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Short communication

Shrinkage of the myenteric neurons of the small intestine in patients with multiple system atrophy

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ABSTRACT

This study aimed to determine whether enteric neurons are involved in multiple system atrophy (MSA). Four-μm-thick slices of small intestine were prepared from 10%-formalin-fixed and paraffin-embedded materials obtained from autopsied cases. Enteric neurons were stained using an anti-peripherin antibody. Immunostaining of phosphorylated α-synuclein was also performed. Areas of the cytoplasm and nucleus that showed nucleoli were measured using computer software. Both areas of myenteric neurons were significantly smaller in MSA cases (n = 3) than in control subjects (n = 3) ($P < 0.0001$); however, no deposits of phosphorylated α-synuclein were observed. These findings suggest that myenteric neurons in MSA are affected independent of α-synuclein accumulation.

1. Introduction

Multiple system atrophy (MSA) is a neurodegenerative disorder characterized by cerebellar ataxia, parkinsonism, pyramidal tract signs, and autonomic dysfunction (Gilman et al., 2008). Patients with MSA frequently have gastrointestinal symptoms (Colosimo et al., 2010). Compromised gastrointestinal motility may underlie the gastrointestinal symptoms in MSA patients (Sakakibara et al., 2004; Tanaka et al., 2012). However, whether the enteric neurons responsible for gastrointestinal motility are involved in MSA remains to be elucidated.

Enteric neurons in submucosal and myenteric plexuses extend neural networks annularly and longitudinally throughout the gastrointestinal tract. The structural features of the enteric nervous system make performing neural cell counts in the gastrointestinal tract difficult during postmortem examinations. Aggregation of phosphorylated α-synuclein, which is the pathological hallmark of MSA in the central nervous system, is absent in the enteric neurons of patients with MSA (Nakamura et al., 2015). Therefore, alternative methods must be used when evaluating pathological changes in the enteric nervous system in MSA.

In this study, the sizes of the neurons in the myenteric and submucosal plexuses in patients with MSA were evaluated to determine whether enteric neurons are involved in the disease process of MSA.

2. Materials and methods

Four-μm-thick slices of small intestine were prepared from 10%-formalin-fixed and paraffin-embedded materials obtained from 3 pathologically confirmed cases of MSA (Table 1). Three age-matched individuals without any neurological disorders were included as controls. Enteric neurons were stained using anti-peripherin antibody (mouse monoclonal antibody, Ncl-Periph, Novo Castra; 1:50), which is a marker for the immunohistochemical study of morphological changes of enteric neurons (Szabolcs et al., 1996). Immunostaining using an antibody against phosphorylated α-synuclein (mouse monoclonal antibody, pSyn#64, Wako; 1:1000) was also performed to identify deposits or inclusions caused by aggregations of α-synuclein. Peripherin-immunolabeled areas of enteric neurons that showed nucleoli were measured with the polygon tool of computer imaging software (cellSens Ver. 1.6, Olympus Corporation, Tokyo, Japan). Statistical analysis was performed using SPSS Statistics ver. 24 (IBM, Chicago, IL, USA). In MSA cases, a semi-quantitative assessment of neuronal cell loss of the dorsal motor nucleus of the vagus nerve, which innervates the upper gastrointestinal tract including the small intestine, was also performed. Neuronal cell loss of the dorsal motor nucleus of the vagus nerve was assessed using sections stained with hematoxylin and eosin, and one of the following four grades was assigned to each case of MSA: absent (0),

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Table 1
Demographic characteristics of pathologically confirmed cases of MSA.

	Case 1	Case 2	Case 3
Age (y)	73	72	66
Sex	F	F	M
Clinical subtype	MSA-P	MSA-C	MSA-C
Disease duration (y)	19	8	13
Constipation	Yes	Yes	Yes
Gastrostomy	Yes	Yes	Yes
Duration of liquid food (y)	10	2	8
Final ADL	Bedridden	Bedridden	Bedridden
Severity of neuronal cell loss in DMV	3+	2+	3+
Pathology subtype[a]	Equivalent SND and OPCA pathology	Equivalent SND and OPCA pathology	Equivalent SND and OPCA pathology

MSA-P, multiple system atrophy with predominant parkinsonism; MSA-C, multiple system atrophy with predominant cerebellar ataxia; ADL, activities of daily living; DMV, dorsal motor nucleus of the vagus nerve; SND, striatonigral degeneration; OPCA, olivopontocerebellar atrophy.

[a] Categorized according to the published criteria (Ozawa et al., 2004).

slight (1+), moderate (2+), or severe (3+).

All study protocols were approved by the Ethics Committee of Niigata University School of Medicine.

3. Results

Enteric neurons in submucosal and myenteric plexuses in MSA and control subjects were stained with anti-peripherin antibody. No deposits or inclusions were immunolabeled by phosphorylated α-synuclein in the intestinal tissues examined in this study. The number of myenteric neurons evaluated was 143 in the 3 MSA cases and 131 in the 3 controls. The number of submucosal neurons evaluated was 187 in the 3 MSA cases and 155 in the 3 controls. The frequency distributions of the sizes of submucosal and myenteric neurons in MSA cases and control subjects are shown in Fig. 1. For submucosal neurons, the peak frequencies of the sizes in the peripherin-immunolabeled area (Fig. 1-A) and the nuclear area (Fig. 1-B) in MSA cases tended to be observed in the smaller size range compared in control subjects. Similarly, in myenteric neurons, the peak frequencies of the sizes in the peripherin-immunolabeled area (Fig. 1-C) and the nuclear area (Fig. 1-D) in MSA cases tended to be observed in the smaller size range compared in control subjects.

Because the data of the peripherin-immunolabeled area and the nuclear area in submucosal neurons and myenteric neurons showed non-normal distributions, a nonparametric test (Mann-Whitney *U* test) was used to compare median values of the peripherin-immunolabeled area and the nuclear area in enteric neurons between MSA cases and control subjects (Fig. 2). For submucosal neurons, the peripherin-immunolabeled area did not differ between MSA cases and control subjects (Fig. 2-A), whereas the nuclear area was smaller in MSA cases than in control subjects ($P = 0.048$) (Fig. 2-B). For myenteric neurons, both the peripherin-immunolabeled area (Fig. 2-C) and the nuclear area (Fig. 2-D) were significantly smaller in MSA cases than in control subjects ($P < 0.001$).

4. Discussion

The morphological characteristics of enteric neurons in the small intestine have been well established in the literature (Furness, 2006); therefore, the small intestine was chosen as a subject of this morphological study of enteric neurons in MSA. Since histological specimens of the small intestine are rarely obtained from living patients, postmortem examination results were used to explore pathological changes of enteric neurons in the small intestine in patients with MSA.

Because of the difficulties in assessing cell loss in the enteric nervous system, measures to assess the size of neurons in the myenteric and submucosal plexuses were adopted in the present study. Although this was a preliminary study, the results suggest that patients with MSA have shrinkage of myenteric neurons in the small intestine. However, no firm conclusion regarding the involvement of submucosal neurons in MSA can be made because the difference in the nuclear area between the MSA patients and control subjects was not robust ($P = 0.048$), and the peripherin-immunolabeled areas in the two groups were of similar size.

Because the dorsal motor nucleus of the vagus nerve is involved in MSA pathology, (Benarroch et al., 2006) vagal control of the gastrointestinal tract is thought to be disrupted in patients with MSA. In the present study, the occurrence of moderate to severe neuronal cell loss was confirmed in the dorsal motor nucleus of the vagus nerve in the MSA patients examined. The disruption of vagal control may insidiously compromise the activities of myenteric neurons in the gastrointestinal tract (Yoon et al., 1996). The cause of shrinkage of myenteric neurons is

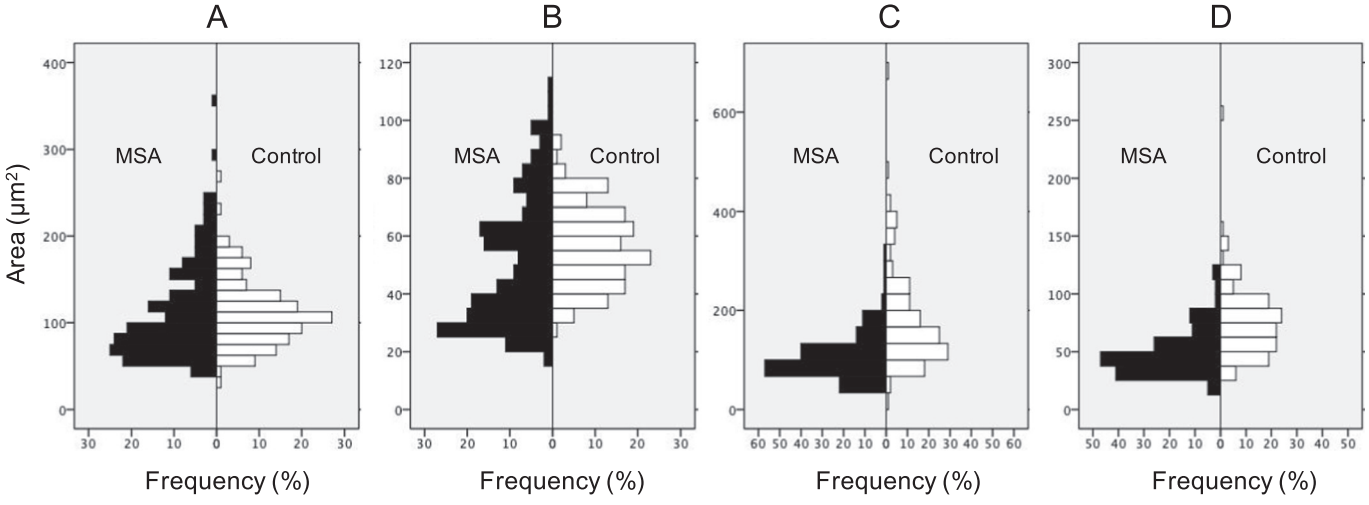

Fig. 1. Frequency distributions of the sizes of submucosal and myenteric neurons in MSA cases and control subjects. For submucosal neurons, the peak frequencies of the peripherin-immunolabeled area (A) and the nuclear area (B) in MSA cases are observed in the smaller size range when compared to those in control subjects. For myenteric neurons, the peak frequencies of the peripherin-immunolabeled area (C) and the nuclear area (D) in MSA cases are observed in the smaller size range when compared to those in control subjects.

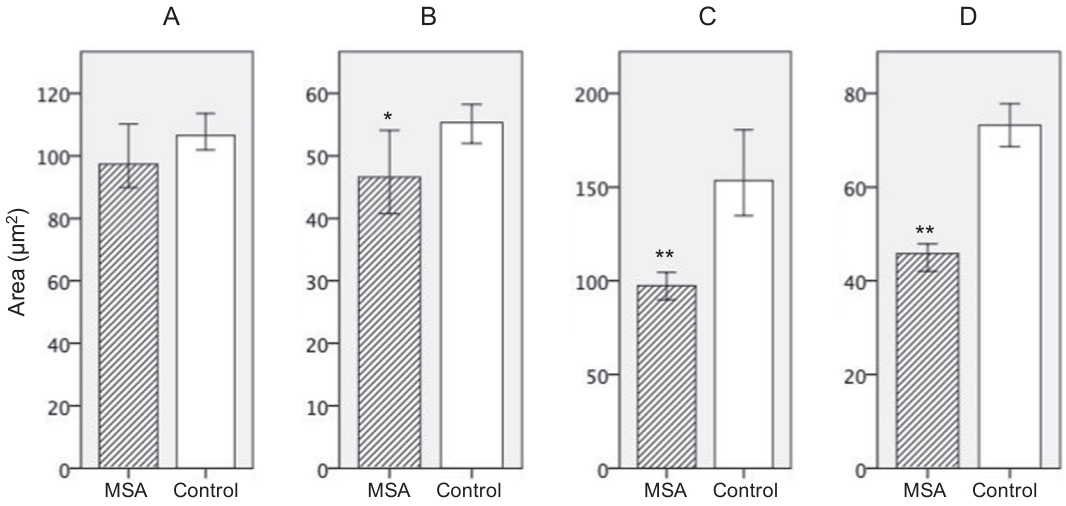

Fig. 2. Comparisons of the peripherin-immunolabeled area and the nuclear area in enteric neurons between MSA cases and control subjects. For submucosal neurons, the peripherin-immunolabeled area does not differ between MSA cases and control subjects (A), whereas the nuclear area is smaller in MSA cases than in control subjects (B). For myenteric neurons, both the peripherin-immunolabeled area (C) and the nuclear area (D) are significantly smaller in MSA cases than in control subjects. Graphs show median values and 95% confidence intervals. $*P = 0.048$ $**P < 0.001$.

unknown; however, the present result points to the need for further investigation to elucidate whether the shrinkage of myenteric neurons occurs secondary to disruption of vagal control of the gastrointestinal tract in MSA.

An alternative possibility is that the relative prevalence of large-sized myenteric neurons is decreased in the small intestine in patients with MSA. Experimental data obtained from guinea pig small intestines indicated that the majority of constituent neurons of the myenteric plexus are Dogiel type I neurons (Furness, 2006). The large-sized Dogiel type I neurons exist only in the myenteric plexus, and they correspond to the ascending interneurons that connect with excitatory motor neurons (Furness, 2006). In the present study, submucosal neurons were relatively preserved in MSA, in line with the possibility that large-sized myenteric neurons are vulnerable in MSA. Further immunohistochemical studies of myenteric neurons may help elucidate the neurochemical aspects of survival of myenteric neurons in patients with MSA.

MSA patients who participated in this study underwent gastrostomy because of dysphagia, and they took liquid food for years (Table 1). In general, taking liquid food may reduce the amount of undigested food residue, thereby decreasing digestive loads to the intestine. However, whether the fluidity of food exerts an effect on the morphological changes of the enteric nervous system remains to be established. The limitation of this study is that it was not possible to evaluate the strength of the effect of taking liquid food on the shrinkage of myenteric neurons in the small intestine.

MSA is categorized as a synucleinopathy based on the finding that α-synuclein accumulates in the central nervous system (Wakabayashi et al., 1998). The neuropathological hallmark of MSA is oligodendroglial cytoplasmic inclusion, (Papp et al., 1989) which is mainly composed of phosphorylated α-synuclein. Generally, in motor systems such as the olivopontocerebellar and striatonigral systems, the severity of neuronal cell loss correlates with increased numbers of oligodendroglial cytoplasmic inclusions (Ozawa et al., 2004). This suggests that the regional accumulation of phosphorylated α-synuclein is linked with the neurodegeneration in the central nervous system of MSA patients. Regarding the α-synuclein pathology in the peripheral nervous system, Nakamura et al. performed an immunohistochemical examination of α-synuclein aggregation in peripheral ganglia and the nervous system of visceral organs including the small intestine in patients with MSA, and they found accumulation of phosphorylated α-synuclein in the cytoplasm of Schwann cells, but not in the enteric nervous system of the small intestine in MSA patients (Nakamura et al., 2015). The present observation that no phosphorylated α-synuclein-immunolabeled deposits or inclusions were observed in the intestinal tissues examined was in keeping with the previous observation by Nakamura et al. Changes in the size of the myenteric neurons seen in the present study may reflect mechanisms independent of regional α-synuclein accumulation.

Further study is needed to determine whether changes in the size of myenteric neurons are linked to neurodegeneration in the enteric nervous system in MSA.

Declaration of competing interest

The authors have no conflicts of interest.

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