Familial amyotrophic lateral sclerosis and parkinsonism-dementia complex — tauopathy without mutations in the tau gene?

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We present the clinical and genetic characteristics of a Japanese patient with neuropathologically confirmed familial amyotrophic lateral sclerosis/parkinsonism dementia complex (ALS/PDC). The 68-year-old proband with an 8-year history of parkinsonism and neurogenic amyotrophy and her three siblings suffering from parkinsonism associated with dementia originated from the Kii Peninsula of Japan. The proband’s brain exhibited mild frontal lobe atrophy, moderate atrophy of the pes hippocampi, decoloration of the substantia nigra and locus coerules, and atrophy of the anterior root of the spinal cord. Microscopic examinations revealed degeneration of the CA1 portion of the hippocampus to the parahippocampus gyrus, substantia nigra, locus coerules and the spinal anterior horn with Bunina bodies. Neurofibrillary tangles (NFTs) were observed in widespread regions of the central nervous system through the cerebral cortex to the spinal cord. The predominant distribution of NFTs in the the third layer of the cerebral cortex was compatible with the characteristic feature of ALS/PDC in Guam. No tau mutation was found in the proband. The lack of mutations in the tau gene not only in this patient but also in earlier reported cases of ALS in the Western Pacific seems to suggest that other genetic factors may be contributing to ALS/PDC.

key words: ALS/PDC, amyotrophic lateral sclerosis, dementia, neurofibrillary tangles, parkinsonism, tau gene, tauopathies

INTRODUCTION

A group of heterogenous dementias and movement disorders characterised neuropathologically by prominent intracellular accumulations of abnormal filaments formed by the microtubule-associated protein tau are collectively known as neurodegenerative tauopathies. Tau protein is abundant in the central nervous system (CNS), where it is expressed predominantly in axons. It is expressed both in axons of the peripheral nervous system neurones and in CNS astrocytes and oligoden-drytes. Tau protein promotes the assembly of microtubules and is involved in neurite outgrowth, axonal development, transport and maintenance. The protein is post-translationally modified by phosphorylation. Several different kinases and phosphatases regulate this process. Abnormal phosphorylation of tau seems to be
This finding, providing direct evidence that parkinsonism linked to chromosome 17 (FTDP-17) [7].

Covered as a cause of frontotemporal dementia and the role of tau in the pathogenesis of tauopathies alone are sufficient to cause neurodegeneration in different tauopathies. Peripherally phosphorylated tau molecules, so-called neurofibrillary tangles (NFTs). The neurodegenerative tauopathies with neurofibrillary lesions in distinct regions of the brain include Alzheimer’s disease (AD), Pick’s disease, corticobasal degeneration (CBD), progressive supranuclear palsy (PSP), and the atypical lateral sclerosis/parkinsonism dementia complex (ALS/PDC). The tau protein is encoded by a single gene located on chromosome 17q21, which consists of 16 exons [1]. In 1998, multiple pathogenic mutations in the tau gene were discovered as a cause of frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17) [7]. This finding, providing direct evidence that tau abnormalities alone are sufficient to cause neurodegenerative disorder, opened up a new direction in studies of the role of tau dysfunction in the mechanisms of brain degeneration in different tauopathies.

Amyotrophic lateral sclerosis (ALS) is defined as an adult onset neurodegenerative disorder, which selectively affects both upper and lower motor neurones. Although clinical signs of the classical type of ALS are basically restricted to the motor neurones, certain types of the disease are caused by lesions in central nervous systems other than the motor neurone system. According to Ikemoto et al. [8], ALS is classified mainly on the basis of differences in clinical and histopathological features into the following distinct subtypes: 1) ALS with dementia, 2) ALS in the Western Pacific, 3) ALS with multi-system degeneration, 4) familial ALS, and 5) superoxide dismutase 1-linked ALS. An extremely high incidence of ALS has been reported in certain areas of the Western Pacific, especially the islands of Guam, the Kii Peninsula in Japan, and Western New Guinea [8, 10, 18]. The patients in these areas frequently show familial occurrence of the same disease, a longer disease duration than ALS in other areas, and occasional mental symptoms (including dementia). Moreover, patients with parkinsonism-dementia complex (PDC) are frequently found in this region. It has been suggested that Guamanian ALS has a common epidemiological background with Guamanian parkinsonism-dementia complex because the distribution of NFTs is similar to that of neuronal loss and also because patients with combined ALS and PDC have been identified clinically as well as pathologically. In addition to this, admixtures of ALS and PDC have been known to occur within one family [3, 16]. Our recent studies disclosed the occurrence of ALS/PDC in the Kii Peninsula of Japan [9, 10, 13]. Here we present the clinical, neuropathological and genetic characteristics of a Japanese patient with familial ALS/PDC. To contribute to our knowledge on the role of the tau gene in the pathogenesis of ALS/PDC, we screened for tau mutations in the case presented.

MATERIAL AND METHODS

Case report

The proband was a 68-year-old woman with an 8-year history of parkinsonism followed by psychiatric symptoms and neurogenic amyotrophy which appeared 5 years after disease onset. She was born and lived until age of 2 years in the Hobara district and then lived in other districts of the same town until the age of 10 years. The Hobara district on the Kii Peninsula is a focus of a Japanese ALS. The proband’s three elder siblings, who also grew up in the Hobara district, showed progressive dementia and parkinsonism after the age of 60. Unfortunately, they were not autopsied. Although their parents had lived in the Hobara district in middle age, no neurological disease developed later in life. There were no family relatives who suffered from neurodegenerative disease. Their ancestors originated far from the Hobara district and were not consanguineous. The proband exhibited tremor of the left upper extremity in 1990 (at 60 years old), and was diagnosed with Parkinson’s disease in 1993. Her neurological condition at our first observation in June 1995 was characterised by: a depressive psychiatric state with mild delirium, normal cognitive function apart from slight difficulty in short-term memory, a moderate masked face and left-side dominant hemi-parkinsonism, which were estimated as Hoehn and Yahr stage 4 disease. Other neurological conditions were normal including the muscular system. A cranial CT study revealed only moderate dilatation of the inferior horn of the lateral ventricles. A daily administration of 300/75 mg of L-dopa/carbidopa reduced her condition to Hoehn and Yahr 3 stage, and 40 mg of mianserin chloride improved her psychiatric symptoms. In November 1995, she showed left side bradykinesia and difficulty in ascending stairs. Her neurological symptoms were L-dopa responsive left dominant parkinsonism, distal dominant muscular atrophy and weakness in the left leg, but no fasciculation or bulbar signs. Deep tendon reflexes were reduced in the left lower extremity, increased in the left upper extremity, and normal in the right upper and lower extremities. No pathological reflexes were observed nor any

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coordination or sensory disturbances. Electromyography showed neurogenic changes and a positive sharp wave was seen in the left anterior tibial muscle. Subsequently her muscular deterioration gradually progressed and she lost her walking ability in November 1997 (at 67 years old). At that time, fasciculations were observed in the left upper extremity, but no bulbar signs. She showed recent memory dysfunction and moderate disorientation (total IQ 98, verbal IQ 109 and performance IQ 85 by WAISR in July 1998). In November 1998, she was admitted for pneumonia. She showed hallucination and delusion, muscular atrophy dominant in the lower extremities, mild tongue atrophy and reduced tendon reflexes. She died of respiratory failure in January 1999.

General pathology revealed bilateral lower lobe bronchopneumonia and congestion of the liver with perivenous necrosis.

**Neuropathology and immunohistochemistry**

The proband’s brain and spinal cord were fixed with 10% formalin and embedded in paraffin. Sections (10 μm thick) were stained by haematoxylin and eosin (H & E), Klüver-Barrera’s (K–B), Holzer’s, Gallyas-Braak’s (G–B), and Bodian’s methods. Sections (4 μm thick) were also examined by immunohistochemistry for ubiquitin, tau protein, paired helical filament tau (PHF-tau) and α-synuclein using the streptavidin-biotin method (LSAG kit, Dako, USA) with rabbit anti-human ubiquitin antibody (Dako), mouse monoclonal anti-human tau (Dako) and anti-human PHF-tau (Immogenetics, USA) antibodies, and goat anti-human a-synuclein antibody (Santa Cruz, USA).

**Tau gene analysis**

Genomic DNA was extracted from a sample of the proband’s blood. Screening for mutation in the tau gene was performed by SSCP analysis of PCR products, as previously described [11]. All 15 exons of the tau gene were amplified separately using primers designed for the flanking intronic sequences. Exons presenting band shifts in electrophoresis were subsequently subcloned into a pAT vector (Invitrogen, La Jolla, CA) and analysed by DNA sequencing using the Biochemicals Sequence kit (Amersham, Arlington, Heights, IL).

**RESULTS**

Neuropathological examination of the proband’s brain, 1040 g in weight, showed atrophy of the frontal lobe and the pes hippocampi, decoloration of the substantia nigra and locus coeruleus, and spinal anterior root atrophy. The microscopic examinations revealed severe cell loss and gliosis of the CA1 portion of the hippocampus to the parahippocampus gyrus, substantia nigra and spinal anterior horn with Bunina bodies (Fig. 1A, 1F). The degeneration was also seen moderately in the locus coeruleus, and mildly in the spinal pyramidal tract. G-B staining showed diffuse NFTs in the cerebral cortex (Fig. 1C), especially in the cortices through the hippocampus to the lateral occipitotemporal gyri. In these cortices, NFTs were abundant in the third layer but less so in the fifth or sixth layers (Fig. 1C). NFTs were positive to both tau and PHF-tau staining (Fig. 1D, 1E). Many ghost tangles were observed in CA1 of the hippocampus (Fig. 1B). There were also senile plaques in the cortex, predominantly in the temporal lobe, in a number compatible with the physiological ageing. Besides the sites mentioned above, NFTs were observed in the putamen, pallidum, subthalamic nuclei, thalamus, superior colliculus, central gray matter of the midbrain, pontine nuclei, raphe nucleus, reticular formation, inferior olive nucleus, dentate nucleus of the cerebellum, anterior and posterior horns of the spinal cord, intermediolateral nucleus and Onufrowitcz nucleus (Fig. 2A, 2B). Lewy bodies were observed in the substantia nigra and locus coeruleus. Neuropil threads were seen in the superior colliculus, central gray matter of the midbrain and inferior olive nucleus. There were no ballooned neurons. In summary, neuropathological examinations of the present case revealed findings characteristic of lower motor neurone dominant ALS and degeneration in the hippocampus, parahippocampus, substantia nigra and locus coeruleus with widespread proliferation of NFTs throughout the CNS. Genetic analysis of the tau gene demonstrated a lack of pathogenic mutation in the patient.

**DISCUSSION**

A large number of neuropathological studies have been performed on Western Pacific ALS. Both Guamanian ALS (G-ALS) and Guamanian ALS/PDC (G-ALS/PDC) show an extensive distribution of NFTs and associated neuronal loss [2, 5, 6]. The immunocytochemical and ultrastructural features of NFTs in G-ALS/PDC are basically identical to those seen in Alzheimer’s disease. In contrast to other neurodegenerative disorders which are histologically characterised by NFTs, such AD, PSP, and CBD, G-ALS/PDC causes not only NFTs, but also glialfibrillary changes in astrocytes and oligodendrocytes [14]. The pathological findings observed in the Japanese patient presented were consistent with those of G-ALS/PDC [15, 19]. Severe changes in the substantia nigra and lower motor neurones typical for ALS coincided with the main clinical symptoms, i.e. muscular atrophy in the course of parkinsonism. Moreover,
Figure 1. Microscopic examinations of the patient’s brain. 

A. Severe cell loss and gliosis of the CA1 portion of the hippocampus and pes hippocampi (H & E); 
B. Ghost tangles of the CA1 portion of the hippocampus (G–B); 
C. NFTs were predominantly located in the third layer of the cortex of the lateral occipitotemporal gyri (G–B); 
D. NFTs in the third layer of the cortex of the lateral occipitotemporal gyri (Tau staining). The cellular nuclei are visualised by the counter staining (haematoxylin); 
E. PHF-tau positive NFTs in the cortex of the temporal lobe pole (counter stained by haematoxylin); 
F. Severe cells loss and melanin depletion in the substantia nigra (K–B, × 25).
widespread NTFs have been demonstrated throughout the brain, including the spinal cord (Fig. 1C, 1D).

To date, over 20 pathogenic mutations in the tau gene responsible for FTDP-17 have been identified [12]. The different mutations have multiple effects on the biology and function of tau. The majority of missense mutations disrupt tau-microtubule interactions and alter tau polymerisation into filaments; while the intronic mutations, located downstream of exon 10 near the 5′ splice donor site, affect alternative splicing of exon 10 [7, 12]. The tau gene seems to be a strong candidate for a genetic factor of ALS/PDC because of extensive brain deposition of tau in affected subjects. In our case of ALS/PDC from the Kii Peninsula, the tau gene exhibited no mutations. Kuzuhara et al. [13] did not observe tau mutations either in the recently reported autopsied PDC case in a pedigree of familial ALS from the Hobara district. Similarly, Perez-Tur et al. [17] did not find abnormalities in the sequence of the tau gene in a cohort of 23 unrelated patients with PDC from Guam island. These data suggest that the involvement of tau is not perhaps a primary cause for the disease. On the other hand, we can not rule out the possibility that tau is an important downstream factor in the process of NFT formation [20]. A familial background of ALS/PDC confirms that the disease has a genetic origin. Therefore, other genes contributing to ALS/PDC should exist somewhere in a human genome. The genes can be modified

Figure 2. Spinal cord. A, Cell loss in the anterior horn and demyelination of the lateral pyramidal tracts in L4 (K–B); B, Bunina body in the anterior horn cell of C8 (H & E).
by as yet unknown environmental factors [4]. In our case of ALS/PDC, the proband’s three siblings, who had spent their childhood in the Hobara district, showed parkinsonism and dementia, while their parents, who resided in the same place in middle age, did not develop the neurological disease. It is possible, that the genetic factors of ALS/PDC are sensitive to environmental factors, especially in the early stages of ontogenesis.

REFERENCES