GIANT AXONAL NEUROPATHY: REPORT OF TWO SIBLINGS WITH ENDOCRINOLOGICAL AND HISTOLOGICAL STUDIES

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Takebe, Y., Koide, N. and Takahashi, G.: Giant axonal neuropathy: Report of two siblings with endocrinological and histological studies. Neuropediatrics 12: 392-404 (1981). Giant axonal neuropathy in two siblings was reported. The fact that two cases are found in the same family supports this disorder is genetically determined and recessively inherited. These two cases, similar to the cases reported in literature, had chronic peripheral neuropathy and CNS symptoms, and also petit mal absence and mental retardation in elder sister (case 1) and precocious puberty in younger sister (case 2). Sural nerve biopsies in both cases disclosed axonal swellings or giant axons filled with aggregated neurofilaments, and that aggregated intermediate-sized filaments were found within cytoplasm of Schwann cells, endothelial cells of intra and extra-neurial capillaries and of extra-neurial arterioles, perineurial cells and endoneurial fibroblasts. Skin biopsies in both cases disclosed that aggregated intermediate-sized filaments were also found within cytoplasm of fibroblasts, Langerhans' cells, melanocytes and endothelial cells of capillaries, lymphatic vessels and arterioles. The diagnosis of giant axonal neuropathy can be made only by the findings in skin biopsy.

Two siblings with giant axonal neuropathy
endocrinological study vitamin B₁₂
fine structures of skin biopsy
intermediate-sized cytoplasmic filament

Introduction

Giant axonal neuropathy (GAN) was first described by Berg et al. and Asbury et al. (1972) in a 6-year-old girl presenting signs of chronic neuropathy and unique pathological findings of sural nerve, i.e., marked enlargement of axon with an increase in number of neurofilaments. Subsequently ten cases with GAN (Car

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The present cases were the second and third children of three siblings of related parents, with negative family history of neuromuscular disorders and with no history of exposure to chemical substances. The first female child died of unknown cause shortly after delivery.

The elder sister (case 1), born in Nov. 1964, was uneventfully delivered at full-term after normal pregnancy. The perinatal period was normal. She could stand up at 10 months of age and could walk at 18 months, when it was already noticed that she tumbled with abnormal frequency, followed by retarded motor development. She could run at the age of 7 years. Since the end of 7 years of age, she was noticed rapidly progressive disability of walking because of muscular weakness of extremities, especially in lower extremities and also noticed poor school performance. Her intelligence quotient (IQ, Suzuki-Binet’s test) was 81 at the age of 9 years. She was admitted to Hirosaki University Hospital at the age of 11 years and 2 months.

Physical examination on admission disclosed the general appearance of slightly dull but cooperative girl. She had tightly curled hair, a feature found only in her younger sister in her family. She was a slender girl, and her height and weight were 125 cm (88 %) and 23 kg (65 %) respectively. There were mild weakness and clumsiness of the upper extremities, and marked weakness, muscular wasting and hypotonia of the lower extremities with equino-deformities and with absent knee and ankle jerks and positive Babinski’s sign. Decreased sensation in the lower extremities, especially of vibration, was noted. Examination of cranial nerves revealed optic nerve atrophy with normal visual acuity, biphasic nystagmus and slurred speech. Cranial nerves under 7th were all normal. Mild dysmetria was present. There were moderate scoliosis and lordosis. Progressive mental retardation was suspected because of IQ 60 at the age of 12 years. She could walk by support with steps at the insides of feet. During follow-up study for four years and six months from her admission, she developed episodes of syncope varying from several times a day to a few times a week from the age of 11 years and 8 months. These seizures disappeared by administration of ethosuximide and phenobarbital soon after the onset. She showed slowly progressive muscular weakness and decreased sensation especially in lower extremities, and became dull and hypoactive in daily life and was restricted only in wheelchair at present (15 years and 8 months of age). She developed pubic hair and hypertrophy.
of the breast at the age of 12 years with menarche at the age of 15 years.

Routine laboratory tests during admission were all normal, including cerebrospinal fluid, serum ceruloplasmin, creatine kinase and aryl-sulfatase A activity in WBC.

The younger sister (case 2), born in Jan. 1969, first visited our pediatric clinic because of generalized muscular weakness, especially in the lower extremities at the age of 6 years and 11 months. The past history revealed uneventful delivery after normal pregnancy and normal development for the first 18 months of life. But milestones of motor development were delayed, e.g., gait soon after 24 months, followed by mild foot deformity of pes cavus of the left foot at the 5 years of age. The inability to walk was noted at the age of 8 years and 10 months because of rapid aggravation of foot deformity, though remaining the ability to waddle fast at 7 years of age. After corrective surgery for the deformed foot, the gait improved, resulting in attending school again. Sexual precocity was noted, i.e., development of pubic hair and hypertrophy of the breast at the age of 8 years and 6 months with menarche at the end of 8 years.

Physical examination at the age of 8 years and 10 months disclosed the general appearance of an alert girl with IQ 125. The height and weight were normal for this age. The visual acuity and optic fundi were normal with biphasic nystagmus. No dysmetria or dysarthria was present. Generalized hypotonia was noted, especially in the lower extremities more distally. The knee and ankle jerks were absent. The Romberg's phenomenon and Babinski's pathological reflexes were positive with touch insensitivity only in the big toes. The position sense was normal. Mild contracture of the right knee joint was present. The spinal column deformity was not present. After the operation, clinically stationary state had persisted for 2 years and 7 months, especially after administration of a large dosage of vitamin B₁₂ at present (11 years and 6 months).

Routine laboratory tests were all normal as in case 1.

Electrophysiological studies

The EEG in case 1 showed paroxysms of generalized high amplitude 3–4 Hz sp–w bursts and disorganized background activity in waking record with normal sleeping record on admission. These seizure discharges disappeared after administration of anti-epileptics at the age of 11 years and 9 months. But subsequently serial EEG records showed reappearance of these seizure discharges without absence seizures.

The EEG in case 2 was normal at the age of 7 years. But the EEG at the age of 8 years and 2 months showed generalized paroxysms of high amplitude 3–4 Hz slow wave bursts with disorganized background activity. And generalized 3–4 Hz sp–w bursts were observed from the end of 9 years and
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Table 1 Nerve conduction studies in siblings with giant axonal neuropathy

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<thead>
<tr>
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<th>Case 1</th>
<th>Case 2</th>
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<tr>
<td><strong>Motor conduction studies</strong></td>
<td></td>
<td></td>
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<tr>
<td>Age at testing</td>
<td>11y2m</td>
<td>13y6m</td>
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<tr>
<td>Rt. median nerve</td>
<td></td>
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<tr>
<td>Amplitude (mV)</td>
<td>3.9</td>
<td>4.6</td>
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<tr>
<td>Terminal latency (m/sec)</td>
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<td>5.8</td>
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<tr>
<td>Velocity (m/sec)</td>
<td>51.4</td>
<td>51.3</td>
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<tr>
<td>Rt. tibial nerve</td>
<td></td>
<td></td>
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<tr>
<td>Amplitude (mV)</td>
<td>0.2</td>
<td>n.d</td>
</tr>
<tr>
<td>Terminal latency (m/sec)</td>
<td>4.6</td>
<td>n.d</td>
</tr>
<tr>
<td>Velocity (m/sec)</td>
<td>40.0</td>
<td>n.d</td>
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**F wave conduction studies (Rt. median nerve)**

- **Velocity (m/sec)**
  - Elbow to wrist: n.d 51.7 50.0
  - Cord to elbow: n.d 40.8 39.0

Sensory potentials of rt. sural and rt. median nerves were not obtained in both cases.
(n.d: not done)

10 months without convulsive episodes or absence seizures so far.

Auditory and visual evoked responses performed at the age of 9 years and 6 months in case 2, and 13 years and 9 months in case 1 were all normal. Somatosensory evoked responses disclosed no primary components and normal secondary components with low amplitude in both cases.

The results of nerve conduction studies were detailed in Table I. The motor nerve conduction velocity of right median nerve was low normal in both cases. The velocity of tibial nerve was moderately low in case 1 and very low in case 2. Sensory potentials were not detectable in both cases. F wave conduction velocity in median nerve from elbow to wrist was 51.7 m/sec in case 1 and 50.0 m/sec in case 2, and that from cord to elbow was 40.8 m/sec in case 1 and 39.0 m/sec in case 2. The velocities of proximal parts were slower than those of distal parts in both cases.

**Endocrinological studies**

Endocrinological studies were performed at the age of 13 years and 1 month in case 1, and at 8 years and 10 months in case 2. Definite high levels were detected in serum prolactin, 75 ng/ml in case 1 and 25 ng/ml in case 2 (normal value for these ages, below 10 ng/ml). Elevated serum levels of triiodothyronine 275 ng/ml (normal, 70–180 ng/ml) and thyroxine 12.3 µg/ml (normal, 5–12 µg/ml), and increased urinary excretion of total human pituitary gonadotropin 32 IU/24 hrs (normal, below 12 IU/24 hrs) were obtained in case 2, while these hormones were within normal limits in case 1. Serum progesterone, and urinary excretion of 17 OHCS and 17 KS were normal in both cases. Abnormal hyper-responses to intravenous luteinizing hormone-releasing hormone loading test (10 µg/kg loaded in both cases) were observed, compared to age-matched control girls (Hoshina et al. 1977), i.e., increased excretion of not only luteinizing hormone and fol-
A large dosage of vitamin B\textsubscript{12} 1500 µg/day per os was tried for therapy of GAN from the time of the loading test on both cases. With the vitamin B\textsubscript{12} therapy, no improvement was observed in case 1 in the course of gradual progressive deterioration, but in case 2 clinical features, i.e., muscular weakness and touch insensitivity, seemed to be stationary without progression from our subjective viewpoint.

**Histological studies**

Sural nerve was biopsied at the age of 11 years and 3 months and skin of inguinal region at the age of 15 years and 3 months in case 1. Sural nerve and adjacent skin were biopsied at the age of 8 years and 10 months in case 2. The small tissue fragments were fixed with 2.5% glutaraldehyde in 0.1 M Na-cacodylate-buffer, postfixed with 2% osmium tetroxide in 0.1 M phosphate-buffer, en block stained in aqueous 1% uranyl acetate and were embedded in Epon. Ultra-thin sections were doubly stained with uranyl acetate and lead citrate. The number and diameter distribution of myelinated fibers of perineurial areas in case 2 were counted on electronmicrographs calibrated carbon grating replica. Some specimens were available for teased fiber preparation in case 2.
The biopsied sural nerve in case 1 disclosed reduction in number of the total myelinated fibers, especially of the large fibers. Ninety-nine percent of the myelinated fibers were under 6 μ in diameter. Onion bulb formations were common and a few fusiform axonal swellings were observed among these myelinated or un-myelinated fibers. The axonal swellings were usually 8 to 12 μ, up to 14 μ in diameter. An enlarged axon thinly myelinated was filled with tightly packed neurofilaments and a few neurotubules which were accumulated in neurofilament-free areas (Figs. 2 and 3). Increased neurofilaments were observed not only within the swollen axons but also within the normalized or even within the small axons. The aggregation with increase in

Fig. 2  Electronmicrograph of thinly myelinated swollen axon filled with tightly packed neurofilaments in longitudinal section of sural nerve. X4100, case 1

Fig. 3  Portion of myelinated swollen axon in cross section. The axon is filled with neurofilaments tightly packed at random in bundles in lower part of this photograph, and with accumulation of neurotubules near the axolemmal membrane. X21000, case 1
Fig. 4  
*Schwann* cells in nerves of skin tissues, showing aggregation with an increase of intermediate-sized filaments within cytoplasm. X11000, case 1

Fig. 5  
Higher magnification of boxed area in Fig. 4. X28000

Fig. 6  
Fibroblast in skin tissues showing markedly increased intermediate-sized filaments within cytoplasm. X8000, case 1

Fig. 7  
Higher magnification of boxed area in Fig. 6. X56000
intermediate-sized filaments was also observed within the cytoplasm of Schwann cells, endoneurial fibroblasts, perineurial cells and endothelial cells of capillaries and of extra-neurial arterioles. Abnormal Schwann cells with filamentous abnormality surrounded normal and/or abnormal axons, and normal Schwann cells did axons with filamentous abnormality. Electron dense granular substances were commonly observed in both swollen and normal-sized axons of myelinated or un-myelinated fibers. These granular substances usually occurred discretely in the aggregated neurofilaments. Markedly aggregated filaments were again observed within un-myelinated swollen axons and the cytoplasm of Schwann cells (Figs. 4 and 5), within the cytoplasm of fibroblasts (Figs. 6 and 7), Langerhans’ cells, melanocytes (Figs. 8 and 9) and endothelial cells of capillaries, lymphatic vessels and arterioles in biopsied skin tissues. The electron dense granular substances were found only in the axons with filamentous abnormality. The aggregation with increase in cytoplasmic filaments was frequently observed mostly in endothelial cells (over half of cells), Schwann cells (half of cells) and melanocytes (one of three to five cells). High magnification of the neurofilaments and filaments within the axons and cytoplasm re-

![Fig. 8](image1) Melanocytes in skin tissues showing cloudy condensation within cytoplasm. X4200, case 1

![Fig. 9](image2) Higher magnification of boxed area in Fig. 8. Filaments are tightly packed at random in bundles. X54000

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Fig. 10  Teased fiber of sural nerve in case 2, showing fusiform axonal swelling, measuring 80 μm in length and 20 μm in diameter, and thin myelin sheath in the swollen axon with myelin ovoid like figures and with segmental demyelination in the other portion.

vealed average 110 Å in axons, Schwann cells and melanocytes, 120 Å in endothelial cells, and 100 Å in fibroblasts.

In case 2, the nerve was slightly hypertrophic and teased fiber preparations disclosed fusiform axonal swellings, measuring 40 to 90 μm in length and 10 to 30 μm in diameter (Fig. 10). Several serial enlargements in the same axon were frequently observed. The myelin sheaths were thin or demyelinated in the swollen axons with myelin avoid like figures and segmental demyelination in the other portion. On electronmicrographs, myelinated nerve fibers were normal in the total number of 9134/mm². But ninety-nine percent of the observed fibers were under 5 μm in diameter (Fig. 11). An enlarged axons or giant axons were a few in number and filled with tightly packed neurofilaments with paucity of the other organelles, and

Fig. 11  Electronmicrograph of sural nerve in cross section. Myelinated fibers are slender and mostly under 5 μm in diameter. X1300, case 2.

Fig. 12  Thinnly myelinated giant axon filled with tightly packed neurofilaments with paucity of other organelles. X4100, case 2. Right lower inset shows boxed area at higher magnification, demonstrating mitochondria and accumulation of neurotubules. X27000
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Fig. 13 Un-myelinated swollen axon covered with processes of Schwann cells. The axon is filled with neurofilaments and with numerous electron dense substances of various size. X6900, case 2

Fig. 14 Higher magnification of boxed area in Fig. 13, showing electron dense substances and accumulated neurotubules in filament-free area. X41000

Fig. 15 Endothelial cell of capillary in skin tissues, showing aggregation with an increase of intermediate-sized filaments within cytoplasm. X8200, case 2

Fig. 16 Higher magnification of boxed area in Fig. 15
were myelinated or un(de)-myelinated as those in case 1 (Figs. 12 and 13). Discrete electron dense granular substances were seen in the axons as seen in case 1 (Fig. 14). Enormous aggregates of filaments were also observed within cytoplasm of following cells as in case 1, i.e., Schwann cells, fibroblasts, perineurial cells and endothelial cells of capillaries and of extra-neurial arterioles in biopsied sural nerve, and fibroblasts, melanocytes, Langerhans' cells and endothelial cells (Figs. 15 and 16) of capillaries, lymphatic vessels and arterioles in biopsied skin tissues.

Discussion

GAN had first been considered to be a unique entity mainly of peripheral neuropathy. Subsequent reports indicate that GAN is neuropathy involving the central nervous system (CNS). The two cases in this paper presented signs and symptoms of CNS involvement, and also sexual precocity in case 2 as in one case previously reported only by Igisu et al. (1976) so far. Peiffer et al. (1977) first clarified in their post-mortem studies that GAN is a generalized disorder of CNS, by demonstrating numerous axonal swellings or spheroids entirely filled with neurofilaments in the spinal cord, brain stem and cerebral cortex, with distended nerve cells in the anterior horn of the spinal cord.

Prineas et al. (1976) demonstrated for the first time that large, discrete masses of cytoplasmic filaments were observed in endoneurial fibroblasts, endothelial cells of endoneurial capillaries, Schwann cells and perineurial cells. They postulated that GAN is a generalized disorder of cytoplasmic filament formation. Such a cytoplasmic filamentous abnormality was also observed in these cells (Bolshauser et al. 1977, Larbrisseau et al. 1979, Koch et al. 1976, Gambarelli et al. 1977) and further in endomysial fibroblasts (Koch et al. 1976), and neurons and post-synaptic bags of myenteric plexus (Gambarelli et al. 1977).

Begeer et al. (1979) recently reported that an increase in cytoplasmic filaments averaging 90 Å in diameter were observed in Schwann cells, endoneurial fibroblasts, capillary endothelial cells and perineurial cells, associated with the increased number of filaments 90 Å in diameter in some axons, in the first sural nerve biopsy of an infant with clinical features of Seitelberger's infantile neuroaxonal dystrophy. And then they also mentioned that only very few endothelial and Schwann cells showed some increase in cytoplasmic filaments, while filaments were absent in endothelial and perineurial cells, in the second sural nerve biopsy after one year from the first one. They speculated that these findings were not a phenomenon exclusively occurring in patients with GAN, suggesting transitory features. We demonstrated first in biopsied skin tissues, that marked aggregation of cytoplasmic intermediate-sized filaments was observed in axons and Schwann cells, and in fibroblasts,
endothelial cells, melanocytes and Langerhans’ cells. In view of these findings, GAN is confirmed again to be a generalized disorder of cytoplasmic filament formation, probably caused by hyperplastic process of unknown origin, and probably associated with multifocal accumulation of neurofilaments in axons. These diffuse involvement of abnormal cytoplasmic filament formation would be a distinctive feature of GAN rather than giant axon by itself. The diagnosis of GAN can be made only by these findings in cutaneous tissue biopsy, not in sural nerve biopsy.

Koch et al. (1977) has proposed that the demyelination seen in the childhood form of GAN was due to both primary and secondary demyelinating process, as might be the cases in diabetic neuropathy (Powell et al. 1977). They demonstrated Schwann cells with increased cytoplasmic filaments and with lipid inclusions, and segmental demyelination associated with normal-appearing axons, in addition to numerous swollen axons with attenuated myelin sheaths and occurrence of focal swellings within short segment of demyelination in teased fiber preparations. These findings were again confirmed by us in part in teased fiber preparations. Peripheral neuropathy in GAN is probably caused by an increase in cytoplasmic filament formation diffusely in nerve cells, Schwann cells and axons. It is speculated in the present study on the basis of these findings that the varieties of signs and symptoms of CNS involvement in previously reported cases including ours may be caused by the degree of corresponding CNS lesions, and that endocrinological disorders in our cases may be caused by the involvement of hypothalamus-pituitary system.

The conduction velocity of F wave in the proximal part was significantly slower than that in the distal part in our cases. Such a result is occasionally observed in patients with diabetic neuropathy (Baba et al., unpublished data). It is also speculated in the present study that the effect of primary demyelination, probably of Schwann cell disease, may probably be more invasive in the proximal part of the peripheral nerves.

The family incidence of GAN was first reported in the present study, supporting autosomal recessive inheritance.

References
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