**Table 1** Classification of ADHD in Japanese Survey

| |
|---|
| 1. Abnormal ganglia (Abnormal histology in HE or AchE staining) |
| Immaturity of ganglia (or Immature ganglionosis: IG) |
| Hypoganglionosis (or Oligoganglionosis : HG) |
| Congenital Hypoganglionosis (or Hypogenesis, Hypoplasia) |
| Acquired Hypoganglionosis |
| Intestinal Neuronal Dysplasia (IND) |
| 2. Normal ganglia (Normal histology in HE or AchE staining) |
| Megacystis microcolon intestinal hypoperistalsis syndrome (MMIHS) |
| Segmental dilatation of intestine (SD) |
| Internal Anal Sphincter Achalasia (IASA) |
| Chronic Idiopathic Intestinal Pseudo-Obstruction (CIIP) |

with abnormalities of ganglion cells and those without abnormalities of ganglion cells. We sympathize the Puri's classification based on pathological findings with or without abnormalities of ganglion cells. Two categories were shown in Table 1: abnormal ganglia, including immaturity of ganglia (IG), hypoganglionosis (congenital and acquired), and intestinal neural dysplasia (IND), and normal ganglia, including megacystis microcolon intestinal hypoperistalsis syndrome (MMIHS), segmental dilatation of intestine (SD), internal anal sphincter achalasia (IASA), and chronic idiopathic intestinal pseudo-obstruction (CIIP).

Okamoto and his colleagues featured ADHD as the main theme and reported the results from nationwide survey at the 24th Congress of Japanese Association of Pediatric Surgeons in 1987 [5]. In these reports, IG was reported that the disease was characterized as normal number and extremely immaturity of ganglion cell in intestinal wall. Since then, more detailed survey was performed by "Clinicopathological studies on the diagnosis, treatment, and pathogenesis of pseudo-Hirschsprung's disease and related disorders" founded by Japan Society for the Promotion of Science from 1991 to 1993. In the reports of this study, IG was reported as follows: meconium ileus without mucoviscidosis, meconium disease-like findings on laparotomy, small and extreme immaturity in both nucleus and cell in ganglion cell of not only narrow small intestine but also enlarged small intestine, maturation of ganglion cells several months after ileostomy with recovering bowel function, and normal function with good prognosis. From another side, pathogenesis of meconium ileus without mucoviscidosis was based on meconium ileus without mucoviscidosis. "Immature ganglionosis" was proposed as the name for this disease group. In conclusion of this study, "Immature ganglionosis" presented the pathological immaturity of fetal period under 5–6 months [6, 7]. After this study, Taguchi et al. reported that the immature ganglion cells matured with time [8]. So prognosis of this disease was so favorable, and the comprehensive survey was not made after that. The assumed representative clinical features of the immaturity of ganglia (IG) were as follows: (1) neonatal onset of ileus symptom, (2) microcolon–small colon findings in contrast enema, (3) negative anorectal reflex in manometry in neonatal period changing normally in infant period, (4) meconium disease-like finding in laparotomy, (5) lesion extending to small intestine, and (6) bowel function recovering on time. Our group performed nationwide survey to clarify the current clinical features in diagnosis and treatment of ADHD in Japan. From this nationwide survey, another ADHD such as hypoganglionosis, IND, and CIIP has already been reported [9–12]. The aim of this study is to clarify the current clinical features in diagnosis and treatment for immaturity of ganglia in Japan.

## Materials and methods

We performed a retrospective cohort study as a two-step nationwide survey. As the first-step survey, preliminary questionnaires, requesting the number of cases of all ADHD (IG, Hypoganglionosis, IASA, CIIP, SD, MMIHS, and IND) seen from January 2001 to December 2010 and the criteria used at each institute to detect the number of all ADHD patients, were sent to the 161 major institutes of pediatric surgery or pediatric gastroenterology, representing the core members of the Japanese Society of Pediatric Surgeons, the Japanese Society of Pediatric Nutrition, Gastroenterology and Hepatology, and the Japanese Study Group of Pediatric Constipation. Therefore, almost all institutes which were treating ADHD were considered to be included. The number of patients, including the definite and the suspected cases, based on the tentative classification of ADHD, was requested. We also asked about the criteria used to diagnose these diseases by each institute, to be answered as free descriptions. The criteria for "definitive" or "suspected" depended on each institute. Assumed pathological diagnostic criteria are as follows: normal number and distribution of ganglion cells and small size of ganglion cell in HE staining.

As the second-step survey, a case report form with a questionnaire for each case was sent and collected as part of a detailed survey. Information on patient background, clinical features, examination findings, drug treatments, and surgical treatments were obtained (Table 2). Survey responses were compiled into a database. As a result, specific independent pathophysiology of IG different from another ADHD was introduced from this survey. This study was performed according to the Ethical Guidelines for Clinical Research published by the Ministry of Health, Labor and Welfare of Japan on July 30, 2003. This study was approved by the ethics committee for clinical research of Kyushu University Hospital (No. 24-163).

**Table 2** Main questionnaires in the nationwide survey on Immaturity of Ganglia in Japan

Day of birth_____(yyyy/mm/dd) Gender □□ Male □□ Female

Gestation _____w_____d_____ Birth weight _____g

Onset □Neonatal(<30d) □Infant (1to12m) □Childhood(<30d) □School age or later

Familial incidence □ Present ( ) □ Absesnt

Initial symptom □ Abdominal distention □ Vomiting □ Abdominal pain
□ Delayed meconium excretion (>24h after birth) □ Chronic constipation
□ Enteritis □ Diarrhea □ Megacyst
□ Prenatal digagnosed abnormalrity (if any ) □ Others (free statement )

Affected lesion □ Stomach □ Duodenum □ Jejunum
□ Ileum □ Appendix □ Cecum
□ Ascending colon □ Transverse colon □ Descending colon □ Sigmoid colon □ Rectum □ Anus

Associated malformation □ Present ( )

Examination

Abdominal X ray (multiple answer allowed)

□ Dilatation □ Niveau □ Free air □ Others

Contrast enema (multiple answer allowed)

□ Normal □ Microcolon □ Megacolon
□ Caliber change □ Unknown □ Others

Anorectal reflex

□ Positive □ Atypically Positive □ Negative

Rectal Suction Biopsy (AChE staining)

□ Normal □ Proliferation of AChE-positive fiber
□ Giant ganglia □ Ectopic ganglia

Laparotomy findings □ Intestinal dilatation □ Caliber change □ Microcolon □ Others

Surgical Procedure

Enterostomy position □ Stomach □ Duodenum □ Jejunum □ Ileum □ Appendix □ Cecum □ Ascending colon
□ Transverse colon □ Descending colon □ Sigmoid colon

Enterostomy type □ Double barel □ Bishop-koop □ Santulli □ tube

Intestinal resection □ Performed □ not performed □ unknown

Re-enterostomy □ Performed □ not performed □ unknown

Closure of enterostomy □ Performed □ not performed □ unknown

Intraoperative rapid pathological diagnosis

□ Normal □ Abnormal (findings )

Final pathological findings from example or biopsy specimine

□ Normal □ Abnormal (findings )

Current nutritional management (multiple answer allowed)

□ Usual diet □ Semidigested diet □ Fomula diet □ Paraenteral nutrition

Clinical outcome □ Alive □ Died (cause of death : )

## Results

As a result of the first-step survey, responses were obtained from 157 out of the 161 institutes (98 %). Ninety-five institutes (61 %) had treated a total of 355 ADHD patients including IG, hypoganglionosis, IND, IASA, SD, CIIP, and MMIHS. These included 28 IG (7.9 %) patients. The second-step survey was performed for 355 ADHD patients. Out of 28 case report forms sent as part of the second-step survey, all 28 IG cases were subsequently collected. These 28 cases included "definitive" or "suspected." Thirteen of 28 cases were excluded because there was no histological evidence for IG at each institute. Finally, 15 cases of "definitive" IG were included in this article. Fifteen definitive cases were diagnosed by pathological examination with hematoxylin–eosin (HE) staining by pathologist of each institute.

### General features

Male–female ratio was 9:6, the mean birth weight for patients with IG was 2473 g, and the mean gestational age was 36 weeks and 3 days. Patients of IG with a positive family history was four (26.7 %), two in other twin baby and two in cousin. The incidence of associated anomalies was 1 (6.7 %) case of mesenteric hernia. No chromosomal anomaly was recognized. No genetic examination was performed.

### Imaging and examination findings

Onset of all 15 patients is during the neonatal period. For diagnosis, abdominal X-ray was performed in 100 %. Abnormal intestinal distention was recognized in 13 cases (86.7 %), niveau in two cases (13.3 %), and free air in two cases (13.3 %). Contrast enema was performed in 12 cases (80.0 %). Microcolon was recognized in seven cases (58.3 %) and caliber change in three cases (25 %). Anorectal manometry was performed in eight cases (53.3 %). Positive anorectal reflex was recognized in four cases (50 %), negative in two cases (25 %), and atypical positive in 2 cases (25 %) (Table 3).

*Operative findings and surgical procedures*

Laparotomy was performed in 13 patents (86.7 %). Abnormal intestinal dilatation is recognized in eight patients (61.5 %), caliber change in eight patients (61.5 %), and microcolon in five patients (38.5 %). An enterostomy was performed in 13 patients (86.7 %), an ileostomy in 9 (69.2 %) patients, a jejunostomy in two patients (15.4 %), and a colostomy in two patients (15.4 %). Type of enterostomy is double barrel in 11 (84.6 %), Bishop-koop in 1 patient (7.7 %), and tube enterostomy in 1 (7.7 %) patient. Resection of intestine was performed in 4 patients (30.8 %). Re-enterostomy was performed in 4 patients (30.8 %). Closure of enterostomy was performed in 13 patients (100 % out of enterostomy patients) (Table 4).

**Table 3** Imaging and examination findings

| | |
|---|---|
| Abdominal X-P | |
| 15 Cases (100 %) | |
| Abnormal intestinal distention | 13 (86.7 %) |
| Niveau | 2 (13.3 %) |
| Free air | 2 (13.3 %) |
| Contrast enema | |
| 12 Cases (80 %) | |
| Microcolon | 7 (58.3 %) |
| Caliber change | 3 (25 %) |
| Normal | 1 (8.3 %) |
| Unknown | 1 (8.3 %) |
| Anorectal manometry | |
| 8 Cases (53.3 %) | |
| Anorectal reflex | |
| Positive | 4 (50 %) |
| Atypical positive | 2 (25 %) |
| Negative | 2 (25 %) |

**Table 4** Operative findings and surgical procedures

| | |
|---|---|
| Laparotomy findings | |
| Abnormal distension on intestine | 8 (61.5 %) |
| Caliber change | 8 (61.5 %) |
| Microcolon | 5 (38.5 %) |
| Surgical procedure type | |
| Enterostomy | 13 (86.7 %) |
| Double barrel | 11 (84.6 %) |
| Bishop-koop | 1 (7.7 %) |
| Tube enterostomy | 1 (7.7 %) |
| Position | |
| Jejunum | 2 (15.4 %) |
| Ileum | 9 (69.2 %) |
| Cecum | 1 (7.7 %) |
| Transverse colon | 1 (7.7 %) |
| Intestinal resection | 4 (30.8 %) |
| Re-enterostomy | 4 (30.8 %) |
| Closure of enterostomy | 13 (100 %)[a] |

[a] Out of 13 enterostomy patients

### Histological examinations and findings

AchE staining was performed in five cases (33.3 %). The staining was normal in three cases (60 %), whereas it

**Table 5** Histological examinations and findings

| | |
|---|---|
| AchE staining | 5 (33.3 %) |
| Normal | 3 (60 %) |
| Increased AchE fibers | 2 (40 %) |
| Intraoperative rapid pathological diagnosis | 6 (40 %) |
| Normal | 2 (33.3 %) |
| Abnormal | 4 (66.7 %) |
| Hematoxylin-eosin (HE) staining | 15 (100 %) |
| Immature ganglion cells | 15 (100 %) |
| Maturation of ganglion cell (specimen from closure of enterostomy) | 4 (26.7 %) |

showed increased AchE fibers in two cases (40 %). Intraoperative rapid pathological diagnosis was performed in six patients (40 %). No abnormality was recognized in two patients (33.3 %). Abnormality in the intestinal ganglion cell was recognized in four patients (66.7 %). HE staining was applied for all 15 patients. The sampling specimen was obtained during enterostomy, laparoscopic biopsy, and rectal full-thickness biopsy. Abnormality was recognized in 15 cases (100 %), of which all the 15 patients (100 %) showed immature ganglion cells in HE staining. Maturation of ganglia was confirmed in four cases (26.7 %) with the specimen from closure of enterostomy (Table 5).

### Other treatments

Probiotics was applied in ten patients (66.7 %), Chinese herbal medicine (Daikenchu-to and Rikkushi-to) in eight patients (53.3 %), gastrointestinal prokinetic in seven patients (46.7 %), and laxative in six patients (40 %). Catheter-related sepsis was recognized in two patients 13.3 %).

*Prognosis and diet*

All the 15 patients survived. Thirteen patients (86.7 %) were treated with an ordinary diet, one patient (6.7 %) with the combination of ordinary diet and elemental diet, and one patient (6.7 %) with the combination of ordinary diet and parenteral nutrition.

## Discussion

IG should be the dependent entity of neonatal non-mechanical functional ileus and included ADHD. From this nationwide survey, we elicited that IG has the specific independent pathophysiology different from another ADHD. In this article, we concluded the clinical features of IG based on definitive pathological diagnosis. Five clinical features of IG such as neonatal onset of ileus, lesion extent to small bowel involvement, symptom improvement, microcolon or narrowing of left colon, and caliber change on laparotomy are candidates for the clinical diagnostic criteria. Favorable prognosis is compatible with previous reports of Okamoto et al. and Taguchi et al. [7, 8].

Pathologists of this ADHD group proposed the pathological criteria with HE staining: (a) immaturity of ganglion cell (small size of ganglion cell) and (b) normal number and distribution. Combination of (a) and (b) should to be necessary for the pathological diagnosis of IG.

Burki et al. reported that immature ganglion cells on rectal biopsy might be an indicator of transient functional immaturity of the intestine [13]. These series were all onset in the neonatal period; almost all cases were treated conservatively after diagnosis of immaturity of ganglia and only two patients underwent enterostomy. They confirmed the maturation of ganglion cells by repeated rectal biopsy. But we think that full-thickness histopathological examination is necessary for the diagnosis of immature and mature Auerbach plexus.

One of the pathological hypotheses is that the maturation process of immature ganglion cells involves both maturation of individual ganglion cells and their growth in size. On the other hand, sample tissue from the colon of IG showed that size of ganglion was often normal size because of more immature cells comparing with normal ganglion. This normal size of ganglion including more immature cells might be due to the mature selection of the immature cells leading to normal number of cells. The important agenda is to find the antibody staining for maturation of ganglion cell. For example, immunohistochemical staining has been reported to be useful for the pathological diagnosis of IG, using neuronal and muscular markers, such as bcl2 for immature neurons. The diagnosis of immaturity was easily made in one case using bcl2 immunostaining [14]. In addition, HuC/D is also one of the candidates for the maturation of neurons. HuC/D localization is not entirely clear and needs to be further investigated [15].

On the other hand, we are examining the method of visualization for ganglion cell maturation. Using confocal laser endomicroscopy (CLE), we expect to establish the rapid and accurate intraoperative alternative method for the detection of enteric ganglia. CLE is a new established endoscopic method providing in vivo histology at subcellular resolution during ongoing endoscopy [16]. CLE could not detect the size of ganglion cell, but detect the number of nucleus of ganglion cells in intestinal wall in the current technical state. In near future, this new imaging approach could be of tremendous diagnostic importance to characterize and better understand the functional and motility disorders of gastrointestinal tract including IG. Cine-MRI provided sufficient dynamic images to assess the motility of the entire small bowel [17]. Cine-MRI is non-invasive

and radiation free, and it can directly evaluate the entire small bowel peristalsis and detect the affected loops at a glance [16]; therefore, it might be extremely useful for the diagnosis and follow-up of IG patients in clinical practice. Using this non-invasive diagnostic modality, both the process of maturation of ganglion cell and improving dysmotility of intestine would be elucidated in in vivo imaging.

In conclusion, during a 10-year period in Japan, 15 cases of IG were definitively diagnosed based on pathological findings, and almost all cases underwent surgical procedures with enterostomy. All cases had favorable prognosis. IG is an extremely rare disease. The number of incidence of IG is estimated to be about 1–2 patients of one million live births in Japan. But IG is an independent disease which has different clinical features in ADHD.

**Acknowledgments** This study was supported by a grant from The Ministry of Health, Labor Sciences Research Grants for Research on intractable disease. The authors thank The Japanese Society of Pediatric Surgeons, The Japanese Society of Pediatric Nutrition, Gastroenterology and Nutrition, and The Japanese Study Group of Pediatric Constipation. They also thank Dr. Brian Quinn for reading the manuscript, and Ms. Masutomi and Ms. Yamazaki of Department of Pediatric Surgery, Kyushu University, for their help in processing the data.

**Compliance with ethical standards**

**Conflict of interest** No competing financial interests exist.

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# Reevaluation of Acetylcholinesterase Staining for the Diagnosis of Hirschsprung Disease and Allied Disorders

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## ABSTRACT

**Objectives:** Acetylcholinesterase (AChE) staining has become the gold standard for definitively diagnosing Hirschsprung disease (HD), although some pitfalls have been reported. We reevaluated a large series at our institute in order to validate the accuracy of AChE staining for detecting HD.

**Methods:** A retrospective study of the rectal mucosal specimens of all of the children with suspected HD during a 13-year period was performed. The specimens were stained according to the modified Karnovsky-Roots method for AChE staining. The final diagnosis, prognosis, and management after the histopathological diagnosis were analyzed with a questionnaire sent to the patient's original hospital.

**Results:** Three hundred and fifty-eight specimens were collected. One hundred twenty-two (34%) specimens were diagnosed as HD, 198 (55%) as nonHD, 25 (7%) as "undetermined," and 13 (4%) as "inappropriate." The non-HD group contained 190 (96%) specimens with a normal appearance and 8 (4%) specimens with suspected intestinal neuronal dysplasia (IND). Three hundred and six of 358 questionnaires were returned. The final diagnosis showed that no specimens first diagnosed as HD were identified as non-HD and vice versa, for a sensitivity and specificity of 100%. Four cases were finally diagnosed as chronic idiopathic intestinal pseudo-obstruction (CIIP) in the non-HD group. All of the patients with HD underwent radical surgery. Most non-HD patients were managed conservatively, although some continued to have constipation.

**Conclusions:** AChE staining is an accurate tool for differentiating between HD and non-HD with high sensitivity and specificity. CIIP can be included in cases of non-HD; therefore, careful follow-up is mandatory.

**Key Words:** acetylcholinesterase staining, chronic idiopathic intestinal pseudo-obstruction, Hirschsprung disease, intestinal neuronal dysplasia, rectal mucosal biopsy

(*JPGN* 2015;60: 606–612)

**What Is Known**

- Acetylcholinesterase (AChE) staining is the most reliable histopathological method for the diagnosis of Hirschsprung disease (HD).
- Otherwise, false-positive and false-negative results have been reported.

**What Is New**

- We validated the availability of AChE staining from our experience by improving our protocol.
- As a result, AChE staining could diagnose HD with high sensitivity and specificity, and could diagnose "suspected" intestinal neuronal dysplasia.
- A few cases of chronic idiopathic intestinal pseudo-obstruction were included among the group that showed normal findings of AChE staining.
- Careful follow-up is mandatory in cases with normal AChE findings.

Constipation is a common condition in children, with an incidence of 3% to 5% among all of the visits to pediatric outpatient clinics (1–3). This condition is identified based on the timing and/or substance of stool, in which the child fails to defecate for several days or experiences hard, large, or painful stools upon defecation. The cause of constipation may be functional or organic (3). Functional constipation can be caused by psychogenic factors, dietary imbalances, dehydration, and other issues, and is common among healthy children (3). It is usually self-limiting, although it may become chronic if inappropriately managed (3). It can be easily treated with noninvasive therapies, such as dietary changes and regular evacuation of the rectum (1–3). Organic constipation includes metabolic and endocrine abnormalities, such as hypothyroidism, diabetes, cystic fibrosis, electrolyte imbalances, neuroanatomical disorders, spinal defects, anatomical defects, including anorectal malformation, and Hirschsprung disease (HD) and its variants (3). Anatomical defects and HD or its variants require surgical management. Because the symptoms of HD are similar to those of functional constipation, it is important to differentiate HD from other causes of constipation in order to avoid mismanagement. Most patients with HD can lead a normal life with proper surgical management if diagnosed accurately (4–7).

HD is one of the most common types of organic constipation caused by aganglionosis. The incidence of HD is 1 in every 5000 newborns (6–9), and such patients present with abdominal distention, bilious vomiting, and delayed passing of meconium (6,7). Aganglionosis of the gastrointestinal tract, located mostly in the

Received September 16, 2014; accepted December 3, 2014.
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The study was partly supported by the Ministry of Health, Labour Sciences Research Grants for Research on Intractable Disease.
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The authors report no conflicts of interest.

DOI: 10.1097/MPG.0000000000000664

rectosigmoid colon extending throughout the entire colon, termed total colonic aganglionosis, even extends to the small intestine. The diagnosis is made using radiological studies and anorectal manometry (6,7) and confirmed based on histological and histochemical examinations with acetylcholinesterase (AChE) staining of rectal mucosal biopsy samples (10).

Since the report by Meier-Ruge et al (10), AChE staining has become the gold standard for diagnosing HD. The diagnosis is made based on findings of the combination of an absence of submucosal ganglion cells (Meissner plexus) and an increased number of AChE-positive nerve fibers in the muscularis mucosae and lamina propria mucosae (10). An increased AChE activity is associated with the presence of extrinsic hypertrophied nerve bundles in the submucosa. Although a high degree of histochemical diagnostic accuracy can be obtained, false-positive and false-negative results have been reported (10–12). False-positive findings are rare and occur because the red blood cell membrane contains a high concentration of AChE, which may increase the rate of false-positive reactions in hemorrhagic specimens. In contrast, false-negative results are usually related to age and the absence of AChE reactions in neonates and infants within the first 3 weeks after birth. The intensity of AChE staining increases with increasing age, and the majority of patients >1 year of age demonstrate the classical AChE staining pattern associated with HD (10).

The advantages of AChE staining of rectal mucosal biopsy samples include the simplicity of the technique, the ability to obtain rapid results, the lack of need for anesthesia, easily interpreted findings, and positive results of an increased AChE activity in the lamina propria mucosa, which cannot be found in the normal mucosa (10–12).

Allied disorders of HD (13), sometimes called variant HD (14,15) or pseudo-HD (16–18), clinically resemble HD, despite the presence of ganglion cells in rectal biopsies (14–18).

Intestinal neuronal dysplasia (IND) is a representative variant of HD (14,15), with an incidence of approximately 1 in 7500 newborns and 0.3% to 40% of all of the rectal suction biopsies worldwide. IND is diagnosed based on AChE staining of rectal mucosal biopsy samples (14,15). The typical findings of IND include an increased AChE activity in the lamina propria and the presence of giant ganglia in the submucosa (19–21).

Chronic idiopathic intestinal pseudo-obstruction (CIIP) has been reported to be a form of pseudo-HD (17,18). Children with CIIP show persistent functional intestinal obstruction, despite the presence of ganglion cells with a normal size and number (22–24).

The aim of this study was to assess the accuracy of AChE staining for the diagnosis of HD and allied disorders in constipated children, and to evaluate the outcomes of non-HD patients diagnosed using AChE staining.

## METHODS

A retrospective study of the rectal mucosal biopsied specimens of children with constipation or intestinal obstruction suspected to be because of HD, collected at Kyushu University Hospital and its branch hospitals between January 2000 and December 2012, was performed.

The mucosal biopsies were conducted at each hospital using the punch biopsy method (25). All of the specimens were snap frozen, sent to our department via a special cool delivery service, and stored in a deep freezer until the examination. The specimens were subsequently stained with AChE according to the modified Karnovsky-Roots method using rubeanic acid as an amplifier (11). Briefly, the AChE solution consisted of 6.5 mL of 0.1 mol/L hydrogen maleate buffer, 0.5 mL of 0.1 mol/L sodium citrate, 1 mL of 30 mmol/L copper sulfate, 1 mL of 5 mmol/L potassium ferricyanide, 1 mL of distilled water, and 10 mg of acetylcholine iodide. The rubeanic acid solution was obtained by mixing 10 mg of rubeanic acid, 10 mL of 100% ethanol, 6.55 g of sodium acetate, and 40 mL of distilled water. The frozen samples were sliced at a thickness of 10 μm using a cryostat and then placed on a microscope slide and stained with the AChE solution for 20 minutes followed by the rubeanic solution for 10 minutes. The samples were then dehydrated and embedded with Canada balsam before being examined under a microscope. The histopathological analysis was performed by 5 authors (I.R.B., S.O., K.Y., Y.Y., and T.T.). All 5 observers had 1 year (I.R.B.), 2 years (S.O., K.Y., and Y.Y.), and >20 years (T.T.) of experience in interpreting AChE-stained sections at Kyushu University. At first, the biopsied specimens were observed histopathologically by observers with 1 or 2 years of experience, followed by checking and diagnosis by a doctor with >20 years of experience. The grading system for assessing the extension of AChE fibers followed that reported in a previous study, as shown in Table 1 (11).

We divided the samples into 4 groups based on the AChE staining results: HD, non-HD, "undetermined," and "inappropriate." The non-HD group contained samples with a normal mucosa and suspected IND. The diagnoses of HD, a normal mucosa, and suspected IND were made based on the findings of AChE staining, as shown in Table 2. Meanwhile, the diagnosis of HD was made based on evidence of obvious AChE fiber extension in the lamina propria and muscularis mucosae with no ganglion cells in the submucosa. In addition, the diagnosis of suspected IND was made based on the following findings (19,20,26): hyperplasia of the submucous plexus, giant ganglia containing >8 ganglion cells, an increased AChE activity in the lamina propria or surrounding submucosal blood vessels, heterotopic neural cells in the lamina propria, and a histopathological diagnosis of IND <1 year of age, as giant ganglia disappear via apoptosis and the maturation of the submucosal plexus in the first year of life (26).

An "undetermined" diagnosis was made in cases in which it was difficult to diagnose HD, a normal mucosa, or suspected IND, although either there were no problems with the quality of the specimen or the specimen did not contain the submucosal layer. An "inappropriate" diagnosis was made based on the poor condition of the sample because of destruction of the specimen as a result of poor freezing conditions or malposition in which the sample was covered by stratified squamous epithelium. A repeat biopsy was recommended in some cases with an "undetermined" or "inappropriate" diagnosis.

The final diagnosis, prognosis, and management after the histological diagnosis were analyzed via a questionnaire sent to the doctor at the patient's original hospital. In particular, a clinical final diagnosis of CIIP was made in few cases involving chronic persistent functional bowel obstruction based on the proposed criteria (22,23). The criteria for CIIP are as follows: chronic persistent or recurrent episodes characterized by abdominal pain and/or distention possibly associated with nausea and vomiting with symptoms mimicking subacute mechanical intestinal obstruction prompting hospitalization; the persistence of symptoms for at least 2 months in cases with a neonatal onset and 6 months in those with a later onset;

TABLE 1. Grade of extension of AChE-positive fibers

| | |
|---|---|
| – | No fiber found |
| +/– | Few fine fibers on the base of lamina propria |
| + | Obvious fibers on the base of lamina propria |
| ++ | Fibers spread to the tip of lamina propria |
| +++ | Communicating fibers forming a network between lamina propria |

AChE = acetylcholinesterase.

TABLE 2. Summary of the findings for each diagnosis

| Diagnosis | Submucosa | Lamina propria and muscularis mucosa |
|---|---|---|
| HD | Ganglion cells (−) | AChE fibers (±) ~ (+++) |
| Normal | Ganglion cells (+) | AChE fibers (−) |
| IND | Ganglion cells (++) | AChE fibers (±) ~ (++) |

AChE = acetylcholinesterase; HD = Hirschsprung disease; IND = intestinal neuronal dysplasia.

the lack of mechanical causes of gut lumen occlusion, as detected on endoscopy and/or radiology; radiological evidence of dilated bowel loops with air-fluid levels during at least 1 episode of acute exacerbation; the absence of recognizable organic, systemic, or metabolic disease underlying the syndrome of CIIP, as detected on a complete diagnostic workup.

This retrospective study was performed according to the Ethical Guidelines for Clinical Research published by the Ministry of Health, Labour and Welfare of Japan on July 30, 2003 (revised 2008) and complied with the Helsinki Declaration of 1964 (revised 2008). All of the parents or patients provided their informed consent at the time of the biopsy.

## RESULTS

### AChE Staining

Three hundred fifty-eight specimens were collected and analyzed. One hundred twenty-two (34%) of 358 specimens were diagnosed as "HD" (Fig. 1). The AChE fiber criteria were ± for 3 samples, 1+ for 33 samples, 2+ for 60 samples, and 3+ for 26 samples, with a mean age for each group of 21, 46, 289, and 347 days, respectively. These findings are similar to those reported in the references, such that the older the patient at the time of biopsy (11), the more obvious the fibers, with further extension and a higher grade. Even in the ± group, the definitive diagnosis of HD was made based on the findings of obvious AChE-positive fibers in the muscularis mucosae and the absence of ganglion cells.

One hundred and ninety-eight (55%) of 358 specimens were diagnosed as "non-HD." In the non-HD group, 190 (96%) samples were histologically "normal" (Fig. 2), 8 (4%) samples exhibited "suspected IND" (Fig. 3), and 25 (7%) samples were classified as "undetermined." The "undetermined" group included 3 cases of "highly suspected HD" and 22 cases of "highly suspected non-HD" based on the findings of the lamina propria and muscularis mucosae. The samples classified as "highly suspected HD" contained obvious thick AChE-positive fibers in the lamina propria and muscularis mucosae (Fig. 4A); all of the cases were confirmed on repeat biopsy a few months later. The initial diagnosis did not change at repeat biopsy. Thirteen (4%) samples could not be analyzed and were classified as "inappropriate" (Fig. 4b); repeat biopsy was recommended in these cases, and the 13 specimens were excluded from the study. Questionnaires were subsequently sent for 345 specimens.

### Final Diagnosis After Assessing the Questionnaires

A total of 306 of the 345 questionnaires were returned. The relation between the results of the initial AChE staining and the final diagnosis is shown in Table 3.

#### *HD*

The mean age at the time of the rectal biopsy in the HD group was 167.5 days (0.46 year) (range 4 days–6 years, including 21 neonatal cases). One hundred and nine of 122 questionnaires confirmed HD as the final pathological diagnosis. Three additional HD cases were identified in the "undetermined" group. All of the 3 samples were initially diagnosed as "highly suspected HD" and then confirmed to be HD on the repeat biopsy. None of the samples diagnosed as HD on AChE staining were classified as non-HD at the final diagnosis.

#### *Non-HD*

The mean age of the non-HD patients was 1260 days (3.5 years) (range 10 days–50 years, including 8 neonatal cases). One hundred and seventy-six questionnaires were returned for all of the 198 non-HD cases diagnosed on AChE staining. The non-HD group was ultimately divided into those with functional constipation, CIIP, and suspected IND based on the results of the questionnaires. One hundred and sixty-nine of the 190 questionnaires for the histologically "normal mucosa" group were returned.

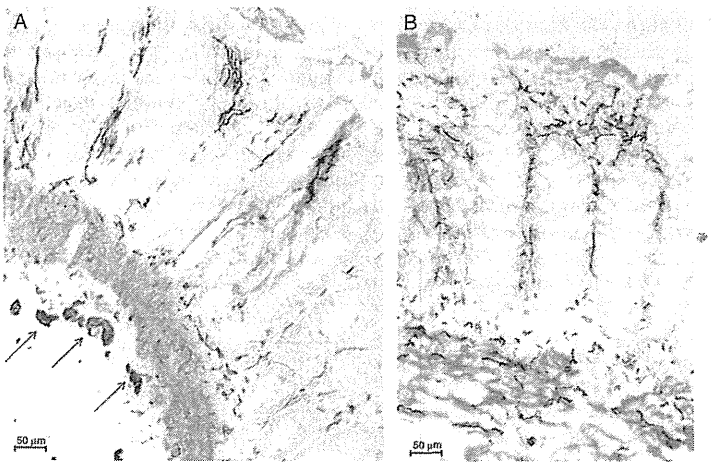


FIGURE 1. HD ([A] a 2-month-old boy, [B] a 15-year-old girl). Numerous thick AChE-positive fibers are seen in the muscularis mucosae and lamina propria mucosa, some of which extend to the tip of villi and form a network. AChE-positive nerve bundles (arrows) are noted in the submucosa. Ganglion cells are absent. AChE = acetylcholinesterase; HD = Hirschsprung disease.

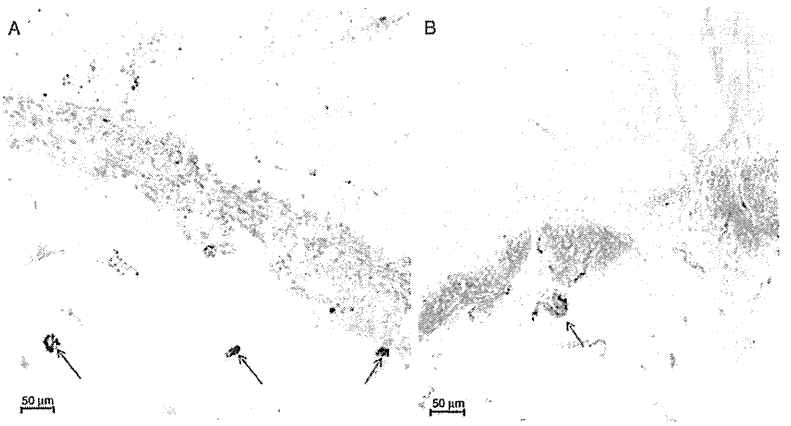


FIGURE 2. Normal mucosa. ([A] a 14-year-old girl, [B] a 2-month old boy). There are normal plexuses including a few ganglion cells in the submucosa (arrows). Neither AChE-positive fibers nor bundles are observed. AChE = acetylcholinesterase.

The final diagnosis showed 166 cases of functional constipation and 3 cases of CIIP. Seven of the 8 questionnaires for cases of suspected IND were returned, and the final diagnosis showed that all of the 7 cases remained "suspected IND," because the patients diagnosed as having suspected IND did not necessarily exhibit a poor clinical course, making it unnecessary to perform a repeat biopsy.

### *"Undetermined" Group*

The mean age of the "undetermined" group was 2042 days (5.6 years; range 1 month–48 years, including no neonatal case). Twenty-one of the 25 questionnaires were returned in this group. The early AChE staining diagnosis included 3 samples with "highly suspected HD," whereas 18 samples were "highly suspected as non-HD." The final diagnosis confirmed that the 3 "highly suspected HD" specimens exhibited HD, and all of the 18 "highly suspected non-HD" specimens were confirmed to be non-HD, resulting in 17 cases of functional constipation and 1 case of CIIP.

## Accuracy of AChE Staining in the HD and Non-HD Groups

The sensitivity and specificity of AChE staining are shown in Table 4; the sensitivity and specificity of AChE staining for detecting HD were both 100%.

## Outcomes in the HD and Non-HD Groups

All of the patients with HD were successfully managed with surgical treatment, primarily transanal endorectal pull-through (27), or Z-shaped anastomosis (28). In the HD group, 58 (53.2%) patients displayed normal bowel motility after undergoing radical surgery, whereas 16 (14.7%) remained constipated and 15 (13.8%) experienced soiling. The details in 20 (18.3%) cases were unknown because the patients were moved to other locations and the data were incomplete.

Most patients in the non-HD group were managed with conservative treatment, including medication, traditional herbal medicine (Kampo), glycerin enemas, and dietary prescriptions,

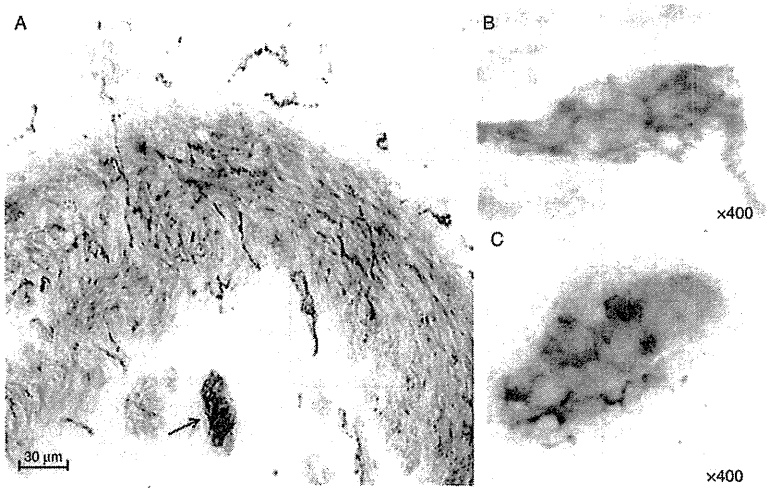


FIGURE 3. Suspected IND (a 3-month-old girl). Several AChE-positive nerve fibers are seen in the lamina muscularis and lamina propria. Giant ganglion cells (arrow) are detected in the submucosa. (B and C) High magnification (original magnification ×400) of a giant ganglia including >8 ganglion cells. AChE = acetylcholinesterase; IND = intestinal neuronal dysplasia.

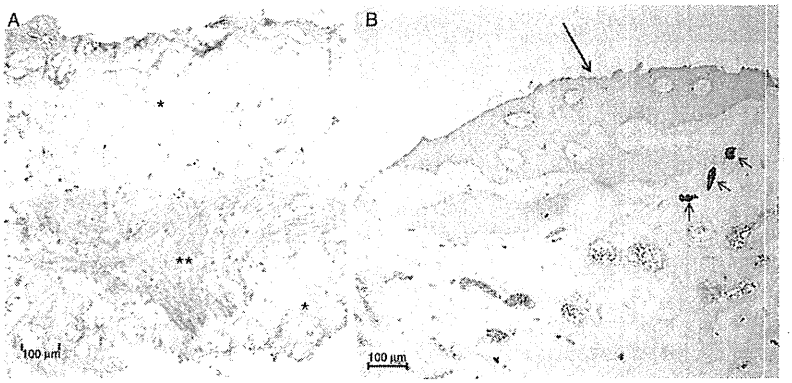


**FIGURE 4.** Examples of "undetermined" and "inappropriate" specimens. (A) This specimen was composed of lamina propria (*) and muscularis mucosae (**). It did not contain the submucosa. (B) Malposition: this specimen is not covered by the rectal mucosa but rather stratified squamous epithelium (long arrow). This specimen was obtained below the dentate line. Some skin appendages showed positive AChE staining (short arrows). AChE = acetylcholinesterase.

with the exception of 6 patients with constipation, and 1 patient with CIIP underwent surgical management for bowel resection because of megacolon.

In the non-HD group, the patients with functional constipation included 90 (49%), 27 (15%), 4 (2.2%), and 1 (0.5%) subjects with a normal bowel motility, persistent constipation, soiling, and death from acute pulmonary failure because of idiopathic pulmonary hemosiderosis with no causal relation with HD, respectively. In addition, 61 (33.3%) patients dropped out from the study.

In the suspected IND group, 4 of the 7 patients (57.1%) exhibited normal bowel motility and 1 patient (14.3%) experienced soiling, whereas no patient (0%) died and 2 patients (28.6%) dropped out.

In the CIIP group, 3 of the 4 patients (75%) displayed normal bowel motility following appropriate management, no patient (0%) experienced constipation, 1 patient (25%) experienced soiling, and no patient (0%) died.

## DISCUSSION

Since the development of rectal suction biopsies and punch biopsies stained with AChE by Meier-Ruge et al (10), the use of rectal mucosal biopsies, which has become the most widely used diagnostic procedure in children with bowel motility disorders, has gradually replaced that of conventional full-thickness biopsies (29) because of the ability to detect the absence of ganglion cells in Meissner plexus and increased AChE activity in the lamina propria

TABLE 3. Relation between AChE staining and the final diagnosis

| Histological diagnosis by AChE staining | Nos | Answers | Final diagnosis | Final nos. |
|---|---|---|---|---|
| Total | 358 | 306 | | 306 |
| HD | 122 (34%) | 109 | HD 109 + 3 = | 112 |
| Non-HD | 198 (55%) | 176 | Non-HD | 194 |
| Normal mucosa | 190 | 169 | Functional constipation 166 + 17 = | 183 |
| Susp IND | 8 | 7 | Susp IND | 7 |
| "Undetermined" | 25 (7%) | 21 | CIIP 3 + 1 = | 4 |
| HD, highly suspected | 3 | 3 | | |
| Normal, suspected | 22 | 18 | | |
| "Inappropriate" | 13 (4%) →excluded | | | |

AChE = acetylcholinesterase; CIIP = chronic idiopathic intestinal pseudo-obstruction; HD = Hirschsprung disease; Susp IND = suspected intestinal neuronal dysplasia.

TABLE 4. Sensitivity and specificity of AChE staining for HD diagnosis

| | | Final diagnosis | | |
|---|---|---|---|---|
| | | HD | Non-HD | Total |
| AChE staining results | HD + highly susp HD | 109 + 3 | 0 | 112 |
| | Non-HD | 0 | 194 | 194 |
| | Total | 112 | 194 | 306 |

Sensitivity: (109 + 3)/112 × 100% = 100%. Specificity: 194/194 × 100% = 100%. Positive predicted value: 112/112 × 100% = 100%. Negative predicted value: 194/194 × 100% = 100%. AChE = acetylcholinesterase; HD = Hirschsprung disease; highly susp HD = highly suspected Hirschsprung disease.

mucosa, findings specific for HD. Alternative diagnostic tools including calretinin or several antibodies against neural tissue including glucose transporter-1 (GLUT-1) are considered as reliable as AChE staining and are used in many laboratories. Calretinin is a useful neural marker, and calretinin immunostaining was related to aganglionosis in case of HD (30). Present reports suggested that calretinin immunostaining is at least as sensitive and specific as AChE staining for the diagnosis/exclusion of HD (31), and calretinin immunostaining would replace AChE enzyme histochemistry as an ancillary method to complement hematoxylin and eosin (HE)-stained paraffin sections (32), whereas false-positive and false-negative results may occur with calretinin immunostaining technique. So, a combination of AChE staining and calretinin immunostaining may be recommended for diagnosis of HD (33,34). GLUT-1 is a marker of perineurium, and the increase in GLUT-1-positive nerves in HD is consistent with intramural extension of extrinsic nerves and includes both abnormally large (hypertrophic) nerves >50 μm and smaller nerves. GLUT-1 as a marker of these fibers may be a useful positive indicator in the diagnosis of HD, whereas GLUT-1 fibers were not found in the cases of total colonic aganglionosis, which usually has a normal AChE pattern and lacks hypertrophic nerves (35).

In the present study, we used the modified Karnovsky-Roots histochemical enzyme method with rubeanic acid as the amplifier (11). Indeed the immunostainings such as calretinin or GLUT-1 may replace AChE staining as an ancillary method to complement HE-stained paraffin sections, whereas the immunostaining methods are time consuming in terms of the time required for paraffin embedding and immunohistological reaction. On the contrary, our method increases the contrast and reduces the staining time to 30 minutes, making it superior to other methods in terms of accuracy, speed, and simplicity (11,25).

Our previous study showed the specificity and sensitivity of AChE staining to be 100% and 91%, respectively (11). Meanwhile, Bagdzevicius et al (29) reported a specificity and sensitivity of 100% and 40%, respectively, in neonates diagnosed as having HD and 100% and 88%, respectively, in infants diagnosed as having HD. After 13 years of experience, we found no discrepancies between the results of AChE staining and the final diagnosis because all of the samples were correctly diagnosed and classified based on AChE staining after the initial biopsy. In addition, none of the samples diagnosed as HD on AChE staining were later classified as non-HD or vice versa. Therefore, the sensitivity and specificity of AChE staining for detecting HD in this study were both 100%. Furthermore, our experience improved the ability to evaluate and diagnose HD based on the results of AChE staining.

The use of "inappropriate" specimens should be avoided. In order to obtain an adequate thickness for the specimen, containing both the mucosa and the submucosa in the accurate position, Bagdzevicius et al (29) recommends the use of endoscopic forceps via endoscopy under anesthesia. In fact, it is sometimes difficult to distinguish HD from IND based on the findings of the lamina propria and muscularis mucosa. Our procedures are usually performed at each branch hospital without general anesthesia if the patient's condition permits (25). Not all of the pediatric surgeons, however, are used to this procedure. Therefore, a rate of "inappropriate" specimens of 4% is considered reasonable.

In the present study, all of the patients with HD were treated surgically and exhibited a favorable outcome, whereas most of the non-HD patients were successfully treated conservatively, with the exception of a few particular cases that required procedures different from HD. A large number of functional constipation group patients were lost to the analysis (61/183), which may have been because of the fact that functional constipation is a self-limiting condition that requires no further treatment.

IND was first described by Meyer-Ruge (36) as a hyperplastic malformation of the enteric plexus. There remains controversy regarding the existence of IND as a distinct histopathological entity, and it has been suggested by several authors that the findings of IND reflect either a variant of normal bowel development (20,26) or a secondary acquired phenomenon caused by congenital obstruction or inflammation (37–39). The fourth international symposium on HD and related neurocristopathies discussed IND, with the following findings (40): almost all of the participants believed that IND does exist; some accepted the presently defined diagnostic criteria, whereas others suggested that the diagnostic criteria are not adequately reliable; some participants questioned whether IND is a truly separate entity or acquired secondary phenomenon related to long-standing constipation and/or chronic obstruction. Therefore, we consider IND to be a variant of HD.

The typical histological features of IND have been reported to include giant ganglia, ectopic ganglion cells, and an increased AChE activity in the lamina propria mucosa and surrounding submucosal blood vessels. The most frequently used diagnostic criteria for IND are as follows: >20% of 25 submucosal ganglia must be giant ganglia containing 9 or more ganglion cells and the patient must be >1 year of age (15,26). Our cases of "suspected IND" showed obviously giant ganglia (Fig. 4), containing >8 ganglion cells with the extension of AChE-positive fibers in the lamina propria and muscularis mucosae. All of these patients, however, were <1 year of age. Therefore, we treated them as having "suspected IND."

The findings of the lamina propria in patients with IND are similar to those observed in patients with HD. If the specimen does not contain the submucosal layer, it is difficult to distinguish IND from HD. Our results show that the thickness of AChE-positive fibers in the lamina propria is greater in patients with HD than in patients with IND. The diagnosis of highly suspected HD in the undetermined cases was based on the findings of obviously thick AChE-positive fibers in the lamina propria and muscularis mucosae, despite the lack of the submucosa. These 3 cases were confirmed to be HD according to the findings of the sequential biopsy performed after a few months. Therefore, the presence of the thick nerve fibers is considered to provide important evidence for the diagnosis of HD, because the nerve fibers in the lamina propria and muscularis mucosae are relatively weak in cases of IND compared with HD.

CIIP has been reported to be an idiopathic form of chronic intestinal pseudo-obstruction (CIPO), excluding myopathy, neuropathy, and collagenopathy (desmosis or fibrosis) (22). Some CIPO cases have been reported to involve adult onset (23). Most myopathy and neuropathy types of CIPO, however, cannot be diagnosed using conventional HE and AChE staining. Furthermore, a decrease in the number of Cajal cells on anti-c-kit antibody immunostaining

has been reported in some cases of CIIP. Therefore, CIIP is a disorder involving recurrent or persistent functional intestinal obstruction with a normal histology on conventional staining with HE and AChE. In our series, 4 cases of CIIP were included among those diagnosed as being non-HD, in which the specimens showed a normal mucosa. Therefore, careful clinical follow-up is mandatory in cases diagnosed as being non-HD because the prognosis of CIIP is not always favorable.

In conclusion, AChE staining of rectal mucosal biopsy samples is indeed an important and reliable tool for differentiating between cases of HD and non-HD with high sensitivity and specificity. Inappropriate specimens, however, can be obtained because of an insufficient thickness, failure to include the submucosa, or the use of an inappropriate site close to the dentate line. It is interesting to note that few cases of CIIP are included among those diagnosed as non-HD on AChE staining. Therefore, careful clinical follow-up is mandatory in cases diagnosed as non-HD.

**Acknowledgements:** The authors thank Brian Quinn for reading the manuscript. The authors also thank the staff of Pediatric Surgery at the branch hospitals of Kyushu University for their kind help in collecting the data.

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Asian Journal of Surgery (2015) xx, 1–6
Available online at www.sciencedirect.com
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ORIGINAL ARTICLE

# The incidence and outcome of allied disorders of Hirschsprung's disease in Japan: Results from a nationwide survey

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Received 26 March 2015; accepted 2 April 2015

**KEYWORDS**
allied disorders;
chronic idiopathic intestinal pseudo-obstruction;
Hirschsprung's disease;
hypoganglionosis;
megacystis microcolon intestinal hypoperistalsis syndrome;
pseudo-obstruction

**Summary** *Background:* Allied disorders of Hirschsprung's disease (ADHD) have been proposed to be the concept of the functional obstruction of the intestine with the presence of ganglion cells in the terminal rectum. They are classified into two categories based on pathology: (1) abnormal ganglia, including immaturity of ganglia, hypoganglionosis (HG), and intestinal neuronal dysplasia; (2) normal ganglia, including megacystis microcolon intestinal hypoperistalsis syndrome (MMIHS), segmental dilatation (SD), internal anal sphincter achalasia (IASA), and chronic idiopathic intestinal pseudo-obstruction (CIIP). Some of these show poor prognosis, therefore, the establishment of criteria and appropriate treatment strategies is required.
*Methods:* The questionnaires were sent to the 161 major institutes of pediatric surgery or gastroenterology in Japan, in order to collect the cases of ADHD during 10 years from 2001 and 2010.
*Results:* In total, 355 cases were collected. They included 28 immaturity of ganglia, 130 HG (121 congenital, 9 acquired), 18 intestinal neuronal dysplasia, 33 MMIHS, 43 SD, three IASA, and 100 CIIP. Of the 95 institutes, 69 (72.6%) had their own criteria for ADHD. Criteria were based on clinical symptoms and signs, and conventional pathological examinations. Prognosis was poor in congenital HG, MMIHS, and CIIP, while the others showed good survival rates.
*Conclusion:* Almost all Japanese cases of ADHD in the past 10 years were collected. Congenital HG and CIIP showed relatively high incidence, whereas acquired HG and IASA were extremely

Conflicts of interest: The all authors declare that they have no financial or nonfinancial conflicts of interest related to the subject matter or materials discussed in the manuscript.

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http://dx.doi.org/10.1016/j.asjsur.2015.04.004


Please cite this article in press as: Taguchi T, et al., The incidence and outcome of allied disorders of Hirschsprung's disease in Japan: Results from a nationwide survey, Asian Journal of Surgery (2015), http://dx.doi.org/10.1016/j.asjsur.2015.04.004

rare in Japan. The criteria of each disorder were also collected and summarized. Prognosis was poor in congenital HG, MMIHS, and CIIP.


## 1. Introduction

Allied disorders of Hirschsprung's disease (ADHD) have been understood as the conditions that clinically resemble Hirschsprung's disease (HD), despite the presence of ganglion cells in the terminal rectum.[1] Patients with Hirschsprung's disease generally present in the newborn period with delayed passage of meconium and abdominal distention or as a young child with severe chronic constipation. Patients with ADHD show similar symptoms and signs to HD, but they can be distinguished from HD by the pathological findings. The term *pseudo HD* was proposed by Ravitch in 1958.[2] They encountered patients referred for treatment of megacolon in whom the difficulty lay elsewhere rather than in the congenital absence of ganglion cells of the myenteric plexuses of a segment of the rectum or of the colon and rectum. Bentley et al[3] summarized *HD and allied disorders* in the *Seminar on Pseudo-Hirschsprung's Disease and Related Disorders*. The main thing to remember was that the various disease patterns were essentially determined by their underlying pathology, irrespective of what we choose to call them. ADHD was classified into two categories based on histology[3]: those with abnormality of ganglion cells and those without abnormality of ganglion cells (Table 1). Puri and Gosemann[4] called this group *variants of HD*, including four disorders: intestinal neuronal dysplasia (IND); isolated hypoganglionosis (HG); internal anal sphincter achalasia (IASA); and megacystis microcolon intestinal hypoperistalsis syndrome (MMIHS) in 2012.[4] They did not treat chronic idiopathic intestinal pseudo-obstruction (CIIP) as one of the variants of HD.

Okamoto and Toyosaka[5] used the term of *pseudo-Hirschsprung's disease* in the Japanese literature. It was defined as a congenital, nonmechanical obstruction of the intestine with presence of intramural ganglion cells in the terminal rectum. They classified them based on histology into two categories for six disorders: immaturity of ganglia (IG); HG; hypogenesis; IND; CIIP; and MMIHS.[5]

According to the literature and Okamoto and Toyosaka's[5] classification, ADHD was classified into two categories depending on the pathological findings (Table 2): (1) abnormal ganglia, including IG, HG, and IND; (2) normal ganglia, including MMIHS, segmental dilatation (SD), IASA, and CIIP. Some of them show poor prognosis; therefore, establishment of criteria, severity, and treatment strategy are required. In order to examine the incidence and criteria of ADHD, a preliminary nationwide survey was planned in Japan.

## 2. Patients and methods

As a nationwide retrospective cohort study, supported by Ministry of Health and Welfare, Japan, the preliminary questionnaires, requesting the number of cases of ADHD from January 2000 to December 2009 and the criteria of each institute, were sent to the 161 major institutes of pediatric surgery or pediatric gastroenterology representing the core members of the Japanese Society of Pediatric Surgeons, the Japanese Society of Pediatric Nutrition, Gastroenterology, and Hepatology, and the Japanese Study Group of Pediatric Constipation. Therefore almost all institutes that are treating ADHD are considered covered. The number of patients, including the definite and suspected cases, based on the classification of ADHD in Japan (Table 1) and the survival rate and clinical outcome were asked. The criteria of each institute were asked to be answered as free descriptions. The criteria for *definitive* or *suspected* were dependent on each institute.

This study was performed according to the Ethical Guidelines for Clinical Research published by the Ministry of Health, Labor, and Welfare of Japan on July 30, 2003. And this study was approved by the Ethical Committee for Clinical Research of Kyushu University Hospital, Fukuoka, Japan (No. 24-163).

## 3. Results

Replies were obtained from 157 of 161 institutes (98%). Out of 157 institutes, 95 (61%) had ADHD. In totally, 355 cases,

**Table 1** Hirschsprung's disease and allied disorders (Ehrenpreis 1966).[3]

| |
|---|
| With abnormalities of ganglion cells |
| Hirschsprung's disease |
| Congenital megacolon |
| Congenital aganglionosis |
| Chagas' disease |
| Acquired megacolon |
| Aperistalsis |
| Hypoganglionossis |
| Immaturity of ganglion cells |
| Without abnormalities of ganglion cells |
| Aetiology obscure |
| Idiopathic megacolon |
| Functional megacolon |
| Psychogenic megacolon |
| Megarectum |
| Chronic constipation |
| Pseudo-hirschsprung |
| Segmental dilatation of the colon |
| Achalasia of distal rectal segment |
| Clear etiology |
| Symptomatic megacolon |
| Secondary megacolon (anal stricture, myxoedema, cerebral atrophy) |

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**Table 2** Classification for allied disorders of Hirschsprung's disease in Japanese survey.

(1) Abnormal ganglia (abnormal histology in hematoxylin–eosin or acetylcholinesterase staining)
- Immaturity of ganglia (or immature ganglionosis)
- Hypoganglionosis (or oligoganglionosis)
- Congenital hypoganglionosis (or hypogenesis, hypoplasia)
- Acquired hypoganglionosis intestinal neuronal dysplasia

(2) Normal ganglia (normal histology in hematoxylin–eosin or acetylcholinesterase staining)
- Megacystis microcolon intestinal hypoperistalsis syndrome
- Segmental dilatation of intestine
- Internal anal sphincter achalasia
- Chronic idiopathic intestinal pseudo-obstruction

including 287 definite cases and 68 suspected cases were collected between 2001 and 2010. More than half of the institutes (53 institutes) had three cases or fewer (Figure 1). The mean number of cases per institute was 3.7 cases. There were 165 of 355 cases (47%) treated in university hospitals, 93 (26%) in children's hospitals, and 97 (27%) in general hospitals. ADHD included 28 IG, 130 HG (121 congenital, 9 acquired), and 18 IND in abnormal ganglia; and 33 MMIHS, 42 SD, three IASA, and 100 CIIP in normal ganglia, and these numbers were compared with those of the previous study in Japan (Table 3).[2]

Of the 95 institutes who experienced ADHD, 69 (73%) had their own criteria. The percentages of institutes that had criteria for each disorder were between 28% and 83% (Table 4). More than 80% of institutes had criteria for congenital HG and CIIP, while only $\leq$ 30% institutes had criteria for acquired HG and IASA. Criteria of each disorder were based on clinical symptoms and signs, examinations including radiography findings, manometric study, and conventional pathological examinations including hematoxylin–eosin (HE; Figure 2) and acetylchoinesterase (AchE). According to answers of the questionnaires, the major criteria listed in each disorder are follows. IG: small ganglion cells, 37/46 (80%); number and distribution of ganglion cells are normal, 19/46 (41%); chronological improvement of clinical symptoms, 8/46 (17%); intestinal obstruction on neonatal onset, 6/46 (13%); normal AchE staining, 3/46 (7%); abdominal distention, 2/46 (4%); and microcolon, 2/46 (4%). Congenital HG: few ganglion cells, 41/55 (75%); few small ganglion cells, 14/55 (25%); intestinal obstruction on neonatal onset, 11/55 (20%); hypoplasia of plexus, 4/55 (7%); normal AchE staining, 4/55 (7%); negative rectospincteric reflex, 4/55 (7%); and delayed meconium pass, 2/55 (4%). Acquired HG: ganglion cells decrease in number after some time, 6/19 (32%); few ganglion cells, 4/19 (21%); normal at birth and symptoms occur after some time, 2/19 (11%); no congenital factors, 2/19 (11%); chronic constipation and persistent bowel dilatation, 2/19 (11%); and normal AchE staining 1 (5%). IND: increased AchE positive fibers in the lamina propria, 17/34 (50%); ectopic ganglion cells, 14/34 (41%); giant ganglia (> 5 ganglion cells per plexus), 13/34 (38%); severe constipation or rectal dysmotility, 9/34 (26%); hyperganglionosis, 6/34 (18%); and dilatation of bowel, 2/34 (6%). MMIHS: megacystis, 39/47 (83%); permanent severe symptoms of intestinal obstruction, 35/47 (74%); microcolon 27/47 (57%); normal histology of intestinal neurons and muscles, 25/47 (53%); neonatal onset, 16/47 (34%); normal AchE staining, 5/47 (11%); and positive rectospincteric reflex 4/47 (9%). SD: persistent segmental dilatation, 36/42 (86%); normal histology of intestinal ganglion cells, 24/42 (57%); no mechanical obstruction distal to dilatation, 13/42 (31%); signs of intestinal obstruction in radiography, 7/42 (17%); complete curability after resection of dilated bowel, 5/42 (12%); abrupt caliber change to the normal intestine, 3/42 (7%); thick or thin muscle layer, 2/42 (5%); and positive rectospincteric reflex, 2/42 (5%). IASA: negative rectospincteric reflex, 9/21 (43%); normal AchE staining, 9/21 (43%); severe constipation since birth, 7/21 (33%); and absence of narrow segment, 4/21 (19%). CIIP: symptoms of intestinal obstruction without mechanical cause, 57/57 (100%); normal histology of intestinal ganglion cells,

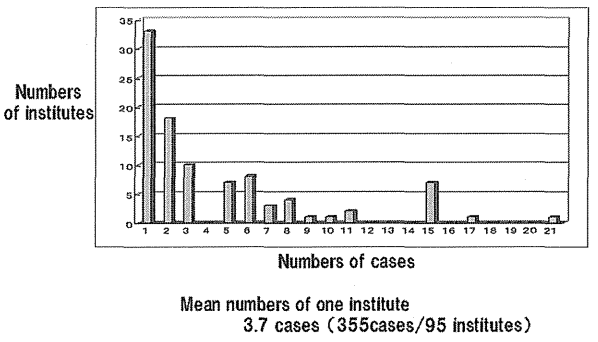


**Figure 1** Number of cases in each institute.

**Table 3** Numbers of patients in each disorder.

| | Definitive | Suspected | Total | Okamoto and Toyosaka[2] |
|---|---|---|---|---|
| **Abnormal ganglia** | | | | |
| IG | 22 | 6 | 28 (7.9) | 26 (24.1) |
| HG | 112 | 18 | 30 (36.6) | 44 (40.8) |
| Congenital | 104 | 17 | 121 (34.1) | |
| Acquired | 8 | 1 | 9 (2.5) | |
| IND | 8 | 10 | 18 (5.1) | 5 (4.6) |
| **Normal ganglia** | | | | |
| MMIHS | 27 | 6 | 33 (9.3) | 9 (8.3) |
| SD | 33 | 10 | 43 (12.1) | NE |
| IASA | 1 | 2 | 3 (0.8) | NE |
| CIIP | 84 | 16 | 100 (28.2) | 24 (22.2) |
| **Total** | 287 | 68 | 355 (100) | 108 (100) |

CIIP = chronic idiopathic intestinal pseudo-obstruction; HG = hypoganglionosis; IASA = internal anal sphincter achalasia; IG = immaturity of ganglia; IND = intestinal neuronal dysplasia; MMIHS = megacystis microcolon intestinal hypoperistalsis syndrome; NE = not examined; SD = segmental dilatation.

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**Table 4** The percentages of institutes that had criteria for each disorder.

| | |
|---|---|
| **Abnormal ganglia** | |
| IG | 46/69 (67%) |
| HG | |
| Congenital HG | 55/69 (80%) |
| Acquired HG | 19/69 (28%) |
| IND | 34/69 (49%) |
| **Normal ganglia** | |
| MMIHS | 47/69 (68%) |
| SD | 42/69 (61%) |
| IASA | 21/69 (30%) |
| CIIP | 57/69 (83%) |

CIIP = chronic idiopathic intestinal pseudo-obstruction; HG = hypoganglionosis; IASA = internal anal sphincter achalasia; IG = immaturity of ganglia; IND = intestinal neuronal dysplasia; MMIHS = megacystis microcolon intestinal hypoperistalsis syndrome; SD = segmental dilatation.

46/57 (81%); abnormality of urinary tract, 13/57 (23%); dilatation of intestine in radiography 9/57, (16%); positive rectospincteric reflex, 8/57 (14%); intermittent or recurrent symptoms, 6/57 (11%); and normal AchE staining, 6/57 (11%). For the diagnosis of IG, immunohistochemical studies using Bcl-2 antibody were performed and shown to be effective in a few institutes (Figure 3).

The survival rates of each entity for which the follow-up data were available are shown in Table 5. Three entities, congenital HG, MMIHS, and CIIP, showed poor survival rate, compared with those of the other five entities. These three entities required long-term nutritional support, including parenteral and enteral nutrition (Table 6). In particular, outcome is extremely poor in MMIHS.

## 4. Discussion

Almost all Japanese cases (~ 98%) of ADHD in 10 years were collected in this nationwide survey. However, the number of cases in each institute was very small, and actually more than half of the institutes (53 institutes) had three cases or fewer. Therefore nation-wide survey is considered to be important.

Okamoto and Toyosaka[5] published a multicenter study of ADHD in the Japanese literature in 1994. They classified ADHD into two categories based on pathology: (1) abnormal histology including IG, HG, hypogenesis, and IND; and (2) normal histology, including CIIP[6] and MMIHS. We followed the classification of Okamoto and Toyosaka[5] in order to compare our results to those of their survey. In addition to Okamoto and Toyosaka's[5] classification, SD and IASA are included in this study, referring to the literature.[1,3]

The presence of immaturity and HG was confirmed and published by Taguchi et al.[7] We proposed there were two types of HG, namely congenital and acquired.[7] *Hypogenesis* is considered to be the initial histological finding of congenital HG at neonatal period. *The 4th International Symposium on Hirschsprung's disease and related neurocristopathies* discussed that diagnosis of HG was difficult and the presence of HG was questionable.[8] However, our

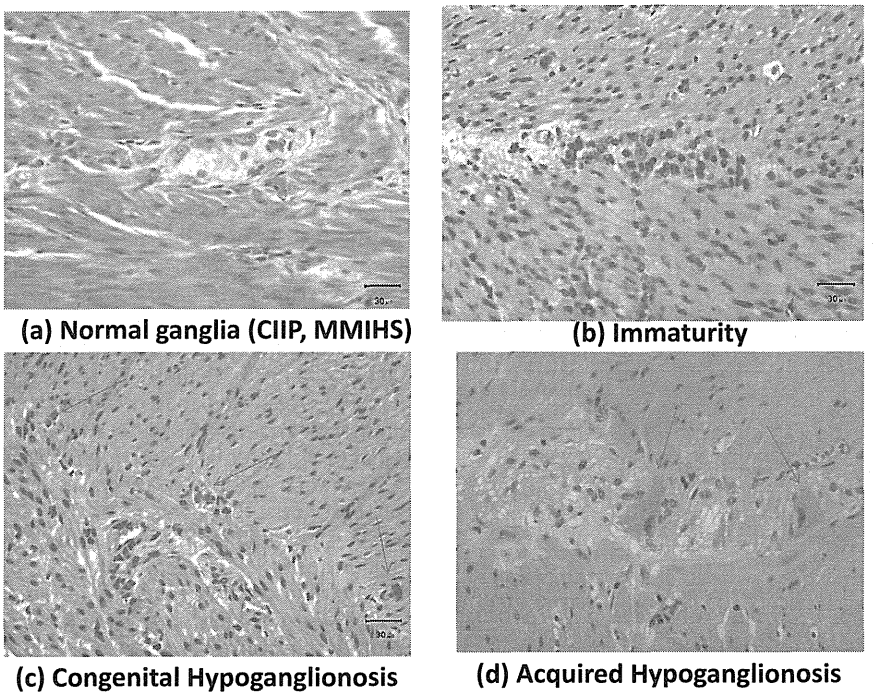


**Figure 2** Typical pathology of allied disorders of Hirschsprung's disease (ADHD) in hematoxylin–eosin staining. (A) Normal ganglia (megacystis microcolon intestinal hypoperistalsis syndrome, MMIHS; chronic idiopathic intestinal pseudo-obstruction, CIIP; segmental dilatation). (B) Immaturity of ganglia. (C) Congenital hypoganglionosis. Arrows indicate small ganglion cells. (D) Acquired hypoganglionosis. Arrows indicate degenerated ganglion cells.

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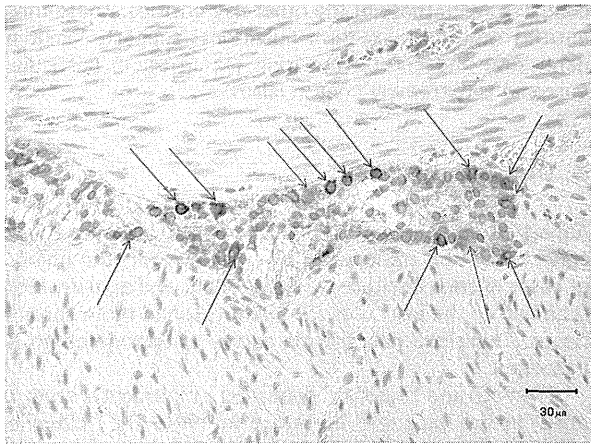


**Figure 3** Bcl-2 immunostaining for immaturity of ganglia (2-day-old boy). Mature ganglion cells (blue arrows) show weekly positive, while immature ganglion cells (red arrows) show strongly positive in Bcl-2 immunostaining. Glial cells are not stained. Therefore, this staining is considered to be able to distinguish mature ganglion cells, immature ganglion cells, and glial cells.

study shows that two types of HG do exist and congenital HG is one of the two main disorders of ADHD in Japan.

*The 4th International Symposium on Hirschsprung's disease and related neurocristopathies* discussed IND and reported as follows[8]: (1) almost all the participants believe that IND does exist; (2) some believe in presently defined diagnostic criteria, whereas others suggest that these diagnostic criteria are not reliable enough; and (3) some participants question if IND is a truly separate entity or an acquired secondary phenomenon related to long-standing constipation or chronic obstruction. Therefore, we decided to include IND as one of ADHD.

The concept of chronic intestinal pseudo-obstruction (CIPO) including myopathy, neuropathy, collagenopathy (desmosis or fibrosis), and idiopathic.[9] Some CIPO patients are reported to be adult onset.[10] However, most myopathy and neuropathy types of CIPO cannot be diagnosed by conventional HE and AChE staining. Furthermore, abnormalities of Cajal cells shown by c-kit immunostaining were reported in some cases of CIIP.[11] Therefore, so far, we decided to treat CIIP as a disorder that shows recurrent or persistent functional intestinal obstruction with normal histology by conventional staining by HE and AchE. Because the diagnosis of Hirschsprung's disease is generally obtained by HE and AchE staining in the most institutes of Japan, the criteria of ADHD are recommended to be based on the conventional histology so far. MMIHS has been considered to be the severe form of CIIP. However, MMIHS can be distinguished from CIIP by clinical characteristics.[12,13]

**Table 5** Survival rate of allied disorders of Hirschsprung's disease.

| Abnormal ganglia | Survival rate |
|---|---|
| IG | 28/28 (100%) |
| HG | |
| Congenital HG | 70/90 (78%) |
| Acquired HG | 8/8 (100%) |
| IND | 11/11 (100%) |
| **Normal ganglia** | |
| MMIHS | 10/19 (53%) |
| SD | 27/27 (100%) |
| IASA | 3/3 (100%) |
| CIIP | 50/56 (89%) |

CIIP = chronic idiopathic intestinal pseudo-obstruction; HG = hypoganglionosis; IASA = internal anal sphincter achalasia; IG = immaturity of ganglia; IND = intestinal neuronal dysplasia; MMIHS = megacystis microcolon intestinal hypoperistalsis syndrome; SD = segmental dilatation.

**Table 6** Dietary status of three poor entities.

| | Survival rate | Normal diet in survivors | Normal diet in all cases |
|---|---|---|---|
| Congenital HG | 70/90 (78%) | 42/69 (60%) | 42/89 (48%) |
| CIIP | 50/56 (89%) | 13/50 (26%) | 13/56 (23%) |
| MMIHS | 10/19 (53%) | 1/10 (10%) | 1/19 (5%) |

CIIP = chronic idiopathic intestinal pseudo-obstruction; HG = hypoganglionosis; MMIHS = megacystis microcolon intestinal hypoperistalsis syndrome.

The entity of SD was proposed by Swenson and Rathauser in 1959.[14] The symptoms and signs of SD, especially sigmoid type of SD, resemble those of Hirschsprung's disease. Therefore, SD is included in ADHD in this study.

The entity of IASA has been considered to be synonymous of ultrashort-segment aganglionosis, which shows normal AchE staining but lacks rectoanal reflex.[15] This entity was discussed in *The 4th International Symposium on Hirschsprung's disease and related neurocristopathies* and was reported to exist.[8] Therefore, we decided that IASA is included in ADHD.

The numbers of cases in each disorder are summarized and compared with Okamoto and Toyosaka's[5] study in Table 2. The distributions of each disorder are similar in these two studies. The numbers of patients as well as the answer rates of criteria were very small in acquired HG and IASA. The rarity of disease is considered to make criteria difficult.

For definitive pathological diagnosis of ADHD, immnunohistochemical staining has been reported to be useful using neuronal and muscular markers, such as: Bcl-2 for immature neurons; CD56 for the size of enteric ganglia; synaptophysin for neuromuscular innervation: S-100 protein for Schwann cells; c-kit for interstitial cells of Cajal; and smooth muscle actin for myopathy.[16] The diagnosis of IG was easily obtained in several of our cases using Bcl-2 immunostaining (Figure 2).

In conclusion, almost all Japanese cases of ADHD for 10 years were collected in this study. Congenital HG and CIIP showed relatively high incidence, whereas acquired HG and IASA were extremely rare. Criteria of each institute were consisted with clinical signs, symptoms, and conventional histological examinations including AchE staining. Congenital HG, MMIHS, and CIIP showed poor survival rate. Further collection of precise data of each case is required to make guidelines for criteria and treatment strategies for ADHD.

Please cite this article in press as: Taguchi T, et al., The incidence and outcome of allied disorders of Hirschsprung's disease in Japan: Results from a nationwide survey, Asian Journal of Surgery (2015), http://dx.doi.org/10.1016/j.asjsur.2015.04.004

## Acknowledgments

This study was supported by a grant from The Ministry of Health, Labor Sciences Research Grants for Research on intractable disease (H23-042, H24-037, H26-045). The authors thank all members of The Japanese Society of Pediatric Surgeons, The Japanese Society of Pediatric Nutrition, Gastroenterology, and Hepatology, and The Japanese Study Group of Pediatric Constipation. The authors thank Dr Bryan Quinn for reading the manuscript and also thank Ms Masutomi and Ms Yamazaki for their help in processing the data.

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■ 特集 腸をもっと知る

# Immaturity of ganglia
## —全国アンケート調査からみた臨床像と今後の診断方法の展望—

家入里志* 三好きな*,** 小幡 聡* 神保教広*
永田公二* 宮田潤子* 小田義直** 田口智章*

### はじめに

Hirschsprung病類縁疾患（H類縁）の一つであるimmaturity of ganglia（IG）に関して，以下のような臨床的・病理学的特徴をもつと考えられている。臨床的特徴としては一般的に，①新生児期からイレウス症状を示し，②Ach-E活性は正常で，③注腸所見ではmicrocolon～small colonを示す，④新生児期では直腸肛門内圧検査では陰性を示すことが多いが，乳児期では正常化する，⑤meconium disease様形態を示すことが多い，⑥病変範囲は小腸に及び，⑦通常回腸瘻で排便機能が得られ，⑧数カ月後には神経細胞の成熟化とともに腸瘻を閉鎖でき良好な予後を示す。以上より，IGは新生児の機能性腸閉塞疾患のなかで独立した疾患としてのentityに分類されるべきと考える。

本疾患に関してわが国では岡本ら[1]が1987年の第24回日本小児外科学会でH類縁を初めてメインテーマとしてとりあげており，全国アンケート調査の結果を報告している。そのなかでIGに関しては，壁内神経細胞の数はあるが著しい未熟性のみを呈する疾患が存在することが報告されている。その後，岡本ら[3]の研究にてさらに詳細な調査研究が行われ，その報告書でもIGに関しては「壁内神経細胞未熟群は，大部分が肉眼的にmeconium ileus without mucoviscidosis，meconium diseaseの形態を示した。病理学的には検索された狭小小腸のみならず，拡張小腸においても壁内神経細胞の核・細胞とも小型で著しい未熟性を示した。この未熟群では回腸瘻造設後，数カ月後には壁内神経細胞の成熟化を示し，腸管運動機能の改善がみられ，小腸瘻閉鎖後はほぼ正常機能を示し，良好な予後を示した。別の面からみると，meconium ileus without muco-viscidosisの病態は壁内神経細胞の未熟性immaturity of gangliaにもとづくことが示唆された。名称として，壁内神経細胞未熟症（immature ganglionosis）が提案された。未熟群では，神経細胞の未熟性は胎生5～6カ月以下の未熟性を示した」と結論づけられている[3]。その後，病理学的に未熟な神経節細胞が経時的に成熟していくことは確認されたが[4]，まとまった臨床研究がなされることはなく，今回岡本班に続いて，田口ら[5]の研究とそれに引き続いて行われた，同じく田口ら[6]の研究のなかにおいて，わが国におけるIGの病態と臨床像を後方視的に詳細に解析・検討するとともに，自験例での病理学的解析をもとに本疾患の今後の診断方法の展望を解説する。

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** 同 形態機能病理学

### Ⅰ．調査研究方法

平成23年度研究班にて平成13～22年の10年間に対して一次調査を行い，症例数を集計した[5]。その後の平成24～25年度の研究班で新たな調査票を策定し，一次調査で回答の得られた施設にさらに詳細な二次調査用紙を郵送し結果を回収し

表 1 疾患別症例数（一次調査結果より）

| | 田口班（2012） | 岡本班（1996） |
|---|---|---|
| **normal ganglia** | | |
| CIPS | 100（28.3%） | 24（22.2%） |
| MMIHS | 33（9.3%） | 9（8.3%） |
| SD | 42（11.9%） | ND |
| IASA | 3（0.8%） | ND |
| **abnormal ganglia** | | |
| immaturity of ganglia | 28（7.9%） | 26（24.1%） |
| hypoganglionosis | 130（36.8%） | 44（40.8%） |
| congenital | 121（34.3%） | |
| acquired | 9（2.5%） | |
| IND | 17（4.8%） | 5（4.6%） |
| total | 353（100%） | 108（100%） |

表 2 症例の概要，男女比，在胎週数，出生体重

| | |
|---|---|
| 確診例 | 15 例 |
| 疑診例 | 13 例 |
| 男児 | 17 例 |
| 女児 | 11 例 |
| 在胎週数 | 37 週未満 11 例 |
| | 37 週～ 17 例（平均 36 週 3 日） |
| 出生体重 | 1,000 g 未満 2 例 |
| | 1,000～1,500 g 未満 4 例 |
| | 1,500～2,000 g 未満 2 例 |
| | 2,000～2,500 g 未満 6 例 |
| | 2,500～3,000 g 未満 6 例 |
| | 3,000 g 以上 8 例 |
| | （平均 2,392 g） |

表 3 発症時期・初発症状・合併奇形

| | |
|---|---|
| 発症時期 | 新生児期 27 例 |
| | 乳児期 1 例 |
| 初発症状 | 腹部膨満 21 例 |
| | 嘔吐 14 例 |
| | 胎便排泄遅延 8 例 |
| | 慢性便秘 4 例 |
| | 腸炎 3 例 |
| | 出生前に異常指摘 4 例 |
| 合併奇形 | なし 26 例 |
| | あり 2 例 |
| | （WEST 症候群＋EVAVS 症候群，腸間膜裂孔ヘルニア） |

表 4 染色体異常・遺伝子検査・家族歴

| | |
|---|---|
| 染色体異常 | 無 14 例 |
| | 未検査・不明 14 例 |
| 遺伝子検査 | 未施行 26 例 |
| | 不明 2 例 |
| 家族歴 | なし 19 例 |
| | あり 7 例 |
| | 双胎他児・いとこに同疾患：4 |
| | 胎便性腹膜炎：1，そのほか：2 |
| | 不明 2 例 |

た[6]。

## II．全国アンケート調査の結果

今回の二次調査で 353 例の H 類縁症例が集計され，28 例の IG 症例が得られた（**表 1**）。その内容としては確診例 15 例，疑診例 13 例であった。今回は疑診例 13 例を加えた全 28 例を対象として詳細な検討を行った（**表 2**）。

### 1．症例の概要

在胎週数は 37 週以後が 17 例，37 週未満が 11 例，出生体重は 1,000 g 未満 2 例，1,000～1,500 g 未満が 4 例，1,500～2,000 g 未満が 2 例，2,000～2,500 g 未満が 6 例，2,500 g 以上が 14 例と低出生体重児と，成熟時の比率は同等であった（表 2）。発症時期は新生児期が 27 例と多く，乳児期が 1 例であった。初発症状は腹部膨満が 21 例ともっとも多く，嘔吐が 14 例と続いた。胎便排泄遅延が 8 例，慢性便秘として発症したものも 4 例あった。合併奇形は少なく，2 例のみに認めた（**表 3**）。家族歴は 7 例に認め，双胎他児といとこに同疾患を認めたものが 4 例あった。染色体および遺伝子異常も無かほとんどが検索されていなかった（**表 4**）。

### 2．検査所見

腹部単純 X 線では腸管異常拡張を 22 例に，ニーボーを 3 例に，free air を 3 例に認めた。注腸造影は 21 例に施行され，micro colon を 13 例に，caliber change を 6 例に，megacolon を 1 例に認めた。直腸肛門内圧検査は 14 例に施行され，陽性 8 例，非定型陽性 2 例，陰性 4 例であった（**表 5**）。直腸粘膜生検は 9 例に施行され，うち 6 例は AchE 線維正常で，2 例に AchE 線維増強を認めた（**表 6**）。