LETTER

Presence of colocalised phosphorylated TDP-43 and TFG proteins in the frontotemporal lobes of HMSN-P

INTRODUCTION

Hereditary motor and sensory neuropathy with proximal dominance (HMSN-P) was originally reported in the Okinawa[1] and Shiga[2] prefectures of Japan. Some families carrying the disease immigrated abroad to countries such as Brazil.[3] The causative gene has been identified as the TRK-fused gene (*TFG*).[4] TFG is localised to the endoplasmic reticulum exit sites and plays an important role in protein secretion.[4] HMSN-P is inherited in an autosomal dominant manner. Its symptoms include weakness and atrophy of the limb, which start from the proximal muscles. Muscle atrophy expands to the tongue and respiratory muscles, and patients develop bulbar palsy and respiratory failure. Additionally, they do not usually develop pyramidal tract signs, and sensory symptoms are minimal. The natural course lasts approximately 20 years or longer. There is only one single case report in which immunohistochemistry using anti-TFG antibody was performed.[4] According to that report, cytoplasmic inclusion containing TFG was found in neurons of the precentral gyrus, spinal anterior horn and dorsal root ganglion (DRG). We present the case of a patient with HMSN-P who died of respiratory failure within 4 years after onset. Part of his clinical course has been described previously.[5] We herein report the findings of his pathological examination.

CASE REPORT

A 38-year-old man presented with weakness in his left upper limbs. He worked as a course maintainer in a golf field. His mother, who had the same symptoms, developed to respiratory failure and died in her 40s. His maternal uncle also had the same disease. He was case V-4 of Family 2 described in Ishiura *et al*'s report.[4] Patients with HMSN-P in his family, including him, had a heterozygous missense mutation, c.854C>T (p.Pro285Leu), in *TFG*. His external ocular movement was normal. There was neither facial weakness nor bulbar palsy. The strength of his deltoid muscle was decreased to 5/4 (right/left) and his left deltoid muscle had atrophied. Moreover, fasciculation was observed in his major pectoris muscle, and the muscle strength of his lower limbs was normal. The tactile and temperature sensations were normal, but vibration sensation in his ankles was decreased. The deep tendon reflex was generally depressed. No pathological reflex was evoked. In the nerve conduction study at the first visit, the sensory nerve action potentials had decreased amplitude; however, the motor conduction was normal. Within 3 years, he became incapable of moving his arms. Fourteen weeks after that, he complained of dyspnoea during conversation. Arterial gas analysis revealed remarkable elevation of carbon dioxide. He refused intubation and artificial ventilation and died at the age of 42 years. Since he did not develop dementia, brain MRI or neuropsychological examination was not performed. His wife signed informed consent for the autopsy.

NEUROPATHOLOGY

Autopsy was performed 5.5 hours after his death. The whole brain weight was 1350 g. Macroscopically, no obvious atrophy was observed in the cerebrum and cerebellum. However, the brainstem and spinal cord was slightly and severely atrophied, respectively. The posterior columns (figure 1A) were pale on Klüver-Barrera (KB) staining. Conversely, the pyramidal tract including the posterior limbs of the internal capsules and the lateral funiculus (figure 1A) were normal on KB staining. We performed immunohistochemistry of neurofilament and CD68 using the spinal cord. The staining pattern of neurofilament was similar to that of KB staining. The posterior columns were heavily damaged. However, aggregates of macrophages positive for CD68 were focally detected in corticospinal tracts as well as the posterior columns. It was suggested that the damage of the axon and myelin sheath was not rapid but gradually occurred. Bunina bodies were not found and neuronal loss was obvious in the anterior horns at all levels of the spinal cord. Group atrophy was found in the iliopsoas muscle (figure 1B), suggesting neuropathic change.

Immunohistochemistry was performed using the anti-phosphorylated TAR DNA-binding protein of the 43 kDa (pTDP-43) antibody (Ab) and/or the anti-TFG Ab as described previously.[4] Surviving neurons in the hypoglossal nucleus (figure 1C), anterior horn of the spinal cord (figure 1D) and the sensory neurons in the DRG (figure 1E) had TFG-immunopositive cytoplasmic inclusions. In the cerebrum, a small number of neurons (figure 1F) in the precentral gyrus were immunopositive for anti-TFG Ab. Interestingly, aggregated TFG was also discovered in the postcentral gyrus (figure 1G). Cytoplasmic granular staining was found in the middle frontal gyrus (figure 1H) and the hippocampus (figure 1I). Colocalisation of TFG (figure 1J–2, K–2, L–2 and M–2) and pTDP-43 (figure 1J–3, K–3, L–3, and M–3) was examined by confocal microscopy using fluorescence Abs. pTDP-43 and TFG were clearly colocalised in the cytoplasm of some neurons in the inferior olivary

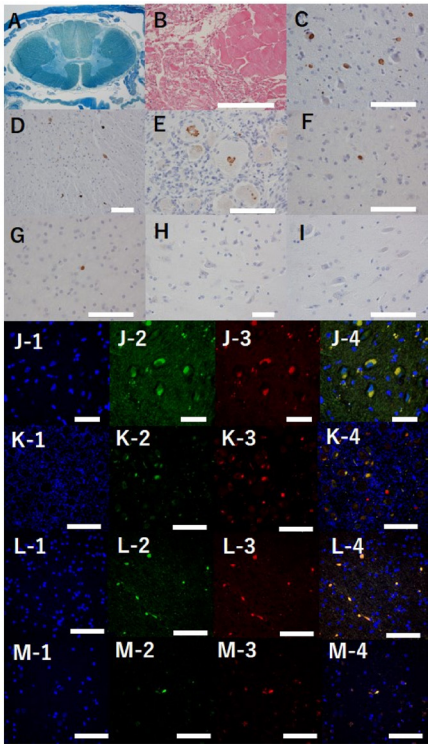

Figure 1 Neuropathological features. (A) Severe myelin pallor of the posterior columns of the cervical cord (KB staining). (B) Remarkable group atrophy of the iliopsoas muscle (H&E staining). (C–I) Immunohistochemistry using anti-TFG antibody coloured by DAB: (C) hypoglossal nucleus; (D) anterior horn of the thoracic spinal cord; (E) DRG; (F) precentral gyrus; (G) postcentral gyrus; (H) middle frontal gyrus; and (I) hippocampus. Aggregated pattern (cytoplasmic inclusions (C–G) and granular pattern (H, I) were observed. (J–M) Double immunohistochemistry using anti-TFG (green) and anti-phosphorylated TDP-43 (red), nuclear staining by DAPI (blue) and merged images (J–M-4). (J) Inferior olivary nucleus; (K) DRG; (L) middle frontal gyrus; (M) hippocampus. Scale bars=500 µm (B); 100 µm (C–I, L, M); 50 µm (J); 200 µm (K). DAB, 3,3′-diaminobenzidine; DAPI, (4′,6-diamidino-2-phenylindole); DRG, dorsal root ganglion; KB, Klüver-Barrera; TFG, TRK-fused gene.

nucleus (figure 1J–4), DRG (figure 1K–4), middle frontal gyrus (figure 1L–4), and hippocampus (figure 1M–4). TFG and pTDP-43 inclusions were also found in small oligodendrocytes as well as neurons. Neurons in the substantia nigra and oculomotor nucleus were positive for TFG, but negative for pTDP-43 (data not shown). We also performed immunohistochemistry of muscles (tongue and iliopsoas muscles) using the same antibodies for pTDP-43 and TFG; however, no positive staining was obtained by these antibodies (data not shown).

DISCUSSION

The pathological approach to HMSN-P after identification of its causative gene is limited to only one patient as reported by Ishiura *et al*.[4] Cytoplasmic aggregation of TFG was shown in the upper motor neurons in the precentral gyrus, the lower motor neurons in the brainstem and the anterior horn of the spinal cord and the sensory neurons in the DRG. TFG was colocalised with pTDP-43. The previously mentioned report[4] clarified the possible overlapping pathogenesis of neurodegeneration to that of amyotrophic lateral sclerosis (ALS). Actually, the fact of neuronal loss in the anterior horn of the spinal cord and DRG suggests that HMSN-P is a neuronopathy, not a neuropathy. In addition, mild loss of Betz cells and gliosis in the precentral gyrus with degeneration of the corticospinal tract were observed in a patient with HMSN-P.[6] Together with the clinical course of progressive muscular atrophy leading to respiratory failure, HMSN-P could be considered as one of the motor neuron diseases. The distribution of TFG pathology within the central nervous system (CNS) has not been fully clarified. We considered the possibility that TFG pathology might be more widely spread than previously expected in the CNS, since another patient with HMSN-P showed semantic dementia with atrophy of the left temporal pole (V-3 of Family 1 in Ishiura *et al*'s report,[4] personal observation) and some patients with TDP-43 proteinopathy show cognitive dysfunction as well as motor symptoms.[7]

The pathological examination of our patient revealed that neurodegenerative processes occurred beyond the upper and lower motor systems and the peripheral sensory system. The 3,3′-diaminobenzidine-staining pattern, in the frontal gyrus and hippocampus (granular), differed from that seen in the tissues classically involved in HMSN-P (aggregated). Although granular staining was observed in a patient who was non-HMSN-P,[4] clear colocalisation of TFG and pTDP-43 suggests pathogenicity. This pathogenicity was proven by experiments using cultured cells expressing mutant TFG.[4] Normally, TDP-43 is localised in the nucleus, while in patients with ALS, pTDP-43 is translocated to the cytoplasm and forms an inclusion body. As our patient died within 4 years after disease onset, the neurons showing granular staining may have formed an aggregated pattern over a long period.[7] Although there is no report of patient with demented HMSN-P,[8] FTD could be present in patients with long survival.

Patients with HMSN-P rarely develop pyramidal tract signs during their lifetime unlike those having homozygous mutations of *TFG*. Although severe neuronal loss in the anterior horn could hide any pyramidal tract signs, lower disturbance of the degenerating neurons in the precentral gyrus could also be responsible for the lack of such signs. Similarly, cortical sensory disturbance can only be confirmed postmortem, since elementary sensory functions are disturbed at the peripheral levels.

In contrast, patients with HMSN-P never show Parkinsonism. Although we have to consider the patient's brief disease duration (4 years), it could be suggested that cytoplasmic inclusions containing both TFG and pTDP-43 might be necessary for neurodegeneration in HMSN-P.

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