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Case Report

Neuropathology of the spinal nerve roots, spinal cord, and brain in the first autopsied case of Charcot–Marie–Tooth disease 4F with a D651N mutation in the periaxin gene

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Charcot–Marie–Tooth disease (CMT) 4F is an autosomal recessive, hereditary peripheral neuropathy, mostly caused by mutations in the periaxin gene (*PRX*). This article reports neuropathological findings of the spinal nerve roots, spinal cord, and brain of a patient with CMT4F and a D651N missense mutation in *PRX*. The patient was a 74-year-old woman who had a history of peripheral neuropathy with onset at the age of 30 years. She also had a history of infantile paralysis at the age of 18 months. The most pronounced autopsy finding was diffuse enlargement of anterior and posterior nerve roots, accentuated at the lumbo-sacral levels. On microscopy, the swollen nerve roots showed a loss of large-diameter myelinated fibers and formation of numerous onion bulbs. Most of the onion bulbs lacked the central, regenerating thin myelin sheaths, and in large-diameter nerve fibers whose axons had been lost, collagen fibers occupied the center of the onion bulbs. Some nerve roots formed glial bundles at the proximal end. The spinal cord showed degeneration of the gracile fascicles, and the lumbar segment anterior horn showed an asymmetric neuronal loss with rarefaction of the neuropil. The brain did not show any notable changes except for multiple foci of a radial microcolumnar arrangement of neurons in the cerebral cortex. Degeneration of the lumbar segment anterior horn is most likely secondary to the anterior radiculopathy, but a localized circulatory disturbance is another possibility.

Key words: anterior horn, Charcot–Marie–Tooth disease 4F, neuronal loss, onion bulb lesion, spinal nerve roots.

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INTRODUCTION

Charcot–Marie–Tooth disease (CMT) is a heterogeneous group of hereditary sensory and motor neuropathies that are subdivided into demyelinating (CMT1, CMT4, and CMTX) and axonal (CMT2) forms based on the patterns of inheritance and electrophysiological findings.[1–5] CMT4 shows autosomal recessive inheritance and consists of several subtypes, from CMT4A to CMT4H.[2–5]Among them, CMT4F, which is mostly caused by putative loss-of-function mutations in the periaxin gene (*PRX*),[2,3,6–10] is a rare disorder and comprises only about 2% of genetically diagnosed cases of CMT.[5]

Tokunaga *et al.* reported three clinical cases of CMT4F with an extensive survey of *PRX* profiles.[10] In one of the three cases, a novel missense homozygous aspartate (Asp) to asparagine (Asn) substitution at codon 651 (D651N) in *PRX* was detected.[10] The patient died after this report was published at the age of 74 years. Although clinical findings, genetic profiles, and pathological findings of peripheral nerves of the patient have already been reported,[10,11] herein we present some neuropathological findings of the spinal nerve roots, spinal cord, and brain of this first autopsied case of CMT4F with D651N mutation in *PRX*.

Although several review articles on the pathological findings of peripheral nerves in CMT have been published,[1–4,12] detailed descriptions of neuropathological findings of the spinal nerve roots and the central nervous system (CNS) in CMT are scarce except for reviews by Hughes and Brownell[13] and Smith *et al.*[14], partly because the disease is not fatal by itself, despite the often very long clinical course.

CLINICAL SUMMARY

Because the clinical history was documented in a previous article (case 1),[10] only a brief summary is provided here. The patient was a 74-year-old woman at the time of death,

and had a past history of infantile paralysis at the age of 18 months although details are unknown. She developed muscle weakness of the legs and distal dominant sensory impairment at the age of 30 years, and the symptoms progressed very slowly thereafter. She became wheelchair-bound when she was 65 years old and died of suffocation due to aspiration of food at the age of 74 years. Throughout the clinical course spanning about 44 years, she did not exhibit any cranial nerve symptoms, cerebellar symptoms, or cognitive impairment. She had never complained of dyspnea, dysarthria, or dysphagia. The chief general pathological findings at autopsy, performed 44 h postmortem, were acute pulmonary edema associated with aspirated materials within the bronchial trees, diffuse adenomatous goiter with chronic lymphocytic thyroiditis (the thyroid gland weight: 43 g), and chronic cystitis. No apparent pathological alterations were noted in peripheral autonomic nerves within the thoracic and abdominal viscera. The diaphragm showed mild neurogenic atrophy.

NEUROPATHOLOGICAL FINDINGS

Spinal nerve roots and spinal cord

The most pronounced finding was diffuse enlargement of both anterior and posterior nerve roots called hypertrophic radiculopathy which was accentuated at the lumbosacral levels where the diameters of nerve roots reached approximately 3 mm (Fig. 1). The proximal end of nerve roots, ranging from 5 to 7 mm, was slightly thinner than the more distal portion. On microscopic examination, nerve fibers composing the roots exhibited marked concentric arrangement and proliferation of Schwann cells associated with an increase in endoneurial collagen fibers, forming onion bulb lesions (Figs 2, 3A). On staining with Luxol fast blue (LFB)-periodic acid-Schiff (PAS), a large proportion of both large- and small-diameter fibers showed demyelination, but the distribution of areas showing myelin loss was not uniform and varied among sites. In the most severely affected areas, almost no myelin sheaths remained (Fig. 3B). In many large-diameter fibers, the axons were lost, and the center of the onion bulbs was occupied by collagen fibers, called denervated onion bulbs. On immunohistochemistry for phosphorylated neurofilament protein (p-NFP) (mouse monoclonal, clone SMI31; BioLegend, San Diego, CA, USA; 1:500), the paucity of large-diameter myelinated fibers could be clearly demonstrated (Fig. 3C), and large-diameter fibers devoid of axons tended to form loose aggregates (Fig. 3D). Immunohistochemistry for glial fibrillary acidic protein (GFAP) (mouse monoclonal, clone EP672Y; Roche Diagnostics, Rotkreuz, Switzerland; prediluted) revealed the formation of glial bundles at the proximal end of some anterior and posterior nerve roots (Fig. 4). No lymphoplasmacytic infiltration was detected within the nerve roots. Although upper segments of the cervical cord were not available for examination, the anterior roots at

Fig 1 Gross findings of the spinal cord. The dorsal aspect of the formalin-fixed lumbo-sacral segments show diffuse enlargement of both anterior and posterior nerve roots. Note that the most proximal portion of each root is slightly thinner than the more distal portion.

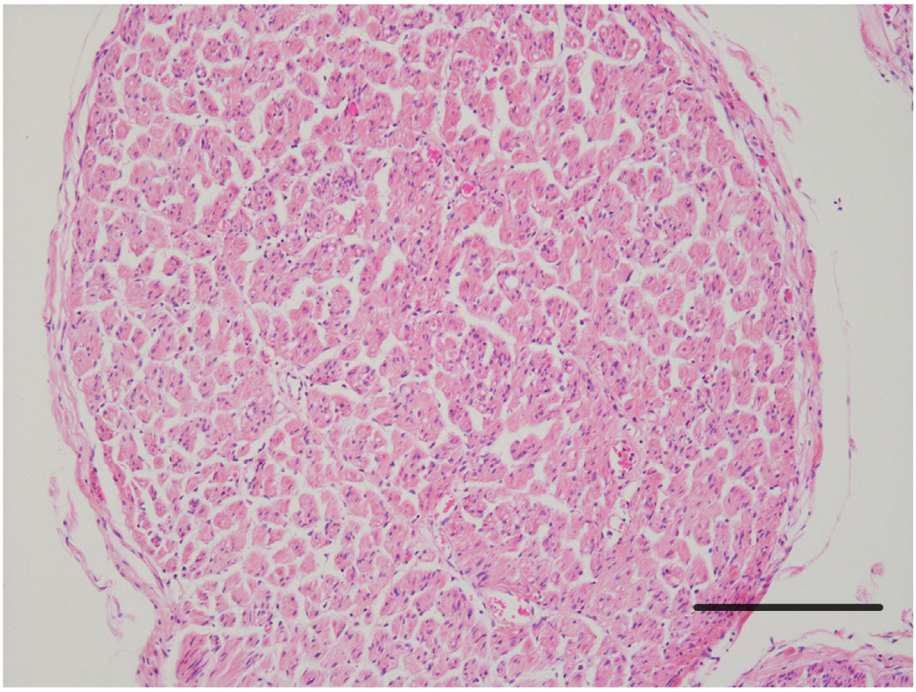

Fig 2 A cross section of a lumbar anterior root. Numerous onion bulbs are observed there. HE staining. Scale bar: 200 μm.

these levels were considered to be involved, based on the fact that the diaphragm showed mild neurogenic atrophy.

On toluidine blue-stained semithin sections of glutaraldehyde-fixed, osmium tetroxide-postfixed, Epon-embedded blocks, the formation of numerous onin bulbs and a marked loss of large-diameter myelinated fibers were observed (Fig. 5A). Regenerating thin myelin sheaths at the center of the onion bulbs were often absent, and the central region

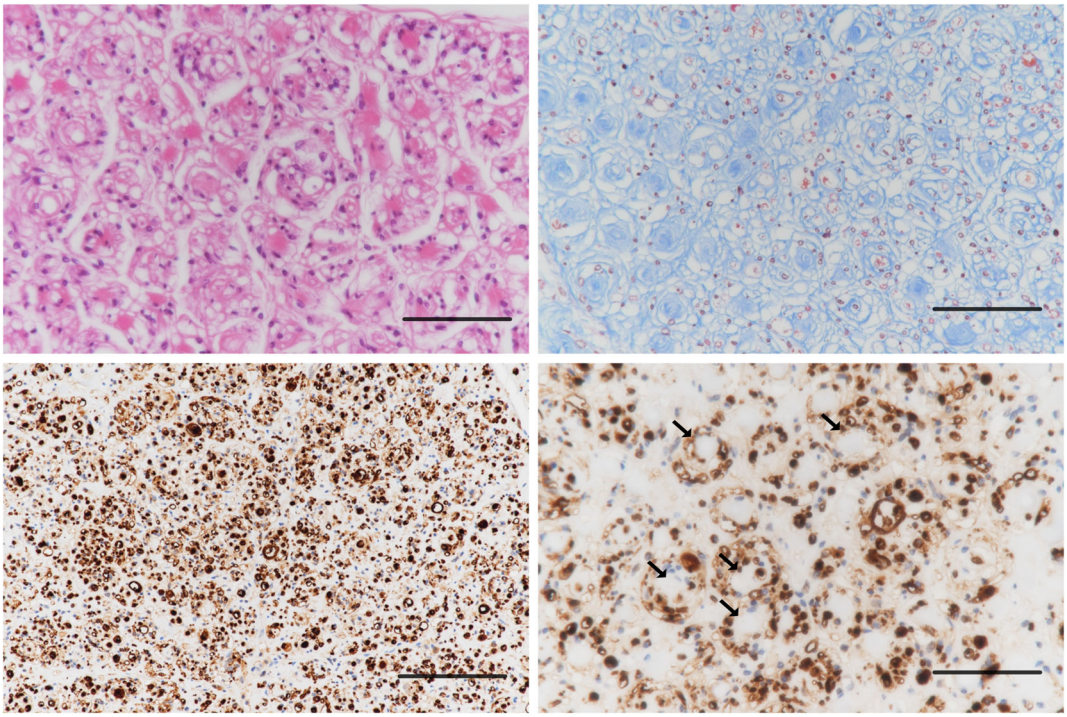

Fig 3 Cross sections of a lumbar anterior root. (A) Marked onion bulbs are seen. The center of some onion bulbs is deeply eosinophilic, suggesting that axonal loss and the replacement by collagen fibers. (B) In this area, myelin sheaths are almost completely lost. (C) The paucity of large fibers is evident. Small nerve fibers are relatively preserved. (D) Many large fibers, whose axons are lost, form loose aggregates. Some denervated onion bulbs are indicated by small arrows. HE (A), LFB-PAS (B), immunohistochemistry for p-NFP (C, D). Scale bars: 50 μm (A, B, D), 100 μm (C).

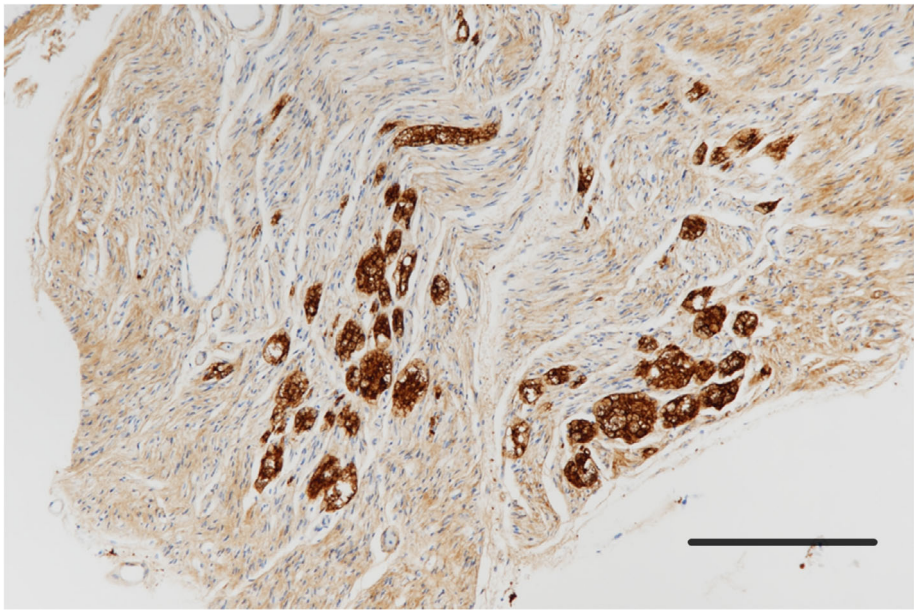

Fig 4 Immunohistochemical observations of GFAP in a spinal nerve root. GFAP-immunoreactive glial bundles are seen at the proximal end of of the root. Scale bar: 200 μm.

was occupied by a demyelinated axon or, when the axons were lost, replaced by dense accumulates of collagen fibers or basal lamina materials (Fig. 5B). It was difficult to identify a border between axons and collagen fibers on the toluidine blue–stained semithin sections. No evidence of the ongoing myelin breakdown, evidenced by myelin ovoid, was observed. Some small fibers showed simple thickening of the myelin sheaths, but no apparent myelin infolding or outfolding was observed. In the areas where most nerve fibers had been lost, cytoplasmic processes of Schwann cells appeared to form fine reticular structures.

The spinal cord showed bilateral degeneration of the posterior columns, especially gracile fascicles. The lumbar cord showed asymmetrical atrophy of the anterior horn that had produced a deformity of the contour of the cord (Fig. 6A). The more severely atrophic side showed a marked loss of anterior horn cells, rarefaction of the neuropil, and the infiltration of macrophages, but reactive astrocytosis was mild (Fig. 6B). None of the chromatolytic neuron, microglial nodule, perivascular lymphocytic infiltrate, or plaque-like gliotic scar was observed. As an incidental finding, a small, round focus of heterotopic gray matter was found in the anterior columns of the upper thoracic cord. Neurons in the dorsal root ganglia (DRG) were well preserved, and no Nageotte's nodule was seen.

Brain

The brain weighed 1290 g and showed no marked change on gross examination. Enlargement of cranial nerves was not observed. On microscopy, neurons in the cerebral cortex multifocally showed a radial microcolumnar arrangement mainly in the broad crown areas of the cerebral gyri in the frontal, temporal, and occipital lobes (Fig. 7A).

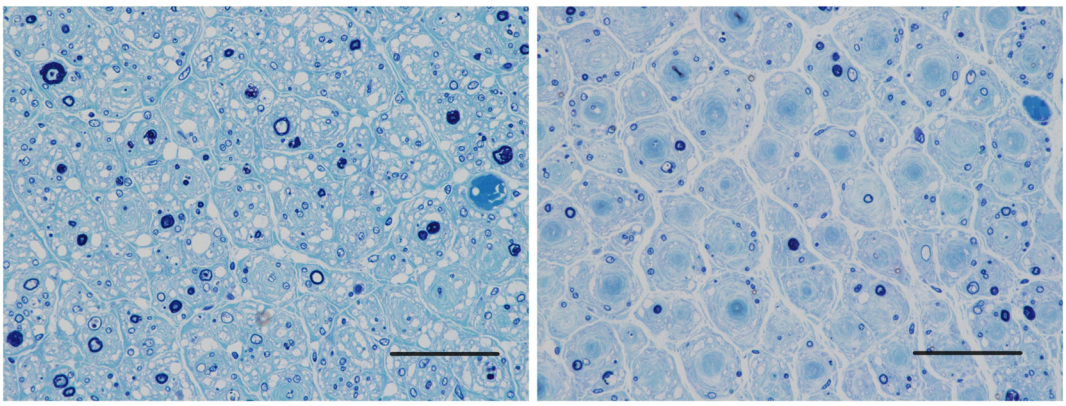

Fig 5 Toluidine blue-stained cross semithin sections of a nerve roots. (A) In the posterior root, myelinated, particularly large-diameter fibers are markedly lost, and numerous onion bulbs are formed. (B) In some large fibers of the anterior root, the axons are lost and replaced by collagen fibers. Scale bars: 50 μm (A, B).

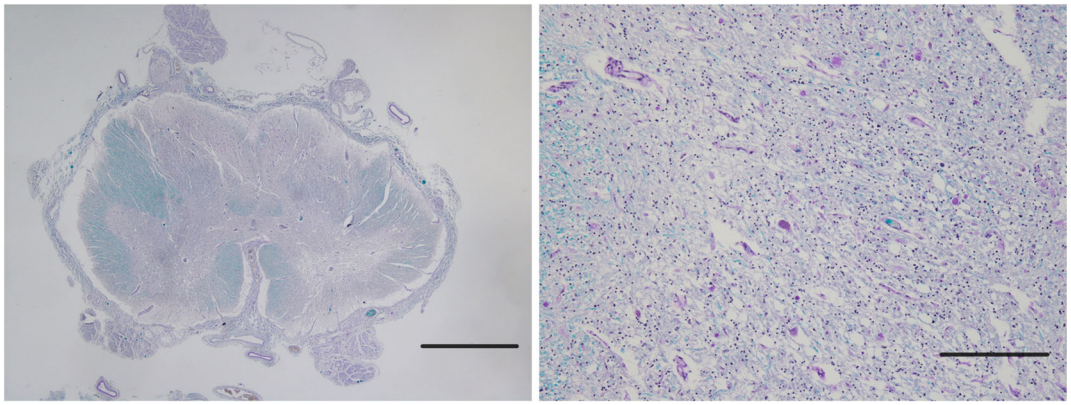

Fig 6 Microphographs of the spinal cord sections stained with LFB-PAS. (A) The lumbar segment demonstrates asymmetric atrophy and degeneration of the anterior horns as well as bilateral degeneration of the posterior columns are observed. (B) The lumbar anterior horn demonstrates a marked neuronal loss with rarefaction of the neuropil. Reactive astrocytosis is mild. Scale bars: 2 mm (A), 200 μm (B).

The polarity of each neuron was well oriented (Fig. 7B), and neither dysmorphic nor ballooned neurons were observed. No heterotopic neurons were present in the subcortical white matter. Mild microvacuolar changes of the neuropil associated with hypertrophic astrocytosis were found in the superficial layers of the parietal and occipital cortices. Neither hypoxic nor senile changes were observed. The basal ganglia, thalamus, and cerebellum showed no significant abnormalities, and the cranial nerve nuclei in the brainstem appeared normal.

DISCUSSION

CMT4F is an autosomal recessive disorder, mostly caused by mutations in *PRX* located on chromosome 19q13 that encodes periaxin.[2,3,6–10] Periaxin is a 147 kDa protein that interacts with dystroglycan complex 2 and links Schwann cell cytoskeleton to the extracellular matrix including the basal lamina.[9,15,16] It is expressed in myelinating Schwann cells,[3,15,17] and its localization dynamically shifts from the adaxonal membranes to the abaxonal membranes, Schmidt–Lanterman incisures, and paranodal membranes during normal myelination as well as remyelination following demyelination.[3,15,17] Mutations in *PRX* cause dissociation of the terminal myelin loops from the axons and loss of transverse bands as the initial changes.[7]

Patients with CMT4F display a variety of distal sensory disturbances, in addition to motor dysfunctions, and the clinical course is usually very prolonged.[2,3,6,8–10] Some patients with *PRX* mutation show a clinical phenotype consistent with Dejerine–Sottas neuropathy.[9,18] In peripheral nerves, onion bulb formation, focal myelin thickening, and hypermyelinated outfoldings associated with axonal compression are described as the characteristic findings.[2,6,8,9] The number of myelinated nerve fibers is markedly reduced, and periaxin is not immunohistochemically detected in the affected nerve fibers, whereas it is present in control samples.[6] However, to the best of our knowledge, pathological findings of the spinal nerve roots or CNS lesions in CMT4F have never been documented in the literature.

The present case is the first autopsied case of CMT4F in which a missense D651N mutation in *PRX* is demonstrated.[10] Previously reported mutations in *PRX* were of

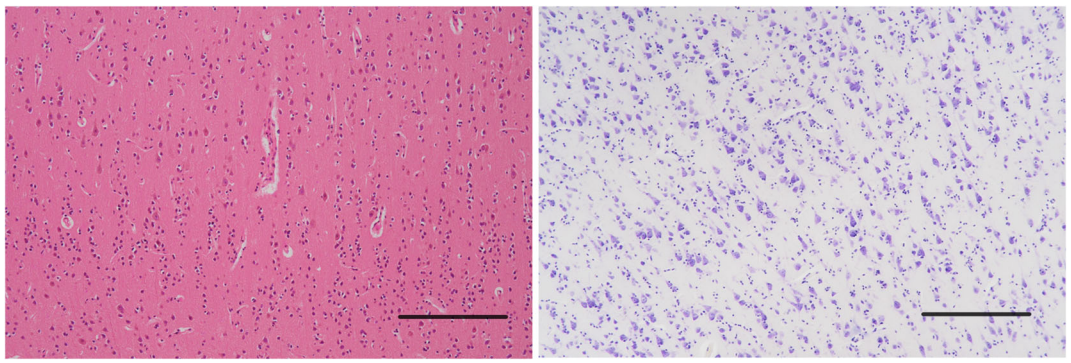

Fig 7 Histological findings of the cerebrum. (A, B) Cortical neurons shows a radial microcolumnar arrangement in some areas of the occipital (A) and frontal (B) lobes. HE ataining (A), Nissl staining (B). Scale bars: 200 μm (A, B).

the nonsense or frameshift type, and a missense mutation such as seen in our case has not been documented.[9,10] In our case, the age at disease onset was late, and the clinical phenotype was milder compared with other patients with CMT4F.[9,10] The main autopsy finding was hypertrophic radiculopathy involving both anterior and posterior roots. Spinal nerve roots showed grossly marked diffuse enlargement and florid concentric arrangement and proliferation of Schwann cells associated with endoneurial fibrosis that formed typical onion bulb lesions. Many large-diameter axons were lost, and axons with regenerating, very thin myelin sheaths were scant. These findings most likely reflect the very long clinical course from the disease onset to death (about 44 years).

CMT is a distal dominant neuropathy, and prominent onion bulb formation in the spinal nerve roots is a rare event. Hughes and Brownell[13] reported four autopsy cases of CMT, in which a marked neuronal loss of the anterior horn and DRG and a secondary degeneration of the posterior column were noted. Although they reported the loss of axons and myelin sheaths associated with fibrosis of the nerve roots, they did not mention the formation of onion bulbs in the spinal nerve roots.[13,19] Onion bulb formation of the spinal nerve roots in CMT was first described by Smith *et al.*[14] in two cases, which accompanied the formation of glial bundles in one case. Onion bulb formation of the nerve roots has also been reported in a few cases of motor neuron disease[20,21] and, in rare instances, in neurologically normal patients.[22] Whether the hypertrophic radiculopathy is a characteristic finding of CMT4F, with this unique mutation in *PRX* (D651N), remains to be elucidated by the further accumulation of similar cases.

The lesions in the lumbar anterior horn in the present case mainly were characterized by asymmetric neuronal loss, which we interpreted to be secondary to severe anterior hypertrophic radiculopathy.[13,14,19] Another possibility can be considered; these lesions represent healed chronic anterior poliomyelitis[23] or postpolio syndrome,[24] based on the fact that the patient had a past history of infantile paralysis, and the anterior horn lesions showed distinct asymmetry. However, the lesions lacked a typical plaque-like focal loss of motor neurons and the neuropil alteration associated with densely fibrous glial scar formation, which characterizes postpolio syndrome.[23,24] Another possibility is that the lesions represent an ischemic injury affecting the lumbar anterior horn.[25]

Degeneration of the posterior column, especially the gracile fascicle, is a finding commonly observed in autopsy cases of CMT and is considered to be Wallerian degeneration secondary to neuronal loss of DRG.[13,19] In the present case, however, neurons in DRG were well preserved. In the central-peripheral transition zone of spinal nerve roots, peripheral nerve fibers and central nerve fibers occupy the outer (surrounding) and inner (axial) compartments, respectively.[26] Given this structure in the transition zone, we supposed another possibility on the pathogenesis of posterior column degeneration; a marked onion bulb formation of the outer compartment of nerve roots resulted in compressing the inner central nerve fibers and vasa nervosum, and caused ischemia at the transition zone and the subsequent Wallerian degeneration of the posterior column.

The brain showed no significant pathological alterations except for a multifocal, radial microcolumnar arrangement of neurons in the cerebral cortex. Radial microcolumnar arrangement is seen in the isolated form or in association with various types of congenital malformations or genetic disorders involving the CNS.[27] It is a potentially epileptogenic lesion and corresponds to focal cortical dysplasia type 1a.[28] It is considered to be a feature suggesting maturation arrest in the histogenesis of the neuronal plate and is usually not accompanied by fibrillary astrocytosis.[27] Although it is a normal but transient feature during the development of the cerebral cortex, it can persist to a mild degree in the normally matured brain, especially in areas where the cortex is tightly curved, such as in the margins of the crowns of gyri or deep in the sulci.[27] In the present case, this finding is most likely not pathological but only represents a normal variation not related to CMT4F, because it did not cause any symptoms, and the expression of *PRK* is restricted to Schwann cells.[15] The possibility that it may represent a mild form of maturation arrest of the cerebral cortex remains.

DISCLOSURE

The authors declare no conflict of interest.

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