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CASE REPORT

**Riki Matsumoto · Nobuyuki Oka**
**Yasuhiro Nagahama · Ichiro Akiguchi · Jun Kimura**

# Peripheral neuropathy in late-onset Krabbe's disease: histochemical and ultrastructural findings

**Abstract** We performed histochemical and ultrastructural studies on a sural nerve biopsy specimen obtained from a 31-year-old man with late-onset Krabbe's disease. Myelin sheaths were uniformly thin for the fiber diameter, with a moderate reduction in the myelinated fiber population. Despite a few small onion-bulb formations, quantitative studies demonstrated uniformly thin myelin sheaths and less variability in internodal lengths. This suggests that the main pathophysiology of the peripheral neuropathy observed in late-onset Krabbe's disease is hypomyelination rather than segmental demyelination as in infantile Krabbe's disease. Ultrastructurally, curvilinear lamellar cytoplasmic inclusions were observed in Schwann cells and fibroblasts.

**Key words** Late-onset Krabbe's disease · Polyneuropathy · Hypomyelination · Cytoplasmic inclusion body

## Introduction

Krabbe's disease, also known as globoid cell leukodystrophy (GLD), is an inherited disorder of myelin metabolism caused by a deficiency of the lysosomal enzyme, galactocerebrosidase. Classic infantile Krabbe's disease is characterized by marked irritability and fluctuating muscle tone appearing within 3–6 months of birth. This is followed within a few weeks by opisthotonic posturing, visual failure, a loss of tendon reflexes, and hypertonic fits. Further psychomotor development ceases and death occurs 1–3 years later [5, 22].

R. Matsumoto (✉)
Department of Neurology, Osaka Red Cross Hospital,
5–53 Fudegasaki, Tenouji-ku Osaka 543, Japan
Tel.: 81-6-771-5131; Fax: 81-6-774-3239

N. Oka · Y. Nagahama · I. Akiguchi · J. Kimura
Department of Neurology, Faculty of Medicine,
Kyoto University, Shogoin Sakyo-ku, Kyoto 606-01, Japan

The peripheral neuropathy associated with infantile GLD has been demonstrated through light and electron microscopic observations of autopsy and biopsy materials [1, 7, 9, 12, 17–19, 21]. Light microscopy reveals segmental demyelination and a reduction in large myelinated fibers with a moderate increase in endoneurial collagenous tissue. Ultrastructurally, characteristic crystalloid and prismatic inclusions are encountered within Schwann cells and histiocytes. These inclusions are usually long and straight with clear contents, but are occasionally made of curvilinear lamellar materials.

Clinically a more heterogeneous, slowly progressive form of GLD has been recently reported and established as "late-onset" GLD with measurement of reduced galactocerebrosidase activity [3, 4, 8, 11, 13, 16, 24]. Clinical signs include pyramidal signs, intellectual impairment, cerebellar ataxia, optic nerve and tract disturbances, sensorimotor polyneuropathy, and pes cavus. In the present report, we described an adult case of late-onset Krabbe's disease where hypomyelination of myelinated fibers is suggested.

## Case report

A 31-year-old man first visited our hospital at the age of 26 years because of difficulty in walking. He had no significant past or neurologic family history. His birth and delivery were uncomplicated and his developmental milestones were normal. However, he had slowly developed a gait disturbance since the age of 9 years. On admission at 30 years in 1994, general physical examination was unremarkable except for scoliosis and pes cavus. Neurologic examination revealed a normal mental status and no cranial nerve abnormalities except for forced laughter. Muscle tone was normal in his upper extremities, but moderately increased in his lower extremities. In addition, muscle strength was mildly decreased in the distal portions of upper extremities and moderately decreased in his lower extremities. Muscle bulk was also diminished in his legs. There was no ataxia or involuntary movements. Deep tendon reflexes were brisk bilaterally with bilateral extensor plantar responses and sustained clonus at both the patellae and ankles. Sensory examination was normal. Additional neurologic abnormalities included spastic gait and spastic bladder. The results of routine hematologic and biochemical investigations were normal. Virus titers, including that for the anti-HTLV-1 antibody, were unre-

markable. The cerebrospinal fluid (CSF) protein concentration was elevated to 104 mg/dl and the CSF IgG concentration to 15.3 mg/dl with no oligoclonal bands. Other CSF values were normal. T2-weighted magnetic resonance images showed high signal intensity in the pyramidal tract and optic radiation. Nerve conduction studies revealed diffuse slowing of both motor and sensory nerves with prolonged F-wave latency. In addition, a focal demyelinating or axonal lesion was found to involve the median or peroneal nerve, respectively. Nerve conduction studies in his parents were normal. He was diagnosed as having GLD on the basis of a marked decrease in the leukocyte galactosylceramidase activity (0.147 nmol/ mg per hour; controls, 1.93–5.58 nmol/mg per hour).

## Materials and methods

After obtaining informed consent from the patient, we performed a sural nerve biopsy under local anesthesia. The resulting specimen was divided into three portions. A portion of the sample was embedded in epoxy resin (Epon) and examined by light and electron microscopy as described previously [10]. Another portion was used for fiber teasing. The remaining portion was fixed in 4% paraformaldehyde and routinely embedded in paraffin. Immunostaining for immunoglobulin deposits using anti-human IgG, IgA or IgM antibodies (Dako) was performed in deparaffinized sections [15]. Relative myelin thickness was assessed by measurement of *g* ratios (axon diameter/fiber diameter). Regression lines for the axon/fiber thickness relationship were calculated for each specimen. Control studies were performed in a sural nerve biopsy specimen from an age-matched control who had been referred for potential vasculitis, but in whom no peripheral nerve abnormality had been detected.

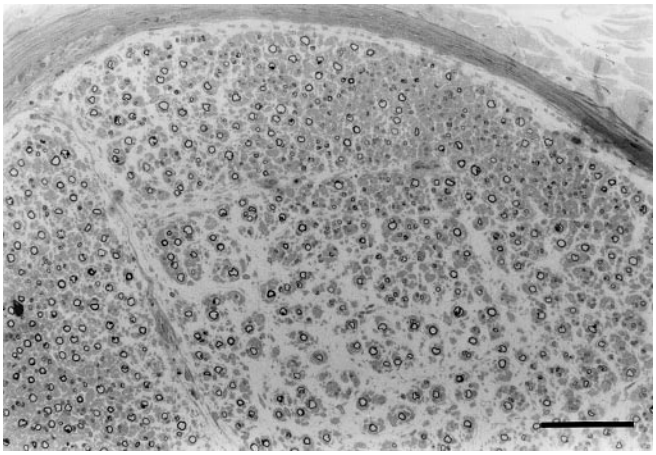

**Fig. 1** Transverse section of the sural nerve. A moderate reduction in myelinated fibers and uniformly thin myelin sheaths characterize the specimen from the patient. Note that the interstitial space in the endoneurium is partly enlarged mainly in the middle portion. *Bar* 100 μm

## Results

### Light microscopy

There was a moderate reduction in the number of myelinated fibers in the patient versus the control subject (2183/mm$^2$; control, 7070/mm$^2$; Fig. 1). A histogram of myelinated fiber diameters showed a unimodal distribution with a peak around 6 mm (Fig. 2). The *g* ratio was plotted against axon diameter, and *g* values were found to be higher than those in the control (Fig. 3a, b).

The teased preparation revealed no fibers undergoing axonal degeneration. Segmental changes in the myelin sheaths were rarely seen (3%). Measurement of the internodal lengths was often difficult in the patient, since most of the fibers had small diameters. However, there were no remarkable variations in individual fibers (Fig. 4), as observed in the control.

The interstitial space in the endoneurium was partly enlarged, mainly in the middle portion (Fig. 1). However, no inflammatory cells were seen and Congo red staining was negative. Immunostaining by antibodies against IgG, IgA or IgM was not present in the endoneurium.

### Electron microscopy

The majority of myelin sheaths were uniformly thin for the fiber diameters. A few small onion-bulb formations were observed (Fig. 5). In contrast, axons were well preserved, and Schwann cells were characterized by the presence of scattered or clustered abnormal cytoplasmic inclu-

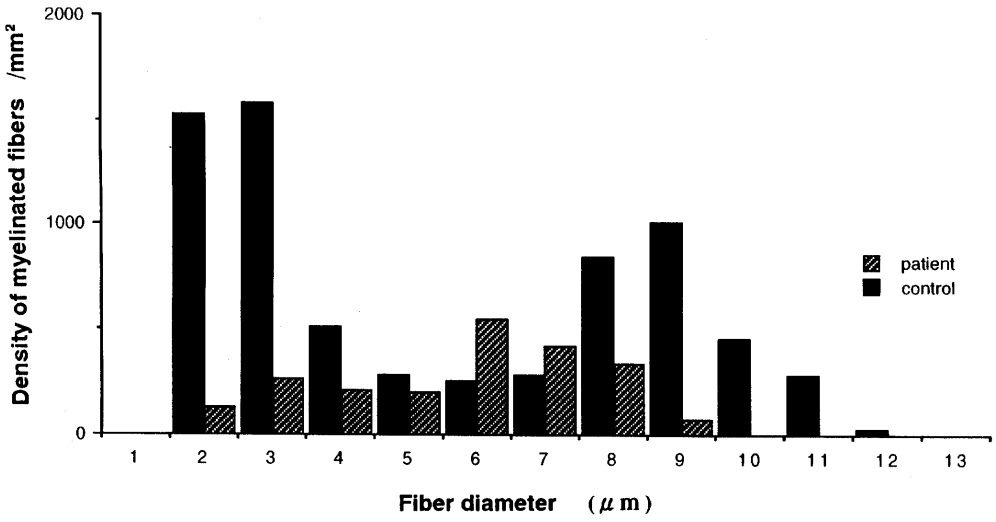


**Fig. 2** Histograms of myelinated fiber diameter measured in 1-mm-thick cross sections, showing a unimodal distribution with a peak around 6 mm. The control displays a bimodal distribution with peaks around 3 and 9 mm

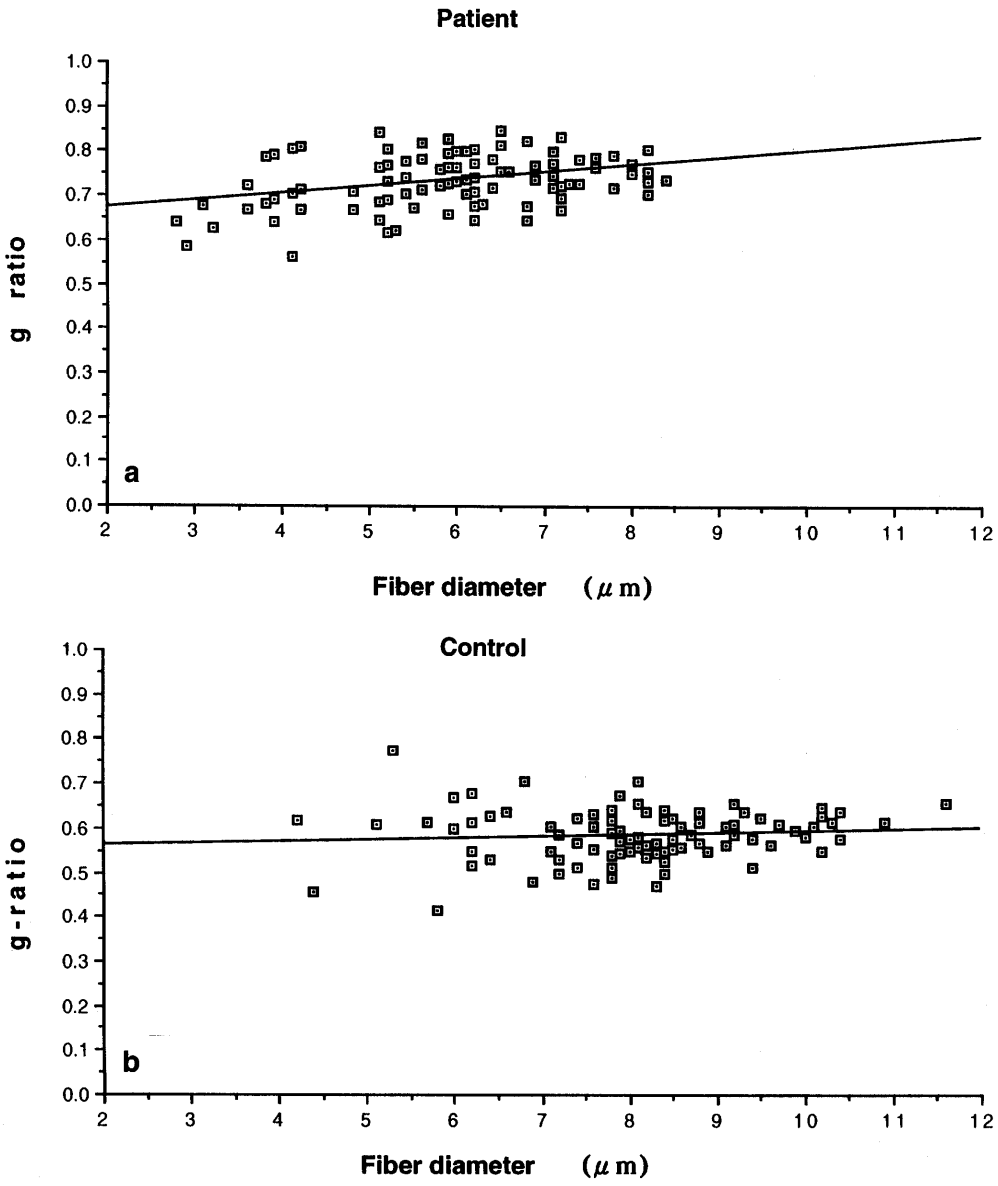


**Fig. 3** Scatterplots of the *g* ratio (axon diameter/fiber diameter) against fiber diameter. Patient *g* values (**a**) were higher than those for the control (**b**)

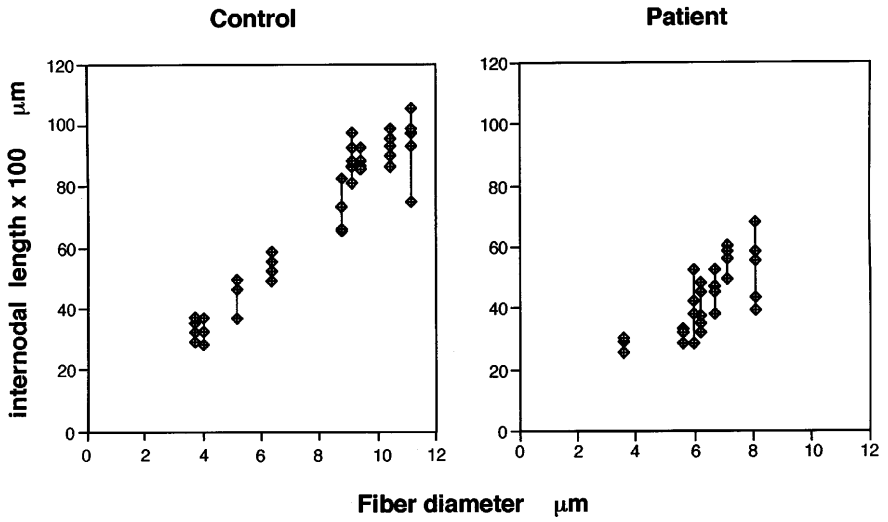


**Fig. 4** Internodal length measured in isolated fibers. *Vertical lines* join internodal lengths from the same fiber. There is a normal linear relationship between internodal length and fiber diameter in the control. A close to normal pattern is observed in the patient

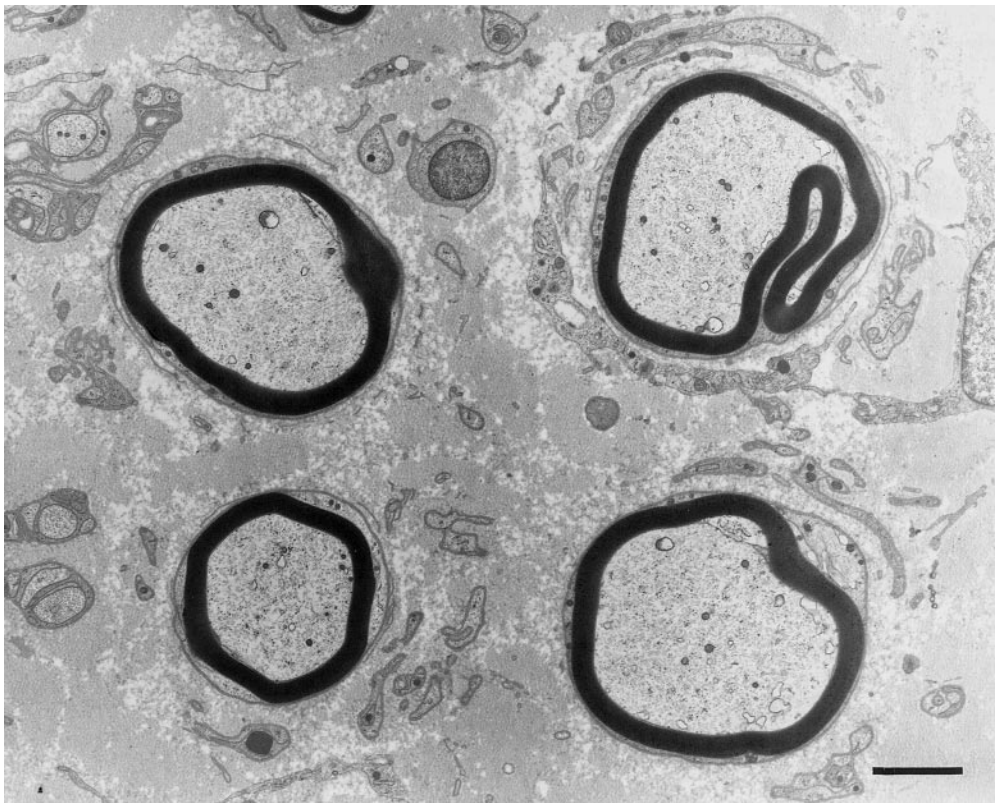

**Fig. 5** Electron micrograph showing small onion-bulb formations. Onion-bulb formations were not as frequently encountered as in infantile globoid cell leukodystrophy cases. *Bar* 1 μm

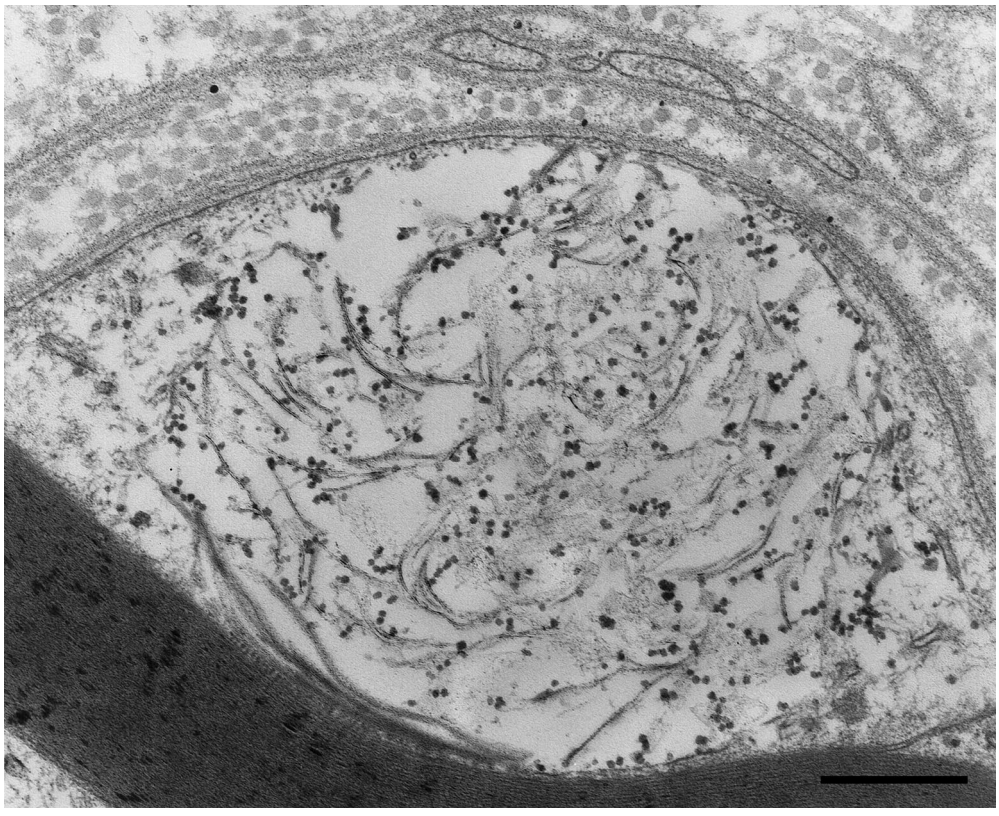

**Fig. 6** Electron micrograph of Schwann cell cytoplasm of a myelinated fiber. The cytoplasm contains inclusions, which are mainly curvilinear lamellar structures, with low-density granular material. *Bar* 0.5 μm

sions. The cytoplasmic inclusions were mainly curvilinear lamellar structures (Fig. 6). Occasionally tubular crystalloid materials were observed. These inclusions also were observed in fibroblasts. The interstitial space was enlarged and amorphous material mixed with fine fibrils was deposited there.

## Discussion

Previous reports of sural nerve biopsy or autopsy in cases of late-onset GLD have demonstrated segmental demyelination, disproportionately thin myelin sheaths with a moderate reduction in the myelinated fiber population, occasional onion-bulb formations, and cytoplasmic inclusion bodies in Schwann cells [2, 6, 8, 11, 13, 24, 25].

In the present case, the typical findings described above also were encountered except that no prominent segmental demyelination was found. The few small onion-bulb formations and the uniformly short internodal length may reflect some demyelination and remyelination. However, hypomyelination appears to be a key process, since all fibers in this chronic neuropathy have inappropriately thin myelin sheaths and none have normal thickness myelin. Even if demyelination occurs, the myelinating process is strongly suppressed. There may be some mechanisms preventing remyelination including that set forth in the psychosine hypothesis [14, 20, 23], stating that the accumulation of psychosine exerts a cytotoxic effect on myelination. This is the first case in which hypomyelination has been quantitatively assessed and reported among infantile and late-onset GLD case reports. The diffuse slowing of nerve conduction in both motor and sensory nerves further supports the presence of hypomyelination.

In previous studies, amorphous material has not been reported in both infantile and late-onset GLD cases except the description of “ground substance” in the extracellular matrix by Bischoff and Ulrich [1] in an infantile case of GLD. Though their”ground substance,” spotty condensations of granular masses, seems similar to the amorphous material we observed, the latter differs in its abundance. However, the endoneurial amorphous material may be a non-specific finding occasionally seen in chronic neuropathies.

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