counts of ganglion cells, differences in section thickness can dramatically affect the perceived percentage of giant ganglia. Similarly, histochemical staining may resolve ganglion cells that are equivocal or deemed nonneuronal with alternative stains (eg, H&E). Therefore, for definitions for giant ganglia from either the original 1990 Frankfurt Consensus criteria or any subsequent modifications to be translated into clinical practice, an identical technique must be used.

The influences of age, site, section thickness, location in the submucosa (deep versus superficial), sample size, and staining method were highlighted by Wester et al,20 who examined the size and density of submucosal ganglia in whole-mount preparations from a series of 29 pediatric cadaveric specimens. They used a combination of Cuprolinic blue (which binds to single-stranded RNA) to visualize all neurons and NADPH diaphorase histochemistry, which labels nitrergic neurons (8%–16% of total), almost all of which are situated in the deep submucosa. They found that the average number of submucosal ganglion cells per ganglion in a given patent ranged from 13.8 to 17.2 and remained stable with age. However, the average number of ganglia per unit area declined more than 3-fold during the first decade of life, most likely because of interstitial growth, and was graded with more ganglion cells in the rectum than the proximal colon. Another potential explanation for this decline is that the population of cells forming the enteric nervous system gradually decreases by apoptosis (vide infra).

Perinatal pathologists encounter abundant clusters of immature ganglion cells and their supporting glial elements in submucosal plexuses of near-term fetuses and neonates, but ganglionic cellularity appears markedly reduced in similar tissues from patients who are a few months older. Part of the apparent change in cellularity is due to age-related changes in the size and shape of ganglion cells and the amount of intervening neuropil. Even if the number of ganglion cells per ganglion remains stable with age, individual ganglion cell somata enlarge,21–23 which can influence the number of cytoplasmic profiles likely to be transected in a section of given thickness. This may be one reason for the age-related decline in submucosal ganglion cell size during infancy and early childhood.24,25 In the context of these dynamic changes in ganglionic morphology, section thickness will also affect ganglion cell counts, and therefore must be standardized.

Although it seems obvious that definitions of giant ganglia based on alternative methods should be validated independently based on appropriate controls, numerous case reports, case series, and studies of IND B violate this principle (Table 2). Others fail to explicitly state what diagnostic criteria or methods were used.

**Lack of Appropriate Controls**

The original diagnostic features of IND B, as developed by Meier–Ruge2 and endorsed at the 1990 Frankfurt Consensus conference,4 appear to have been based entirely on rectal biopsies from symptomatic patients. In none of this early work is reference made to asymptomatic controls. Instead, as pointed out in elegant critiques by Lumb and Moore in 1998,26,27 until that time the largest series of “normal” biopsies used to justify thresholds for giant ganglia appeared

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**Table 2. Varied Published Methods and Diagnostic Criteria Used to Diagnose Intestinal Neuronal Dysplasia Type B**

<table>
<thead>
<tr>
<th>Tissue Preparation</th>
<th>1990 Frankfurt Consensus</th>
<th>2006 Meier-Ruge</th>
<th>Other</th>
<th>Inadequately Specified or Entirely Subjective</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frozen sections with lactate dehydrogenase histochemistry</td>
<td>5, 7, 12, 18, 25, 42, 59, 60, 69–71</td>
<td>28, 72</td>
<td>8–10, 13, 73</td>
<td>74, 75</td>
</tr>
<tr>
<td>Paraffin section +/- immunohistochemistry</td>
<td>39</td>
<td>39</td>
<td>76</td>
<td>77–81</td>
</tr>
<tr>
<td>Other or not specified</td>
<td>15, 31, 64, 82–84</td>
<td>85, 86</td>
<td>87</td>
<td>65–67, 88–92</td>
</tr>
</tbody>
</table>

*The table excludes examples cited by Csury and Pena,34 who published a similar analysis of publications prior to 1995.*
to have been from symptomatic patients who had been deemed not to have IND B or any other diagnostic alteration. Lumb and Moore illustrate how this type of control group is highly vulnerable to selection bias, because patients with many large submucosal ganglia will receive a diagnosis of IND B and those without large submucosal ganglia will be considered “normal.” Using this approach, any variable (eg, height greater than the 95th percentile) could be used to subdivide the disease state and would consistently segregate from the remaining “normal” subset of patients.

Collecting the necessary normative data from appropriate controls is a significant challenge. Ideally, controls should be large numbers of age-, sex-, and site-matched samples collected and handled in the same manner from individuals with normal intestinal motility. Matched biopsies from a population of controls with no motility issues are impractical because IND B is most frequently diagnosed by suction rectal biopsy of distal rectum in young pediatric patients. Outside the context of excluding HSCR or surgery for anorectal rectal malformations, rectal submucosa is seldom removed from infants or young children with normal motility. One alternative is cadaveric rectal samples, acknowledging that autolysis may compromise enzymatic or immunohistochemical staining, and that readily available archival samples will be full thickness (not limited to superficial submucosa) and may not include distal rectum, the site routinely sampled to exclude HSCR but also the site where ganglion cell density declines relative to more proximal rectum.

Kobayashi et al15 collected control data from 23 patients (17 boys and 6 girls, ages 1 day to 12 years), including 4 colostomy closures for imperforate anus, 6 colon samples collected during bladder augmentation, and 13 postmortem examinations conducted less than 4 hours after death. Ten of the specimens were from rectum. Only AChE, not LDH, histochemistry was performed on 10-μm-thick frozen sections, and the presence/absence of any giant ganglia (>7 ganglion cells) was reported. Giant submucosal ganglia were observed in 2 of 23 of these controls, but none of the following information was provided: the percentage of giant ganglia (critical to formal diagnostic criteria for IND B), the location of giant ganglia in the submucosa (colostomy will contain deeper submucosal ganglia than suction rectal biopsies), and details (age, location, etc) about the 2 “affected” controls.

Prior to 1997, Meier-Ruge and colleagues8 used more than 3% to 5% giant submucosal ganglia as a diagnostic threshold for IND B. In 1997, they conducted a morphometric analysis designed to more rigorously define this threshold.12 The published results of this study contain some internal inconsistencies (eg, the methods denote giant ganglia as those with >7 ganglion cells, but their key in Figure 4 reads “>8”). The study included 20 “normal” controls (10 younger than 1.5 years, with a mean age of 13 months; 10 older than 1.5 years, with a mean age of 38 months) but observed no differences between the 2 age groups. The clinical characteristics that led to biopsy of these individuals were never provided, and the average prevalence of giant ganglia (expressed per unit length of bowel) was only reported for 10 of the 20 controls. Regardless, the conclusion from this study changed the cutoff value for IND B to more than 20% giant ganglia (>7 ganglion cells per ganglion) and a minimum of 4 giant ganglia. Support for the new threshold came from a follow-up study of rectal biopsies from 37 healthy adults, none of which exceeded 20% giant ganglia.28

Perhaps the best normative data related directly to IND B were published by Coerdt et al25 in 2004. Multiple locations were sampled from colons obtained at autopsy (postmortem intervals not specified) from 32 individuals with no clinical history of constipation or other intestinal dysfunction. Meier-Ruge’s method of sectioning and staining was used, and morphometric studies were performed to quantify, among other things, how the frequency distribution (in percent) of submucosal ganglia correlated with ganglion size (number of ganglion cells per ganglion). The controls were analyzed as mean values obtained from each of 4 different age groups, including term birth to 1 year (9 individuals) and 1 to 14 years (4 individuals). Their data suggest that the percentage of giant ganglia (>6 ganglion cells) declines with age (32.7% in preterm infants to 11.2% in adults). Limitations to the Coerdt et al study include relatively small numbers of individuals in each age group and a focus on mean values as opposed to range. Unfortunately, neither the control population from this study nor that of any other similar study is large enough to provide sufficient insight into the frequency of submucosal giant ganglia to establish reliable diagnostic norms. The development of valid diagnostic thresholds for the percentage of giant ganglia requires knowledge of the range of percentages observed in controls. Mean values may obscure wide population variation, which could conceivably include normal individuals with more than 20% giant submucosal ganglia.

Interobserver Variability

Reliable assessment of the number of ganglion cells in a histologic section of an individual submucosal ganglion is essential to IND B diagnosis. However, many studies have shown that counting ganglion cells, like many quantitative exercises in surgical pathology, is extremely vulnerable to interobserver bias.20,21,29 In 1999, a group of pathologists, which included Meier-Ruge, Coerdt, and other experienced IND B diagnosticians, examined their own interobserver variability.1 They observed that their “k values were close to the zero value expected by chance for the diagnoses normal and IND B” and concluded that “with current knowledge, rectal biopsy for diagnostic purposes should only be performed in constipated children for diagnosis of Hirschsprung’s disease.” Poor interobserver agreement is not surprising when one considers that only 1 among many sections from each ganglion is counted, boundaries of individual submucosal ganglia and individual ganglion cells are often ill-defined, even anuclear histochemically positive portions of ganglion cell cytoplasm are counted, and the total number of ganglia in a submucosal biopsy may be low, such that differences in interpretation of only 1 or 2 giant ganglia may determine whether the 20% threshold is met.

Changes in Diagnostic Criteria

As discussed above (see History of IND B), the contemporary diagnostic criteria for IND B are far more conservative than the original Frankfurt Consensus criteria. Some of the most obvious differences include (1) elimination of an increased number of AChE-positive nerve fibers around submucosal blood vessels as an obligate diagnostic criterion, (2) stipulation that a giant ganglion contains more than 8 ganglion cells, (3) the requirement that more than 20% of at least 25 ganglia be giant ganglia, and (4) diagnostic exclusion of patients younger than a year. In
the interval between the original and contemporary versions, many alternative schemes existed, as summarized elsewhere (see Table 2 in Kapur30). Existence of these conflicting criteria confounds the literature relevant to this field. For example, in 2017, investigators from Brazil proposed a long-term follow up study of patients with IND B31 that would rely on the 1990 Frankfurt Consensus criteria.

A NEED FOR EVIDENCE-BASED PRACTICE

Each of these significant issues related to the diagnosis of IND B has been recognized by others, including diagnosticians who consider IND B an established clinical-pathologic entity14,32 and skeptics who regard IND B as a questionable histopathologic phenotype with uncertain clinical significance.26,33,34 This difference of opinion is responsible for dramatically different rates of IND B diagnosis,35 which range from 0.3% (7 of 2420)36 to 62% (45 of 115)37 of patients evaluated by rectal biopsy. The highest rates are reported from relatively few locations, particularly parts of Europe and Japan, whereas IND B is seldom diagnosed in North America, England, or many other parts of the world. To a large degree, these geographic trends appear to correlate with the practices of a relatively small number of influential diagnosticians, including Meier-Ruge (Switzerland/Austria/Germany), Prem Puri (Dublin), and their trainees. Interested readers are encouraged strongly to consult publications written by these individuals to gauge their response to concerns about IND B.14,32 The 1998 review by Puri and Wester19 is an interesting example. In this paper, the authors acknowledge many of the same issues discussed above, provide no evidence-based rebuttal for most of them, and yet conclude “the new staining techniques have proven that IND is a distinct histopathologic disorder.” In our opinion, although alterations in the submucosal plexus captured by some of the diagnostic rubrics for IND B may represent significant deviations from the norm, the problems that plague diagnosis of IND B have not been addressed sufficiently to justify acceptance as a neuropathologic cause of dysmotility. The latter opinion was shared by an International Working Group on Gastrointestinal Neuromuscular Pathology (Meier-Ruge was a member), which classified IND B as morphologic finding that “may or may not be causally related to other observed clinical entities.”38

Shortcomings in the diagnostic criteria for IND B have had a catastrophic effect on the quality of published research in this field, and have added to the confusion and potential mismanagement of patients who receive the diagnosis. Examination of “A Critical Appraisal of the Morphological Criteria for Diagnosing Intestinal Neuronal Dysplasia Type B” by Terra et al39 illustrates how these difficulties are perpetuated. The explicit aim of the study was to “analyze the morphological characteristics in a series of cases previously diagnosed as intestinal neuronal dysplasia type B by the 1990 Frankfurt Consensus” and verify the “applicability of the numerical criteria proposed by Meier-Ruge et al in 2004 and 2006.” The investigation used 29 endorectal pull-through resections from patients who received a previous diagnosis of IND B based on the 1990 Frankfurt Consensus. Lactate dehydrogenase histochemistry is neither described nor reported, and it is unclear whether the initial diagnoses were established using this approach, as opposed to extrapolation of the 1990 Frankfurt Consen-
from controls and patients were performed by the same observer and that under these circumstances IND B-like submucosal ganglion cell hyperplasia is present in the ganglionic bowel of some patients with HSCR. This investigation, using a different method to examine submucosal ganglion cell hyperplasia, provides support for the existence of HSCR-associated IND B. However, the study was not designed appropriately for us to understand either the pathogenesis or clinical significance of this pattern of ganglion cell hyperplasia.

**PATHOGENESIS OF IND B**

One interpretation offered for the pathologic features of IND B is that they are acquired secondary to other processes that compromise bowel motility. In HSCR, for example, obstructive physiology in the aganglionic segment is associated with upstream distension, inflammation, and smooth muscle hypertrophy, and could conceivably promote submucosal ganglion cell hyperplasia. Observational and experimental data regarding IND B-like pathology proximal to other obstructive lesions have been inconsistent. In humans, IND-like pathology has been reported in association with strictures due to necrotizing enterocolitis or Crohn disease, congenital intestinal atresia, volvulus, anorectal malformations, and intussusception. However, experimental bowel stenosis in rats, aces, and guinea pigs did not produce a similar response. Kobayashi et al speculate that inflammation, as opposed to distension alone, may be required to induce submucosal neuronal hyperplasia. Response to injury could also explain the IND-like hyperplasia of submucosal ganglia observed in intestinal allografts and fibrosing colonopathy.

Inherent in models of acquired IND B is the hypothesis that enteric neural progenitors persist after ganglia are formed and can be recruited later to form submucosal ganglion cells. During embryologic development, submucosal ganglion cells form from the descendants of progenitors located in the myenteric plexus, which migrate centrifugally toward the bowel lumen. The existence of enteric neural stem cells in the postnatal or adult gut was once considered heretical but is now well documented, at least in animal models. Most of the basic research has focused on myenteric ganglia and murine models. According to a recently published study, enteric neurons undergo postnatal homeostatic apoptosis and replacement by stem cells, although this finding is controversial. Stimuli shown to drive postnatal enteric neurogenesis include tissue injury, whereas neurogenesis in response to obstruction/distension alone has not been documented.

Alternative to the secondary submucosal ganglion cell hyperplasia is the hypothesis that IND B represents a primary malformation, possibly influenced by molecular factors known to be associated with normal enteric neurodevelopment. Some support for this suggestion comes from 2 putative genetic murine models for IND B, Ednrb-mutant rats and Hox11L1-mutant mice. Ednrb mutations in rodents and other species model HSCR with distal aganglionosis. In rats, the sl allele is a 301-bp deletion in Ednrb, which causes long-segment congenital aganglionosis in sl/sl homozygotes; sl/+ heterozygotes do not have aganglionosis. Von Boyen et al studied the ganglionic bowel in 2-week-old sl/sl pups and their 4-week-old sl/+ and +/- littermates. The mean number of ganglion cells per submucosal ganglion in the small intestines of sl/+ rats was more than double that of +/- controls. Myenteric ganglia were not affected, and, surprisingly, hyperganglioniosis was not present in the ganglionic bowel of sl/sl animals. Ednrb encodes an endothelin receptor that regulates enteric neuroprogenitor proliferation during embryogenesis. The IND-like phenotype of sl/+ rats is presumed to represent a primary malformation because the animals are otherwise healthy, have no intestinal distension, and exhibit normal life spans. Hox11L1 is a transcription factor expressed in enteric neurons, and Hox11L1+/− mice demonstrate signs of lethal intestinal pseudo-obstruction. Initial descriptions indicated that myenteric ganglion cell hyperplasia (submucosal ganglia were not studied) was part of the phenotype, although this was not confirmed by another group. Searches for mutations in ENDRB, HOX11L1, or other genes implicated in HSCR (eg, RET) mutations in humans with IND B yielded negative results.

Unequivocal submucosal ganglion cell hyperplasia (ganglioneuromatosis) is often encountered in humans with hamartomatous genetic disorders, such as multiple endocrine neoplasia type 2B (MEN 2B), neurofibromatosis type 1, and PTEN-hamartoma syndromes. In each of these conditions, the hyperplasia of neuroglial elements in the enteric nervous system typically involves not only the submucosa, but also the myenteric plexus and lamina propria. The severity of neural hyperplasia is usually much more dramatic than in IND B and may form grossly obvious tumors. The onset of hyperplasia in these syndromes is difficult to determine, but significant growth of the lesions can occur postnatally, even in adulthood, which suggests an "acquired" neurogenic component. The genetic alterations that underlie each of these conditions affect some of the same molecular pathways that are involved in normal enteric neurodevelopment. By analogy, the same pathways may be involved in the pathogenesis of IND B, and their investigation may improve our ability to unravel the pathogenesis of the IND B phenotype.

**CLINICAL IMPLICATIONS OF IND B**

Difficulties with the diagnosis of IND B have hampered clinical characterization and vice versa. Assuming that at least 1 diagnostic feature of IND B represents more than part of the spectrum of normal developmental enteric neuroanatomy, it remains to be established whether the histopathologic phenotype is a cause or an effect of intestinal dysmotility. As discussed above, selection bias (only patients with symptoms undergo biopsies) could lead to the misconception that IND B is the basis for the clinical findings. Unfortunately, the clinical findings of IND B patients are not particularly distinctive. They overlap with those of HSCR, functional constipation, and many other disorders characterized primarily by significant constipation, bowel distension, etc. Patients, particularly infants, often have spontaneous resolution of their symptoms with conservative medical management.

A few studies have evaluated retrospectively the clinical outcomes of patients receiving a diagnosis of IND B. Although the diagnostic criteria used in each of these investigations vary, conclusions regarding management are generally relatively uniform. Conservative medical therapy with laxatives or other measures is used and surgery is avoided unless more aggressive treatment is unavoidable. Anal sphincter myectomy appears to be helpful, if not
curative, in some patients; only rare patients require partial or complete colectomy.

Gillick et al44 reported follow-up data from 33 patients who received a diagnosis of IND B based on a modified set of criteria that included an unspecified frequency of giant submucosal ganglia (>7 ganglion cells per ganglion) and at least one of the following: (1) ectopic ganglion cells in the lamina propria, (2) increased AChE activity in the lamina propria, or (3) increased AChE activity around submucosal “plexus” (presumably “vessels”). Nearly half of the patients were younger than 1 year at diagnosis, and follow-up periods ranged from 1 to 8 years. A total of 64% (21 of 33) of patients responded to “conservative treatment” and at follow-up required no or only intermittent laxatives. After failed conservative management, the remaining 12 (36%) underwent internal sphincter myectomy, with a good response (normal bowel habits and continence) in 7.

In 2014, Taguchi et al39 reported survey results from 161 institutions in Japan and identified 18 cases of IND B (diagnosed between 2001 and 2010), 13 of which were believed to satisfy the “original diagnostic criteria” (which appear to be from the 1990 Frankfurt Consensus, with AChE histochemistry but not LDH histochemistry). Only 4 of these cases met the 2006 Meier-Ruge criteria. One from the latter group was successfully treated with enemas and laxatives. The other 3 patients went on to bowel resection and pull-through, 1 patient doing so after sphincterotomy was ineffective. Long-term follow-up of the surgical patients indicated that 2 patients still required enemas or suppositories and the third required a permanent colostomy. Hence, a long-term benefit of surgery in this small series was not apparent.

Given the diagnostic and prognostic challenges posed by IND B, it seems imprudent to base management decisions on the presence or absence of IND B–like pathology. Nonetheless, colonic resection for IND B appears to be a frequent practice at 1 institution, where at least 29 pediatric patients underwent surgery to treat isolated IND B.39 An algorithm that has been published from a different center suggests that before a pull-through procedure is completed, patients with HSCR should undergo serial biopsies of their ganglionic colon to exclude IND B.66,67 We believe that insufficient evidence exists to support either of these practices and we also believe that, despite the fact that it has been nearly 5 decades since IND B was first described, it is still best regarded as an inconsistently defined histopathologic phenotype with uncertain clinical relevance.

**FUTURE DIRECTIONS**

In some respects, IND-B can be regarded as a phenotype of relative enteric neural immaturity that may only be recognized with confidence after age 1 year, often disappears spontaneously by age 4 years, and is not by itself an indication for corrective surgery. However, IND B will remain a controversial diagnosis until rigorous, well-controlled scientific studies are conducted to establish reproducible and reliable diagnostic criteria that translate easily from one laboratory to another. If submucosal ganglion cell hyperplasia is the principal anatomic feature, diagnosis should not depend on enzyme histochemistry and frozen sections, but should be possible with conventional formalin-fixed, paraffin-embedded tissues. This would allow for the examination of large numbers of archival cadaveric or other control specimens, including from different ages and intestinal locations, so that valid upper limits of normal can be established. The recent introduction of choline transporter immunohistochemistry as a formalin-fixed, paraffin-embedded compatible surrogate for AChE enzyme histochemistry68 may facilitate studies of this subject. With good norms and reproducible methodology, meaningful studies can be designed to address the frequency and clinical significance of IND B–like histology. Such efforts will likely require a multi-institutional research effort. Unless simpler, possibly automated, means of assessing submucosal ganglion cell hyperplasia are developed, the morphometric analyses required to reliably quantify giant submucosal ganglion cells will be limited to specialized reference laboratories. However, if compelling data arise to demonstrate that IND B is an important diagnosis with actionable implications, simpler diagnostic methods and universal acceptance are likely to follow. Until then, we encourage pathologists and other caregivers to regard IND B with healthy skepticism.

**References**


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