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ORIGINAL ARTICLE

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# Acetylcholinesterase distribution and refractory constipation – a new criterion for diagnosis and management

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**Abstract** To describe manifestations of acetylcholinesterase (AchE) activity in the bowel of patients presenting with refractory constipation and correlate them with outcome, rectal biopsy specimens (RBS) from 165 patients who presented with refractory constipation between 1988 and 1999 were examined. Age at biopsy ranged from 4 days to 17 years; 45 subjects were excluded because they satisfied diagnostic criteria for Hirschsprung's disease, intestinal neuronal dysplasia, or hypoganglionosis. Thirty-five autopsy subjects were used as controls. All RBS were compared and AchE activity was assessed in the lamina propria (LP), muscularis mucosae (MM), and around the submucosal vessels (V). Variations in AchE distribution were classified as grade I (no AchE-positive nerve fibers in the LP or MM), grade II (some positive fibers in the LP or MM), grade III (moderate positive fibers in the LP or MM), grade IV (many positive fibers in the LP, MM, or V), or grade V (fibrillar, foamy, or amorphous staining for AchE). All grade I (11/120) and V (12/120) subjects achieved normal bowel control with laxatives alone and all grade II subjects (58/120) did with laxatives and enemas. Grade III subjects (34/120) required addition of cisapride. All grade IV subjects (5/120) were unresponsive to conservative management and 4/5 were found to have a megarectum, which was treated surgically. AchE distribution correlated well with eventual outcome and requirement for surgery. AchE distribution could also be used to classify bowel motility disorders, and we suggest the term AchE-positive disease be used to describe them.

**Keywords** Hirschsprung's disease · Intestinal neuronal dysplasia · Constipation · Acetylcholinesterase

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## Introduction

Refractory constipation is a common problem among children [9]. Almost all patients with constipation can be successfully managed conservatively with laxatives and/or enemas [4]. Nevertheless, it is generally accepted that some types of chronic constipation in childhood need to be treated surgically in order to relieve symptoms [12]. Patients are referred for rectal biopsy to exclude Hirschsprung's disease (HD) and HD-allied bowel motility disorders such as intestinal neuronal dysplasia (IND) and hypoganglionosis (HP) [5]. Some actually have HD or HD-allied bowel motility disorders that are due to a congenital abnormality of the intestinal neurons, while others do not have such disorders, but have congenitally impaired intestinal motor function that resembles HD.

The development of acetylcholinesterase (AchE) staining has facilitated the diagnosis of HD and HD-allied bowel motility disorders [13]. Some authors have reported that abnormal AchE activity can present with chronic constipation [12, 14]. The aim of this study was to describe manifestations of AchE activity in patients with constipation, and correlate them with clinical outcome.

## Materials and methods

We examined rectal biopsy specimens (RBS) from 165 patients who presented with refractory constipation to the Department of Pediatric Surgery, Juntendo University School of Medicine, and the Department of Surgery, Hokota Hospital, between 1 January 1988 and 31 December 1999.

All patients were diagnosed on the basis of RBS findings.

Forty-five were excluded because they satisfied diagnostic criteria for HD (n = 37), IND (n = 5), or HP (n = 3). The remaining 120 had normal ganglion cells in submucosal plexuses on hematoxylin and eosin (H & E) staining and normal or abnormal AchE activity on histochemistry. Each patient had a clinical assessment and a barium enema with delayed studies at 24 and 48 hours. Thirty-five autopsy subjects aged 1 day to 12 years with no evidence of gastrointestinal disease or tissue autolysis were used as controls.

Age at biopsy ranged from 4 days to 17 years. One RBS taken 2 cm above the dentate line from each patient was fixed in 10% phosphate-buffered formalin and embedded in paraffin wax. Forty serial sections (5 μm) were cut from each biopsy and stained with H & E for routine histologic examination. Another RBS, also taken 2 cm above the dentate line, was snap-frozen and a pair of cryostat sections (5 μm) was cut. AchE activity was demonstrated using the method reported by Lake et al. [8]. Rectal specimens from controls were taken at autopsy less than 4 hours after death.

AchE activity was assessed in the lamina propria (LP), muscularis mucosae (MM), and around submucosal vessels (V). Variations in AchE distribution were classified as grade I (no AchE-positive nerve fibers in the LP or MM, Fig. 1a and b); grade II (some AchE-positive fibers in the LP or MM, Fig. 2a and b); grade III (moderate AchE-positive fibers in the LP or MM, Fig. 3a and b); grade IV (many AchE-positive fibers in the LP, MM, or V Fig. 4a–c), and grade V (fibrillar, foamy, or amorphous staining for AchE, Fig. 5a and b).

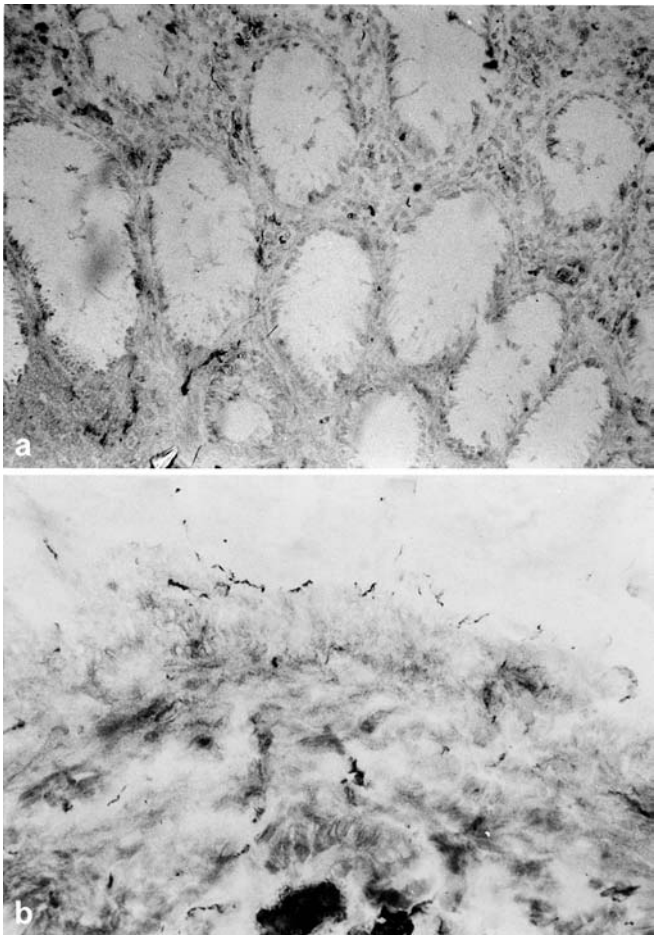


**Fig. 1a, b.** Grade I acetylcholinesterase (AchE) staining: no AchE-positive nerve fibers in **a** lamina propria (LP) and **b** muscularis mucosae (H & E, ×200)

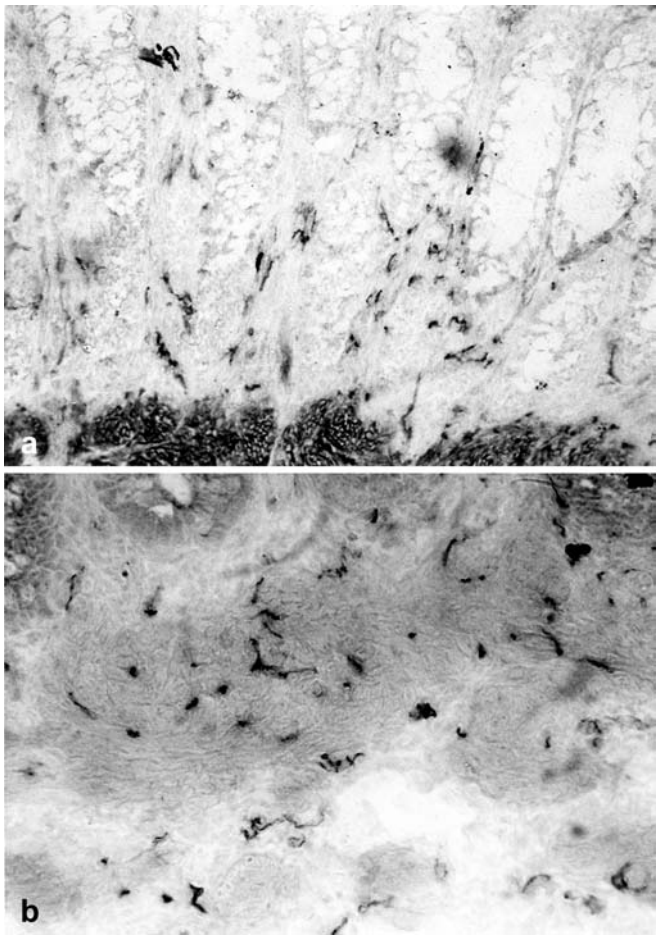


**Fig. 2a, b.** Grade II AchE staining: some AchE-positive fibers in **a** lamina propria, and **b** muscularis mucosae (H & E, ×200)

## Results

Of the controls, 7/35 had grade I AchE distribution, 23/35 had grade II, and 5/35 had grade V. In the 120 patients with constipation, AchE distribution was grade I in 11, grade II in 58, grade III in 34, grade IV in 5, and grade V in 12.

All subjects were treated initially with laxatives only, and then enemas were added. If still unresponsive after an arbitrary period of 8 months, cisapride was added.

Follow-up assessment showed that grade I patients achieved normal bowel control after a mean of 3.2 ± 2.1 months with laxatives alone and grade II patients did after a mean of 4.3 ± 2.4 months with laxatives and enemas. AchE distribution in grade I and II patients was similar to controls; barium enema findings were normal. Of 34 grade III subjects, 26 achieved normal bowel control within 8 months with laxatives and enemas; were given cisapride and had normal control within 1 year; 2 are still under treatment with laxatives, enemas, and cisapride. Barium enemas in these 2 subjects (3 and 13 years old) showed a dilated rectum and delayed transit time in excess of 48 hours.

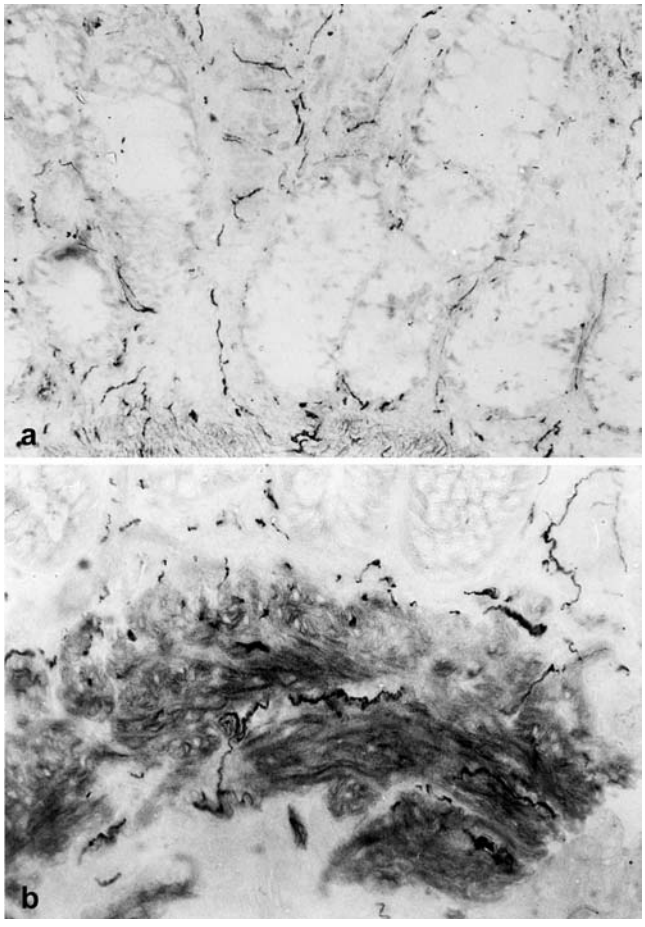


**Fig. 3a, b.** Grade III AchE staining: moderate numbers of AchE-positive fibers in **a** lamina propria and **b** muscularis mucosae (H & E, ×200)

All 5 grade IV subjects were refractory to medication and barium enemas showed a megarectum (Fig. 6) in 4 (13, 13, 16, and 17 years old). Three developed enteritis and abdominal distention. Megarectum subjects were treated surgically (internal sphincter myectomy in 3 and pull-through in 1). All are currently medication-free. One grade IV subject had a delayed transit time on barium enema without a megarectum and still requires aggressive medical management. This child is currently 5 years old. All 12 grade V subjects had only mild symptoms easily controlled by laxatives alone.

## Discussion

The differential diagnosis of patients with refractory constipation is made on the basis of characteristic clinical, radiologic, and histologic findings. However, a definitive diagnosis can only be made using histologic findings. Rectal biopsy is a well-accepted diagnostic procedure for identifying HD and HD-allied bowel motility disorders [13]. AchE immunohistochemistry gives valuable additional information for diagnosing bowel motility disorders. A markedly elevated AchE reaction in the mucosal layer can be used to make a

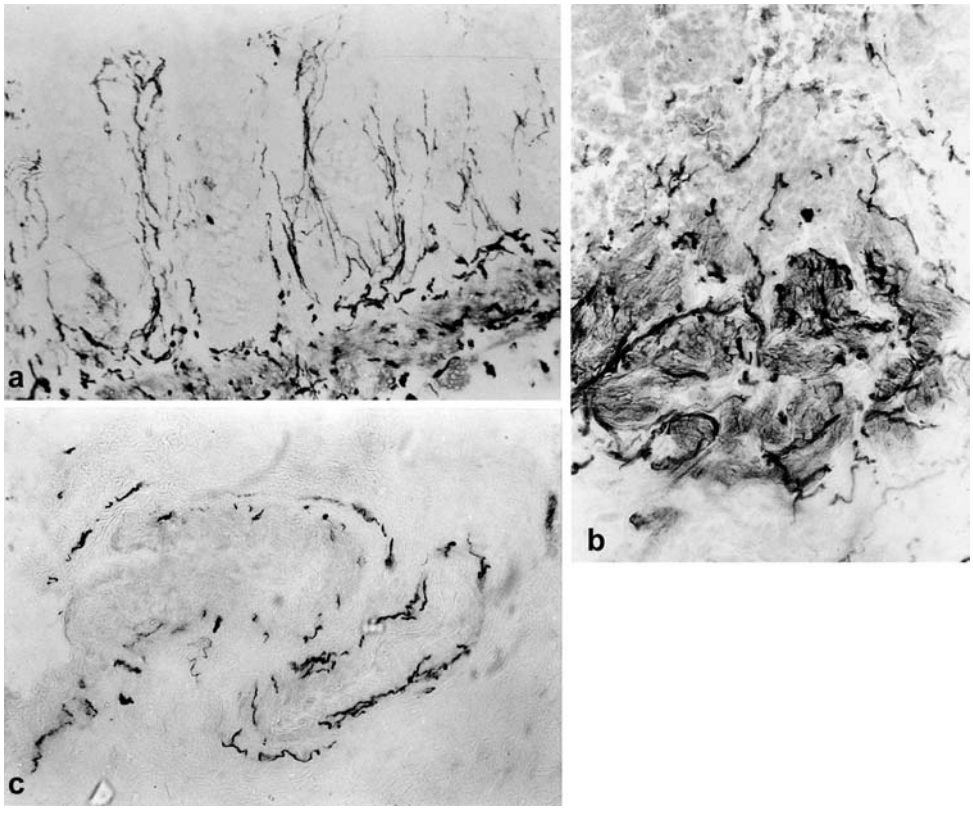


**Fig. 4a–c.** Grade IV AchE staining: many AchE-positive fibers in **a** lamina propria, **b** muscularis mucosae, and **c** around submucosal vessels (H & E, ×200)

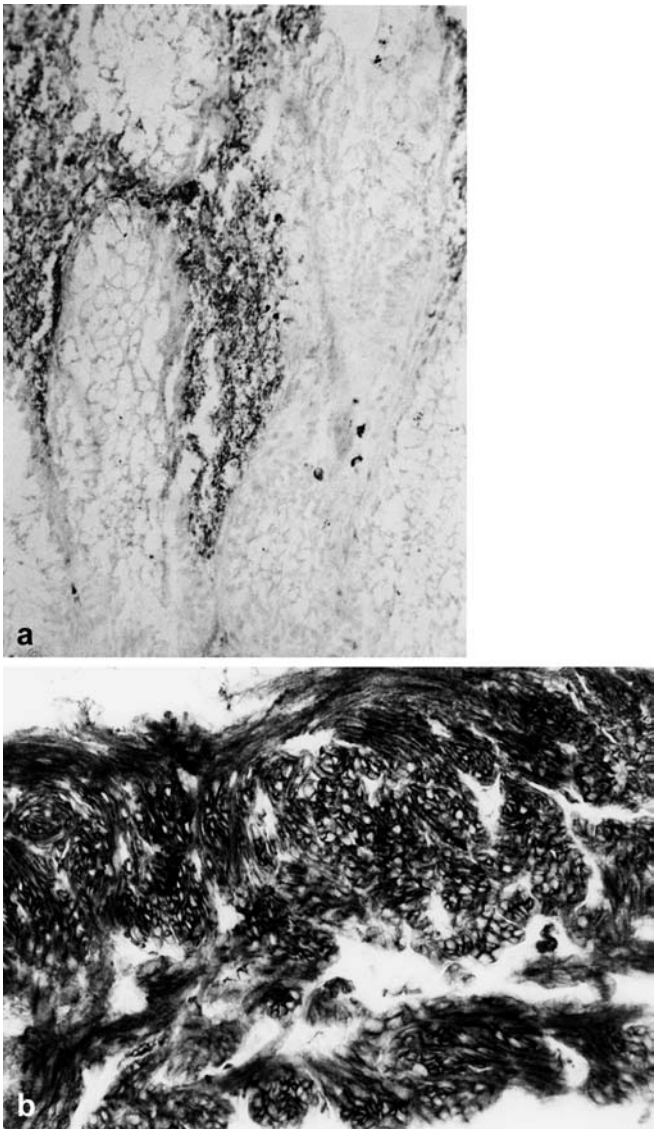


**Fig. 5a, b.** Grade V AchE staining: fibrillar, foamy, or amorphous staining in the **a** lamina propria, and **b** muscularis mucosae. (H & E, ×200)

diagnosis of HD and is strongly suggestive of IND [1, 11].

In our subjects with refractory constipation, 81/120 (67.5%) showed similar AchE staining patterns to control findings, i.e., grades I, II, or V. However, patients with AchE-positive staining such as grades III and IV had symptoms that were more severe and persistent compared with grades I, II, or V. In fact, 4/5 grade IV subjects required surgical management and 2 grade III subjects and the other grade IV subject are being considered for surgery.

Interestingly, although there was increased AchE activity around submucosal blood vessels (V) in grade IV patients, their clinical course was similar to that of IND patients published in the literature [10], and recently Borchard et al. showed increased AchE activity around V and recommended this as the most reliable

**Fig. 6.** Barium enema finding from grade IV patient with megarectum

diagnostic criterion for IND [2]. Although the clinical course of our grade III and IV patients was similar to that of IND patients published in the literature [3, 7], and could even be regarded as IND, we could not identify any IND-diagnostic histopathology such as giant ganglia and hyperganglionosis in the submucosa [3, 7, 10].

Many investigators have suggested that an increase in intestinal cholinergic activity may increase gut contractility, which in turn impairs gut motility, and we believe this is why grade III and IV patients had severe symptoms [6]. The question remains whether the abnormally increased AchE in grades III and IV is a primary or a secondary phenomenon. This question is often an issue in IND, and can only be answered by further investigation.

In conclusion, AchE distribution correlated well with eventual outcome and requirement for surgery. In particular, grade III and IV cases with a megarectum should be treated surgically even if ganglion cells are normal. AchE distribution could even be used to classify bowel motility disorders, and we suggest that the term AchE-positive disease be used to describe them.

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