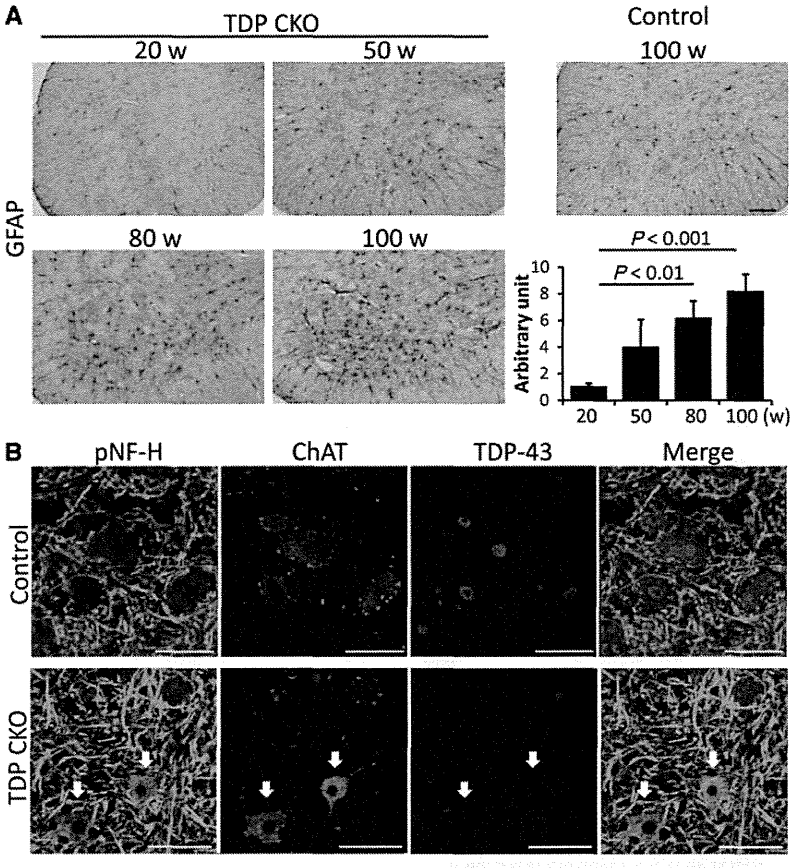

Figure 5 *Astrogliosis and neuronal accumulation of phosphorylated neurofilament.* (**A**) *Immunohistochemistry against GFAP in the ventral horn and a time course analysis of astrogliosis. Error bars indicate SD (n = 3 for each age).* (**B**) Immunofluorescent staining against pNF-H (green), ChAT (red) and TDP-43 (blue). pNF-H was accumulated in the cell bodies of TDP-43-lacking motor neurons of TDP CKO mice (arrows). Scale bars: **A** = 100 μm; **B** = 50 μm.

motor dysfunction and develops motor neuronal loss earlier than 10 weeks of age (Wu *et al.*, 2012). However, given that the Cre-mediated recombination using the *HB9* promoter began at the developmental stage E9.5 (Arber *et al.*, 1999), this model possibly reflects the loss-of-function of TDP-43 in the motor neuron development. By contrast, because the Cre expression in VAChT-Cre.Fast mice is mediated by the VAChT promoter, the number of Cre-expressing motor neurons in VAChT-Cre.Fast mice is scarcely detected at prenatal stages, but becomes maximum in number at 5 weeks (Misawa *et al.*, 2003). We also confirmed that TDP-43 was not excised in spinal motor neurons of TDP CKO mice at post-natal Day 2, but knocked-out in ~50 % of motor neurons of the 10-week-old mice. This temporal pattern of Cre expression appears to contribute to the late-onset progressive motor dysfunction in our TDP CKO mice and enable the assessment of loss of TDP-43 functions in mouse motor neurons at the postnatal stage. As far as we investigated, TDP-43 was knocked-out in spinal motor neurons beginning at 10 weeks, but the function and morphology of motor neurons were unexpectedly preserved for 1 year in TDP CKO mice, suggesting that the loss of TDP-43 was compensated in motor neurons of young mice, but triggered neuronal vulnerability with the ageing process. Given that ALS is an age-related neurodegenerative disease and that the disease develops after middle age even in inherited cases with TDP-43 mutations (Gitcho *et al.*, 2008; Kabashi *et al.*, 2008; Sreedharan *et al.*, 2008; Yokoseki *et al.*, 2008), our TDP CKO mice appear to be a model that recapitulates the age-dependent phenotypes of ALS. However, as TDP CKO mice lack some aspects of human ALS pathology, such as cytoplasmic inclusions of TDP-43 and the involvement of upper motor neurons, the use of this model for therapeutic research needs further validation.

In the histopathological analyses, TDP CKO mice exhibited the atrophy of motor neurons, degeneration of large motor axons, denervation of neuromuscular junctions and grouped atrophy of skeletal muscles, all of which are common to the pathology of human motor neuron disease. The disruption of retrograde labelling in TDP-43-lacking motor neurons suggests that TDP-43 depletion directly induces neuronal dysfunction. Interestingly, the axonal degenerations were evident in the ventral root of TDP CKO mice at 50 weeks of age, when the morphology of lumbar motor neurons was not altered. These findings are compatible with the fact that ALS pathology initially manifests at the axon

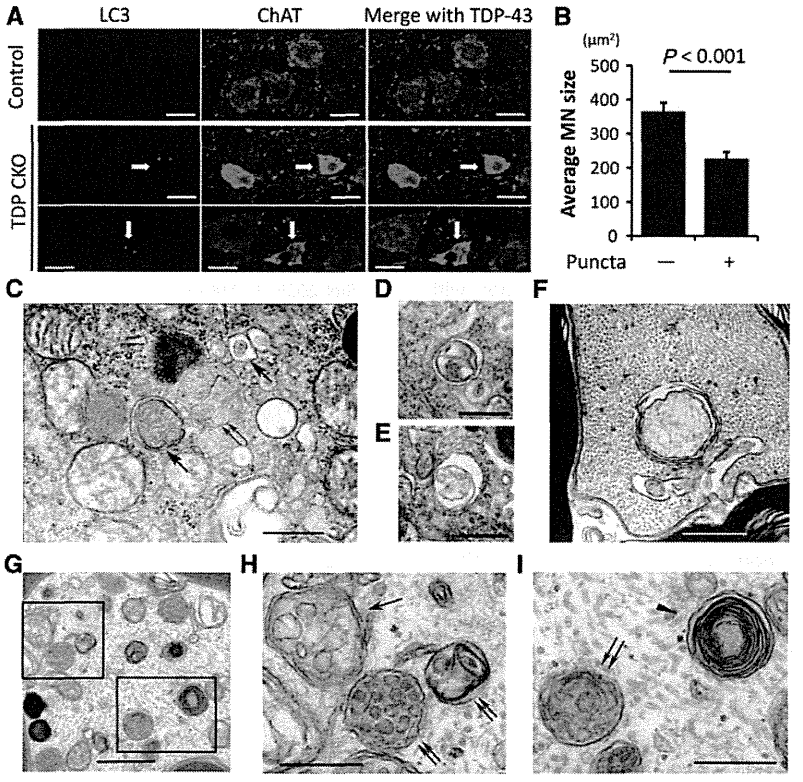

Figure 6 Formation of autophagosomes in motor neurons of TDP CKO mice. (**A**) Immunofluorescent analysis (LC3, green; ChAT, red; TDP-43, blue) revealed LC3-positive cytoplasmic puncta in TDP-43-lacking motor neurons of 100-week-old TDP CKO mice. (**B**) The average size of TDP-43 lacking motor neurons (MN) with ($n = 25$) and without ($n = 48$) LC3-positive puncta. Error bars indicate SEM. (**C–I**) Ultrastructural analysis of 100-week-old TDP CKO mice. Autophagosomes (arrows) and an autolysosome (double arrows) (**C**), autolysosomes surrounded by a single membrane containing mitochondria (**D**) and autophagosomes containing ribosome-like structures (**E**) were observed in the cell bodies of the motor neurons. An autophagic structure in the proximal motor axon (**F**). Accumulation of organelles containing mitochondria, autophagosomes (arrows), autolysosomes (double arrows), and autophagic structure with a multi-lamelleted structure (arrowhead) in the sciatic nerve (**G**) and its enlarged images (**D**, **E**, **H** and **I**). Scale bars: **A** = 20 μm; **C** and **G** = 1 μm; **F**, **H** and **I** = 500 nm.

(Fischer *et al.*, 2004). The increase of small myelinated fibres accompanied by the decrease of large myelinated fibres in the ventral root of 100-week-old TDP CKO mice corresponds to the morphological change in the cell body of the motor neurons, and similar observations were also reported in the patients and mouse models of ALS (Bradley *et al.*, 1983; Zhang *et al.*, 1997). TDP CKO mice also exhibited several features that are shared with patients with sporadic ALS: the involvement in the cranial motor nuclei such as the hypoglossal nucleus, preserved morphology in the extraocular motor neurons, accumulations of phosphorylated neurofilament in motor neurons and astrogliosis in the spinal ventral horn. Dysphagia due to the involvement of the hypoglossal nucleus might enhance the weight loss in aged TDP CKO mice through decreased oral intake. In ALS, extraocular motor neurons are resistant to degeneration compared with other somatomotor neurons, and differences in calcium buffering capacities have been proposed as a possible reason for this selective vulnerability (Alexianu *et al.*, 1994; Reiner *et al.*, 1995; Laslo *et al.*, 2000). Because RNA-seq analysis demonstrates that depletion of TDP-43 affects the calcium signalling pathway in mouse striatum (Polymenidou *et al.*, 2011), it is possible that dysregulation of calcium buffering underlies the pathogenesis of TDP CKO mice.

Our immunofluorescent analysis also demonstrated LC3-positive cytoplasmic puncta in TDP-43-depleted motor neurons, and the presence of these puncta was associated with shrinkage of motor neurons. This finding was confirmed by electron microscopy that revealed the presence of autolysosomes and autophagosomes in the motor neuronal cell bodies and axons of TDP CKO mice, suggesting that TDP-43 depletion resulted in dysregulation of the autophagic pathway. In addition, the accumulation of autophagic structures in the sciatic nerve and the disruption of retrograde labelling in TDP-43-lacking motor neurons suggest that the disruption of retrograde axonal transport may underlie the motor neuronal dysfunction in TDP CKO mice. Although the disruption of constitutive autophagy is shown to instigate the degeneration of certain types of neurons (Komatsu *et al.*, 2006), the causative role of the autophagic dysregulation in the pathogenesis of motor neuron diseases remains controversial. A recent work

demonstrates that motor neuron-specific knockout of the proteasome subunit Rpt3, but not autophagy mediator Atg7, leads to motor neuron degeneration in mice (Tashiro *et al.*, 2012), suggesting that the disruption of autophagic pathway in motor neurons may not be the primary cause of the neurodegeneration. However, accumulation of autophagosomes and autolysosomes was observed in the motor neurons of mice with mutant SOD1 (Li *et al.*, 2008; Tian *et al.*, 2011) and patients with sporadic ALS (Nakano *et al.*, 1993; Sasaki, 2011). In addition, mice carrying mutations of dynein or dynactin exhibit motor dysfunction with accumulation of autophagosomes in the motor neurons (Ravikumar *et al.*, 2005; Laird *et al.*, 2008). These lines of evidence may suggest a possible link between the increased autophagosomes and the process of motor neuron degeneration (Pasquali *et al.*, 2009; Chen *et al.*, 2012), although it remains unclear whether the accumulation of autophagosomes in neurodegenerative diseases results from activation of autophagy, disruption of retrograde transport or decreased lysosome fusion (Shintani and Klionsky, 2004; Baehrecke, 2005; Perlson *et al.*, 2010). Further investigation with regard to the linkage among loss of TDP-43, retrograde axonal transport and dysregulation of autophagy might contribute to our understanding the pathogenesis of ALS.

In conclusion, TDP CKO mice exhibited age-dependent motor impairment and morphological alterations in the motor neuron system that recapitulate several features of sporadic ALS neuropathology, including the accumulation of autophagosomes. These findings suggest that TDP-43 plays an essential role in the long-term maintenance of motor neurons, and that loss of TDP-43 function contributes to the pathogenesis of ALS.

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Supplementary material

Supplementary material is available at *Brain* online.

References

Alexianu ME, Ho BK, Mohamed AH, La Bella V, Smith RG, Appel SH. The role of calcium-binding proteins in selective motoneuron vulnerability in amyotrophic lateral sclerosis. Ann Neurol 1994; 36: 846–58.

Arai T, Hasegawa M, Akiyama H, Ikeda K, Nonaka T, Mori H, et al. TDP-43 is a component of ubiquitin-positive tau-negative inclusions in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Biochem Biophys Res Commun 2006; 351: 602–11.

Arber S, Han B, Mendelsohn M, Smith M, Jessell TM, Sockanathan S. Requirement for the homeobox gene Hb9 in the consolidation of motor neuron identity. Neuron 1999; 23: 659–74.

Ayala YM, Misteli T, Baralle FE. TDP-43 regulates retinoblastoma protein phosphorylation through the repression of cyclin-dependent kinase 6 expression. Proc Natl Acad Sci USA 2008; 105: 3785–9.

Ayala YM, Pantano S, D'Ambrogio A, Buratti E, Brindisi A, Marchetti C, et al. Human, *Drosophila*, and *C.elegans* TDP43: nucleic acid binding properties and splicing regulatory function. J Mol Biol 2005; 348: 575–88.

Baehrecke EH. Autophagy: dual roles in life and death? Nat Rev Mol Cell Biol 2005; 6: 505–10.

Bradley WG, Good P, Rasool CG, Adelman LS. Morphometric and biochemical studies of peripheral nerves in amyotrophic lateral sclerosis. Ann Neurol 1983; 14: 267–77.

Buratti E, Brindisi A, Giombi M, Tisminetzky S, Ayala YM, Baralle FE. TDP-43 binds heterogeneous nuclear ribonucleoprotein A/B through its C-terminal tail: an important region for the inhibition of cystic fibrosis transmembrane conductance regulator exon 9 splicing. J Biol Chem 2005; 280: 37572–84.

Buratti E, De Conti L, Stuani C, Romano M, Baralle M, Baralle F. Nuclear factor TDP-43 can affect selected microRNA levels. FEBS J 2010; 277: 2268–81.

Chen S, Zhang X, Song L, Le W. Autophagy dysregulation in amyotrophic lateral sclerosis. Brain Pathol 2012; 22: 110–6.

Chiang PM, Ling J, Jeong YH, Price DL, Aja SM, Wong PC. Deletion of TDP-43 down-regulates Tbc1d1, a gene linked to obesity, and alters body fat metabolism. Proc Natl Acad Sci USA 2010; 107: 16320–4.

Feiguin F, Godena VK, Romano G, D'Ambrogio A, Klima R, Baralle FE. Depletion of TDP-43 affects *Drosophila* motoneurons terminal synapsis and locomotive behavior. FEBS Lett 2009; 583: 1586–92.

Fischer LR, Culver DG, Tennant P, Davis AA, Wang M, Castellano-Sanchez A, et al. Amyotrophic lateral sclerosis is a distal axonopathy: evidence in mice and man. Exp Neurol 2004; 185: 232–40.

Gitcho MA, Baloh RH, Chakraverty S, Mayo K, Norton JB, Levitch D, et al. TDP-43 A315T mutation in familial motor neuron disease. Ann Neurol 2008; 63: 535–8.

Igaz LM, Kwong LK, Lee EB, Chen-Plotkin A, Swanson E, Unger T, et al. Dysregulation of the ALS-associated gene TDP-43 leads to neuronal death and degeneration in mice. J Clin Invest 2011; 121: 726–38.

Iguchi Y, Katsuno M, Niwa J, Yamada S, Sone J, Waza M, et al. TDP-43 depletion induces neuronal cell damage through dysregulation of Rho family GTPases. J Biol Chem 2009; 284: 22059–66.

Kabashi E, Lin L, Tradewell ML, Dion PA, Bercier V, Bourgouin P, et al. Gain and loss of function of ALS-related mutations of TARDBP (TDP-43) cause motor deficits *in vivo*. Hum Mol Genet 2011; 19: 671–83.

Kabashi E, Valdmanis PN, Dion P, Spiegelman D, McConkey BJ, Vande Velde C, et al. TARDBP mutations in individuals with sporadic and familial amyotrophic lateral sclerosis. Nat Genet 2008; 40: 572–4.

Katsuno M, Adachi H, Kume A, Li M, Nakagomi Y, Niwa H, et al. Testosterone reduction prevents phenotypic expression in a transgenic mouse model of spinal and bulbar muscular atrophy. Neuron 2002; 35: 843–54.

Katsuno M, Adachi H, Minamiyama M, Waza M, Tokui K, Banno H, et al. Reversible disruption of dynactin 1-mediated retrograde axonal transport in polyglutamine-induced motor neuron degeneration. J Neurosci 2006; 26: 12106–17.

Komatsu M, Waguri S, Chiba T, Murata S, Iwata J, Tanida I, et al. Loss of autophagy in the central nervous system causes neurodegeneration in mice. Nature 2006; 441: 880–4.

Kraemer BC, Schuck T, Wheeler JM, Robinson LC, Trojanowski JQ, Lee VM, et al. Loss of murine TDP-43 disrupts motor function and plays an essential role in embryogenesis. Acta Neuropathol 2010; 119: 409–19.

Laird FM, Farah MH, Ackerley S, Hoke A, Maragakis N, Rothstein JD, et al. Motor neuron disease occurring in a mutant dynactin mouse

model is characterized by defects in vesicular trafficking. J Neurosci 2008; 28: 1997–2005.
Laslo P, Lipski J, Nicholson LF, Miles GB, Funk GD. Calcium binding proteins in motoneurons at low and high risk for degeneration in ALS. Neuroreport 2000; 11: 3305–8.
Lee EB, Lee VM, Trojanowski JQ. Gains or losses: molecular mechanisms of TDP43-mediated neurodegeneration. Nat Rev Neurosci 2011; 13: 38–50.
Li L, Zhang X, Le W. Altered macroautophagy in the spinal cord of SOD1 mutant mice. Autophagy 2008; 4: 290–3.
Misawa H, Nakata K, Toda K, Matsuura J, Oda Y, Inoue H, et al. VAChT-Cre. Fast and VAChT-Cre.Slow: postnatal expression of Cre recombinase in somatomotor neurons with different onset. Genesis 2003; 37: 44–50.
Nakano I, Shibata T, Uesaka Y. On the possibility of autolysosomal processing of skein-like inclusions. Electron microscopic observation in a case of amyotrophic lateral sclerosis. J Neurol Sci 1993; 120: 54–9.
Neumann M, Sampathu DM, Kwong LK, Truax AC, Micsenyi MC, Chou TT, et al. Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Science 2006; 314: 130–3.
Pasquali L, Longone P, Isidoro C, Ruggieri S, Paparelli A, Fornai F. Autophagy, lithium, and amyotrophic lateral sclerosis. Muscle Nerve 2009; 40: 173–94.
Perlson E, Maday S, Fu MM, Moughamian AJ, Holzbaur EL. Retrograde axonal transport: pathways to cell death? Trends Neurosci 2010; 33: 335–44.
Polymenidou M, Lagier-Tourenne C, Hutt KR, Huelga SC, Moran J, Liang TY, et al. Long pre-mRNA depletion and RNA missplicing contribute to neuronal vulnerability from loss of TDP-43. Nat Neurosci 2011; 14: 459–68.
Ravikumar B, Acevedo-Arozena A, Imarisio S, Berger Z, Vacher C, O'Kane CJ, et al. Dynein mutations impair autophagic clearance of aggregate-prone proteins. Nat Genet 2005; 37: 771–6.
Reiner A, Medina L, Figueredo-Cardenas G, Anfinson S. Brainstem motoneuron pools that are selectively resistant in amyotrophic lateral sclerosis are preferentially enriched in parvalbumin: evidence from monkey brainstem for a calcium-mediated mechanism in sporadic ALS. Exp Neurol 1995; 131: 239–50.
Sasaki S. Autophagy in spinal cord motor neurons in sporadic amyotrophic lateral sclerosis. J Neuropathol Exp Neurol 2011; 70: 349–59.
Sephton CF, Cenik C, Kucukural A, Dammer EB, Cenik B, Han Y, et al. Identification of neuronal RNA targets of TDP-43-containing ribonucleoprotein complexes. J Biol Chem 2011; 286: 1204–15.
Sephton CF, Good SK, Atkin S, Dewey CM, Mayer P III, Herz J, et al. TDP-43 is a developmentally regulated protein essential for early embryonic development. J Biol Chem 2010; 285: 6826–34.
Shan X, Chiang PM, Price DL, Wong PC. Altered distributions of Gemini of coiled bodies and mitochondria in motor neurons of TDP-43 transgenic mice. Proc Natl Acad Sci USA 2010; 107: 16325–30.
Shintani T, Klionsky DJ. Autophagy in health and disease: a double-edged sword. Science 2004; 306: 990–5.
Sreedharan J, Blair IP, Tripathi VB, Hu X, Vance C, Rogelj B, et al. TDP-43 mutations in familial and sporadic amyotrophic lateral sclerosis. Science 2008; 319: 1668–72.
Stallings NR, Puttaparthi K, Luther CM, Burns DK, Elliott JL. Progressive motor weakness in transgenic mice expressing human TDP-43. Neurobiol Dis 2010; 40: 404–14.
Strong MJ, Volkening K, Hammond R, Yang W, Strong W, Leystra-Lantz C, et al. TDP43 is a human low molecular weight neurofilament (hNFL) mRNA-binding protein. Mol Cell Neurosci 2007; 35: 320–7.
Swarup V, Phaneuf D, Bareil C, Robertson J, Rouleau GA, Kriz J, et al. Pathological hallmarks of amyotrophic lateral sclerosis/frontotemporal lobar degeneration in transgenic mice produced with TDP-43 genomic fragments. Brain 2011; 134: 2610–26.
Tashiro Y, Urushitani M, Inoue H, Koike M, Uchiyama Y, Komatsu M, et al. Motor neuron-specific disruption of proteasomes, but not autophagy, replicates amyotrophic lateral sclerosis. J Biol Chem 2012; 287: 42984–94.
Tian F, Morimoto N, Liu W, Ohta Y, Deguchi K, Miyazaki K, et al. *In vivo* optical imaging of motor neuron autophagy in a mouse model of amyotrophic lateral sclerosis. Autophagy 2011; 7: 985–92.
Tollervey JR, Curk T, Rogelj B, Briese M, Cereda M, Kayikci M, et al. Characterizing the RNA targets and position-dependent splicing regulation by TDP-43. Nat Neurosci 2011; 14: 452–8.
Tsai KJ, Yang CH, Fang YH, Cho KH, Chien WL, Wang WT, et al. Elevated expression of TDP-43 in the forebrain of mice is sufficient to cause neurological and pathological phenotypes mimicking FTLD-U. J Exp Med 2010; 207: 1661–73.
Uchida A, Sasaguri H, Kimura N, Tajiri M, Ohkubo T, Ono F, et al. Non-human primate model of amyotrophic lateral sclerosis with cytoplasmic mislocalization of TDP-43. Brain 2012; 135: 833–46.
Wang HY, Wang IF, Bose J, Shen CK. Structural diversity and functional implications of the eukaryotic TDP gene family. Genomics 2004; 83: 130–9.
Wegorzewska I, Bell S, Cairns NJ, Miller TM, Baloh RH. TDP-43 mutant transgenic mice develop features of ALS and frontotemporal lobar degeneration. Proc Natl Acad Sci USA 2009; 106: 18809–14.
Wils H, Kleinberger G, Janssens J, Pereson S, Joris G, Cuijt I, et al. TDP-43 transgenic mice develop spastic paralysis and neuronal inclusions characteristic of ALS and frontotemporal lobar degeneration. Proc Natl Acad Sci USA 2010; 107: 3858–63.
Wu LS, Cheng WC, Hou SC, Yan YT, Jiang ST, Shen CK. TDP-43, a neuro-pathosignature factor, is essential for early mouse embryogenesis. Genesis 2010; 48: 56–62.
Wu LS, Cheng WC, Shen CK. Targeted Depletion of TDP-43 Expression in the spinal cord motor neurons leads to the development of Amyotrophic Lateral Sclerosis (ALS)-like phenotypes in mice. J Biol Chem 2012; 287: 27335–44.
Xu YF, Gendron TF, Zhang YJ, Lin WL, D'Alton S, Sheng H, et al. Wild-type human TDP-43 expression causes TDP-43 phosphorylation, mitochondrial aggregation, motor deficits, and early mortality in transgenic mice. J Neurosci 2010; 30: 10851–9.
Yokoseki A, Shiga A, Tan CF, Tagawa A, Kaneko H, Koyama A, et al. TDP-43 mutation in familial amyotrophic lateral sclerosis. Ann Neurol 2008; 63: 538–42.
Zhang B, Tu P, Abtahian F, Trojanowski JQ, Lee VM. Neurofilaments and orthograde transport are reduced in ventral root axons of transgenic mice that express human SOD1 with a G93A mutation. J Cell Biol 1997; 139: 1307–15.
Zhou H, Huang C, Chen H, Wang D, Landel CP, Xia PY, et al. Transgenic rat model of neurodegeneration caused by mutation in the TDP gene. PLoS Genet 2010; 6: e1000887.

Original Investigation

Lower Motor Neuron Involvement in TAR DNA-Binding Protein of 43 kDa–Related Frontotemporal Lobar Degeneration and Amyotrophic Lateral Sclerosis

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IMPORTANCE TAR DNA-binding protein of 43 kDa (TDP-43) plays a major role in the pathogenesis of frontotemporal lobar degeneration (FTLD) and amyotrophic lateral sclerosis (ALS). Although a pathological continuity between FTLD and ALS has been suggested, the neuropathological changes of the lower motor neuron (LMN) systems have not been assessed in TDP-43–associated FTLD (FTLD-TDP), to our knowledge.

OBJECTIVE To investigate a pathological continuity between FTLD-TDP and ALS by comparing their respective neuropathological changes in the motor neuron system.

DESIGN AND SETTING A retrospective clinical medical record review and a semiquantitative neuropathological evaluation of the cranial motor nerve nuclei and spinal cord were conducted at autopsy. We included 43 patients with sporadic FTLD-TDP, type A, B, or C, from 269 consecutively autopsied patients with TDP-43 proteinopathy. Patients were categorized as having FTLD without ALS, FTLD-ALS (onset of FTLD symptoms/signs preceded those of ALS), or ALS-FTLD (onset of ALS symptoms/signs preceded those of FTLD).

MAIN OUTCOMES AND MEASURES Neuronal TDP-43 pathological changes and neuronal loss.

RESULTS Forty-three patients were included in the clinical analysis, and 29 from whom spinal cords were obtained were included in the neuropathological analysis. Survival time was significantly shorter in the FTLD-ALS and ALS-FTLD groups than in the FTLD without ALS group ($P < .001$). At neuropathological examination, 89% of patients in the FTLD without ALS group showed aggregations of TDP-43 in the spinal motor neurons. The LMN loss was most severe in ALS-FTLD, followed by FTLD-ALS and FTLD without ALS. All the patients with type A or C FTLD-TDP were included in the FTLD without ALS group, and all those with type B pathological changes were in the FTLD-ALS or the ALS-FTLD group. Lower motor neuron loss and TDP-43–positive skeinlike inclusions were observed in all pathological subtypes.

CONCLUSIONS AND RELEVANCE The LMN systems of FTLD-TDP frequently exhibit neuropathological changes corresponding to ALS. Thus, a pathological continuity between FTLD-TDP and ALS is supported at the level of the LMN system.

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Frontotemporal lobar degeneration (FTLD) is a sporadic or familial neurodegenerative disease that clinically encompasses frontotemporal dementia, language disorder, and motor symptoms.[1] Immunohistochemical profiles show that approximately half of patients with FTLD present with tau-positive disease, but the other half primarily exhibit an accumulation of TAR DNA-binding protein of 43 kDa (TDP-43), referred to as FTLD-TDP.[2-5] Currently, the cortical TDP-43 pathological changes in sporadic FTLD-TDP are classified into 3 subtypes: A, B, and C.[6-8]

TAR DNA-binding protein of 43 kDa is also a major disease protein in amyotrophic lateral sclerosis (ALS), which is characterized by upper motor neuron and lower motor neuron (LMN) involvement.[9] The pathological features of LMN involvement in ALS include neuronal loss, gliosis, TDP-43-positive neuronal inclusions with skeinlike or round shapes and glial inclusions, and Bunina bodies.[10]

Some patients exhibit symptoms of both ALS and FTLD, and the cerebral cortices of patients with FTLD and ALS almost always show type B TDP-43 changes.[7,11-13] Thus, a pathological continuity between FTLD and ALS has been proposed based on brain TDP-43 pathological findings. Studies of the cerebral cortex, including the motor cortex, and subcortical gray matter have shown common TDP-43 pathological findings in FTLD, FTLD with ALS, and ALS.[12,14-17] However, the neuropathological features of LMN systems in FTLD-TDP have not been investigated comprehensively, particularly in the spinal cord, although characterization of these features is necessary to confirm the pathological relationship between FTLD and ALS.

In this study, we investigated LMN pathological findings in patients with sporadic FTLD-TDP who clinically demonstrated FTLD, FTLD with ALS, or ALS. We also investigated the correlation between TDP-43 pathological subtypes (type A, B, and C) and LMN involvement to further elucidate the continuity of FTLD and ALS.

Methods

Study Patients

We enrolled 269 consecutively autopsied patients with sporadic and adult-onset FTLD, FTLD with ALS, or ALS in which pathological aggregation of TDP-43 was confirmed at the Department of Neuropathology, Institute for Medical Science of Aging, Aichi Medical University, from 1988 to 2012. All patients had been clinically evaluated by neurological experts in the affiliated hospitals of Nagoya University School of Medicine or Aichi Medical University. Permission to perform an autopsy and archive the nervous system tissues for research purposes was obtained from family members after death. The clinical data on the included patients were obtained from case notes made at diagnosis and at an advanced stage of illness. We initially excluded 216 of the 269 patients because they did not present clinical FTLD symptoms. In 53 patients, FTLD or FTLD with ALS was diagnosed according to the diagnostic criteria of FTLD and ALS.[1,18] Moreover, we subclassified FTLD with ALS into FTLD-ALS (onset of FTLD symptoms/signs preceded those of ALS) and ALS-FTLD (onset of ALS symptoms/signs preceded those of FTLD) groups.

The enrolled patients were categorized into 3 groups: FTLD without ALS, FTLD-ALS, and ALS-FTLD. The FTLD symptoms were categorized into 2 groups: behavior-variant frontotemporal dementia and language impairments.[14] The LMN symptoms/signs were defined by progressive muscular weakness, muscular atrophy, fasciculation, or electromyographic findings. We excluded patients with Alzheimer disease-associated neurofibrillary pathological abnormalities that were more advanced than Braak stage IV,[19] those with argyrophilic grain disease, and those with invalid clinical data. Finally, 43 patients were included in the clinical analysis (11 with FTLD without ALS, 9 with FTLD-ALS, and 23 with ALS-FTLD). For comparison, we prepared 13 age-matched controls (mean [SD] age at death, 68.2 [6.9] years) who had no diagnosis of any neurodegenerative disease, dementia, or cerebrovascular disease.

Clinical Analyses

The information regarding sex, age at onset, disease duration, and duration between onset of FTLD and ALS was collected from clinical notes. Causes of death were classified as respiratory failure due to respiratory muscle weakness, pneumonia, or other. The last category comprised systemic diseases other than respiratory failure or pneumonia, including cancer, ileus, infections, and renal failure. Information on the subtypes of dementia, clinical data on motor symptoms, and electromyographic results were also collected.

Pathological Evaluations

For the neuropathological analysis, we excluded patients who had received a respirator/tracheotomy (n = 11) or whose spinal cord was not available (n = 3). In total, 29 patients were included in the neuropathological analysis and divided into 3 groups: FTLD without ALS (n = 9), FTLD-ALS (n = 8), and ALS-FTLD (n = 12). The tissues were fixed in 20% neutral-buffered formalin. The paraffin-embedded tissue blocks were cut at a thickness of 4.5 μm. We evaluated sections from the spinal cord and whole brain. The whole spinal cord was examined at each segmental level, but only the cervical cord was available in 3 patients and the sacral cord was not available in another 3.

For routine neuropathological examinations, the sections were stained with hematoxylin-eosin and Klüver-Barrera. Immunohistochemical studies were performed using a standard polymer-based method with the EnVision Kit or anti-goat immunoglobulin (Dako). As primary antibodies, we used antibodies to the following: anti-ubiquitin (ubiquitin, monoclonal mouse, 1:250; Millipore), anti-TDP-43 (TARDBP, polyclonal rabbit, 1:2500; ProteinTech), anti-phosphorylated TDP-43 (pTDP-43 ser409/410, polyclonal rabbit, 1:2500; CosmoBio), anti-phosphorylated tau (AT-8, monoclonal mouse, 1:4000; Innogenetics), anti-β-amyloid (β-amyloid 6F/3D, monoclonal mouse, 1:100; Dako), anti-CD68 (CD68, monoclonal mouse, 1:200; Dako), anti-cystatin C (cystatin C, polyclonal rabbit, 1:200; Dako), p62 N-terminal (p62N, polyclonal

guinea pig, 1:100; Progen), anti-ubiquilin 2 (UBQLN-2 5F5, monoclonal mouse, 1:5000; Abnova), and anti-choline acetyltransferase (ChAT, polyclonal goat, 1:100; Millipore). Diaminobenzidine (Wako) was used as the chromogen.

Antigens were retrieved with trypsin for anti-CD68 immunohistochemistry and with 95°C 3 mmol/L citrate buffer at 95°C for 20 minutes, followed by 5-minute incubation in 98% formic acid for anti-p62N, anti-TDP-43, anti-pTDP-43, and anti-ChAT immunohistochemistry. To confirm the presence of TDP-43-positive inclusions within the cholinergic motor neurons, we performed double immunohistochemistry using anti-pTDP-43 and anti-ChAT antibodies. Spinal cord specimens were prepared from 3 patients with type A, 3 with type B, and 2 with type C. Initially, the specimens were immunostained with the anti-ChAT and anti-goat immunoglobulin antibodies and diaminobenzidine. The anti-ChAT antibody was inactivated in distilled water at 100°C for 20 minutes, followed by immunohistochemistry with pTDP-43 and violet pigmentation using a VIP Peroxidase Substrate Kit (SK-4600; Vector).

For the semiquantitative neuropathological analysis, 2 investigators (Y.R. and M.Y.) observed the specimens containing the facial and hypoglossal nuclei and the anterior horn of the spinal cord. They evaluated the severity of LMN neuropathological changes that are indicative of ALS (neuronal loss, gliosis, aggregation of macrophages, TDP-43-immunopositive neuronal inclusions, and Bunina bodies) and graded neuronal loss and gliosis using Klüver-Barrera and hematoxylin-eosin staining. The investigators also evaluated the aggregations of macrophages rather than rod-shaped microglia using anti-CD68 immunohistochemistry and identified Bunina bodies using hematoxylin-eosin staining and anti-cystatin C immunohistochemistry. They scored the severity of neuronal loss and gliosis as grade 0 (none), 1 (mild), 2 (moderate), or 3 (severe) (eFigure 1 in Supplement. The appearance of TDP-43-positive inclusions was scored as grade 0 (none), grade 1 (1-5 neuronal inclusions per 5 fields; ×20 objective), grade 2 (6-10 inclusions), or grade 3 (≥11 inclusions) using anti-pTDP-43 immunohistochemistry.

Pathological cortical TDP-43 subtypes were identified according to current neuropathological criteria, using specimens from the frontal lobes, temporal lobes, and hippocampus.[5] For FTLD-TDP, type A was defined as the presence of neuronal cytoplasmic inclusions predominantly in the neocortex layer 2 and short dystrophic neurites; type B, as a predominance of neuronal cytoplasmic inclusions in all cortical layers; and type C, as a predominance of long dystrophic neurites in layer 2 and cytoplasmic inclusions in the dentate granular cells of the hippocampus. Our patient series did not include type D, which is characterized by numerous short dystrophic neurites and neuronal intranuclear inclusions in association with valosin-containing protein gene mutations. We also evaluated pathological changes in the upper motor neuron systems that include the primary motor cortex and corticospinal tract (CST). We evaluated the presence or absence of neuronal loss and gliosis in the primary motor cortex and myelin pallor, as well as the aggregation of macrophages in the CST.

Immunohistochemical Screening of Hexanucleotide Repeat Expansion Sequence in Chromosome 9 Open Reading Frame 72

Our study focused on sporadic FTLD-TDP, and patients with familial histories of FTLD or ALS, dementia, or other neurodegenerative diseases were excluded. However, FTLD or ALS associated with chromosome 9 open reading frame 72 (C9ORF72) hexanucleotide expansion exhibits pathological aggregation of TDP-43 and, in some cases, low penetration,[20,21] although these mutations are extremely rare in Japan.[22] It was recently reported that the pattern of ubiquilin abnormalities in ALS and FTLD corresponds well with the presence of C9ORF72 hexanucleotide expansion.[23] The UBQLN-2-positive, p62-positive, but TDP-43-negative thick dystrophic neurites are abundantly present in patients with C9ORF72 hexanucleotide expansion, predominantly in the hippocampus and cerebellum. Because the materials for a genetic study were not available for a large proportion of our patients, we histologically screened C9ORF72 hexanucleotide expansion with the absence of UBQLN-2 and p62N-positive thick dystrophic neurites in the temporal lobes and cerebella of all patients.

Statistical Analysis

The Mann-Whitney test was applied to continuous variables between 2 groups, and the Kruskal-Wallis test was applied to the analysis of continuous variables among 3 groups. The χ^2 test was used for categorized variables among 3 groups. Spearman rank correlation coefficient analyses were applied to univariate correlations between the clinical groups and severity of pathological changes. Survival curves were constructed using the Kaplan-Meier method. The end point of clinical course was defined as death or the introduction of a respirator or tracheotomy. The significance level for all comparisons was set *at* $P < .05$. All statistical tests were 2 sided and were conducted using the PASW 18.0 program (IBM SPSS).

Results

Clinical Analysis

Patient characteristics are summarized in the **Table**. The mean (SD) time from symptom onset to death or respirator or tracheotomy administration was 50.5 (58.4) months across all patients. The survival time from symptom onset did not differ significantly between the FTLD-ALS and ALS-FTLD groups but was significantly shorter for the FTLD without ALS group than for the FTLD-ALS or ALS-FTLD group (**Figure 1** and Table; $P < .001$). The most common cause of death for the ALS-FTLD and FTLD-ALS groups was respiratory failure, but patients with FTLD without ALS commonly died of other systemic diseases ($P < .001$). Frequencies of dementia subtypes did not significantly differ between the clinical groups.

With regard to motor symptoms/signs, 3 patients in the FTLD without ALS group had hyperreflexia, 1 had the Babinski sign, and 1 had spasticity, but none had a clinical diagnosis of progressive lateral sclerosis (PLS) according to the published diagnostic criteria of PLS.[24] Patients with FTLD-ALS or

Table. Clinical and Demographic Patient Characteristics

Characteristic	Patient Group			*P* Value
	FTLD Without ALS (n = 11)	FTLD-ALS (n = 9)	ALS-FTLD (n = 23)	
Sex, No. female/male[a]	8/3	3/6	10/13	.16
Age at onset, mean (SD), y[b]	62.4 (9.4)	58.2 (11.3)	61.2 (9.5)	.79
Clinical duration without respirator or tracheotomy, median (range), mo[c]	84.0 (47.0-360.0)	28.0 (7.0-60.0)	22.0 (7.0-71.0)	<.001
Duration between FTLD and ALS, median (range), mo[d]		18.0 (4.0-48.0)	19.0 (0-60.0)	.92
Patients with tracheotomy, No. (%)[a]	0	0	2 (9)	.40
Patients with respirators, No. (%)[a]	0	1 (11)	8 (35)	.047
Duration with respirator or tracheotomy, median (range), mo		30.0	39.0 (1.0-141.0)	...
Causes of death, No. (%)[a]				
Respiratory failure	0	6 (67)	20 (87)	<.001
Pneumonia	3 (27)	3 (33)	3 (13)	.37
Other	8 (73)	0	0	<.001
Subtypes of dementia, No. (%)				
Behavior-variant FTD	7 (64)	7 (78)	19 (83)	.29
Language impairments	4 (36)	2 (22)	4 (17)	.62
Motor symptoms/signs, No. (%)				
Muscle weakness[a]	0	9 (100)	23 (100)	<.001
Muscle atrophy	0	7 (78)	22 (96)	<.001
Fasciculation	0	4 (44)	15 (65)	.002
Hyperreflexia	3 (27)	5 (56)	17 (74)	.009
Babinski sign	1 (9)	4 (44)	3 (13)	.08
Spasticity	1 (9)	0	1 (4)	.63
Electromyography, No. (%)				
Total examined	2 (18)	4 (82)	21 (91)	...
Active denervation[a]	0	3 (75)	12 (57)	.054

Abbreviations: ALS, amyotrophic lateral sclerosis; ALS-FTLD, onset of ALS symptoms/signs preceding those of frontotemporal lobar degeneration (FTLD); ellipses, not significant; FTD, frontotemporal dementia; FTLD-ALS, onset of FTLD symptoms/signs preceding those of ALS.

[a] χ^2 Test.

[b] Kruskal-Wallis test.

[c] Log-rank test.

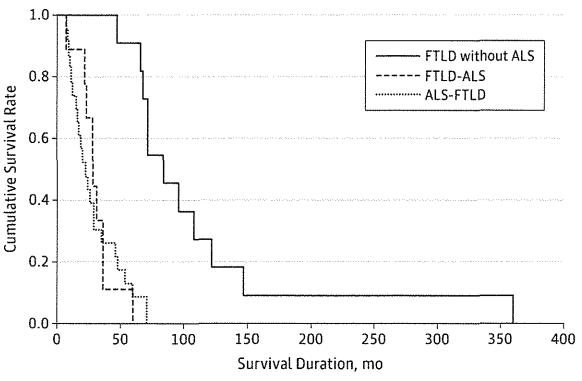
[d] Mann-Whitney test.

ALS-FTLD generally exhibited both upper motor neuron and LMN symptoms/signs except for 3 who exhibited only LMN symptoms/signs. Based on the electromyographic data, active denervation potentials (positive sharp waves and fibrillation potentials[18]) were identified in 3 patients with FTLD-ALS and 12 with ALS-FTLD but not in any of those with FTLD without ALS.

Pathological Evaluations of the LMN System

The results of semiquantitative pathological evaluations of the 3 clinical groups are summarized in **Figure 2**. In the FTLD without ALS group, 8 of 9 patients (89%) showed pTDP-43-positive neuronal inclusions. In addition, neuronal loss and gliosis in the spinal anterior horns were observed in 5 of 11 patients (45%) and Bunina bodies were present in 4 (36%). The pathological changes in LMN systems were most severe in the ALS-FTLD group, followed by the FTLD-ALS group, and were rather mild in the FTLD without ALS group. Among control patients, 1 had a pTDP-43-positive glial inclusion in the lumbar anterior horn, but this patient did not show neuronal loss, gliosis, or Bunina bodies (eFigure 2 in Supplement).

Figure 1. Survival by Clinical Group



Kaplan-Meier plot showing the survival rates of patients with frontotemporal lobar degeneration (FTLD) without amyotrophic lateral sclerosis (ALS) (solid line; n = 11), those in whom the onset of FTLD symptoms/signs preceded those of ALS (FTLD-ALS) (dashed line; n = 9), and those in whom the onset of ALS symptoms/signs preceded those of FTLD (ALS-FTLD) (dotted line; n = 23). Survival times were significantly shorter in patients with FTLD without ALS than in those with FTLD-ALS or ALS-FTLD ($P < .001$).

Figure 2. Semiquantitative Evaluations of Pathological Changes by Clinical Group

	FTLD Without ALS									FTLD-ALS								ALS-FTLD												*P* Values	*r* Values
Patients	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29		
Clinical duration, mo	47	66	84	108	147	360	68	96	122	22	23	28	28	31	36	36	60	7	8	9	10	16	20	22	24	26	54	60	71		
Neuronal loss																															
Facial nuclei	+	-	-	-	+	-	-	-	-	++	NA	+	+	+	+	++	+	+	++	+	+	++	++	NA	++	+	+++	+++	+	<.01	0.730
Hypoglossal nuclei	-	-	-	-	-	-	-	-	-	+++	NA	++	+++	++	+	+++	++	+++	+	+	+++	+++	+++	+++	+++	++	+++	+++	+++	+++	<.01	0.823
Anterior horn of Cx	+	++	-	-	+	-	-	-	+	++	++	+	++	++	+	++	+	+++	++	++	++	++	++	++	NA	+++	+++	++	+++	++	<.01	0.804
Anterior horn of Tx	+	+	NA	+	+	-	NA	-	+	+	++	++	++	++	++	+	++	++	+	+++	++	++	+++	++	++	+++	+++	+++	++	<.01	0.768
Anterior horn of Lx	-	-	NA	+	+	-	NA	-	-	+	+	+	+	+	+	++	+	++	++	+++	+	++	++	NA	++	+++	++	++	++	<.01	0.856
Anterior horn of Sx	-	-	NA	+	-	-	NA	-	-	+	NA	+	+	+	+	++	+	++	++	NA	NA	++	++	NA	++	++	+++	NA	+	<.01	0.880
Gliosis																															
Facial nuclei	++	-	-	-	+	-	-	-	-	++	NA	+	+	+	+	++	+	++	++	+	++	++	++	NA	+	++	+++	+++	++	<.01	0.768
Hypoglossal nuclei	-	-	-	-	-	-	-	-	-	+++	NA	++	++	++	+	+++	++	+++	+	+	+++	+++	+++	+++	++	+++	+++	+++	++	<.01	0.828
Anterior horn of Cx	+	++	-	-	+	-	-	-	+	++	++	+	+++	++	+	++	+	+++	+	++	++	++	++	NA	++	+++	++	+++	+++	<.01	0.730
Anterior horn of Tx	+	+	NA	-	+	-	NA	-	+	+	++	+	++	++	++	+	++	++	+	+++	++	++	+++	++	+	+++	+++	+++	++	<.01	0.740
Anterior horn of Lx	-	-	NA	+	+	-	NA	-	-	+	+	+	++	+	+	++	+	++	++	+++	++	++	++	NA	+++	+++	++	++	++	<.01	0.878
Anterior horn of Sx	-	-	NA	+	-	-	NA	-	-	+	NA	+	++	+	+	++	+	++	++	NA	NA	++	++	NA	+++	+++	+++	NA	++	<.01	0.904
pTDP-43-positive neuronal inclusions																															
Facial nuclei	+	-	-	-	+	-	-	-	-	++	NA	+	+	+	+	+	+	+	++	+	++	+++	+	NA	+	++	+	+	++	<.01	0.729
Hypoglossal nuclei	-	+	+	-	-	-	+	-	-	+	NA	+	+	+	+	++	+	+	+	-	+	+++	+	-	+	+	-	+	+	.08	0.348
Anterior horn of Cx	+	+	+	+	+	-	+	+	-	++	++	+	+	+	+	+	++	++	+++	+	+++	++	++	NA	+	+	+	+	+	<.01	0.511
Anterior horn of Tx	+	+	NA	-	+	-	NA	+	+	+	+	++	+	+	+	+	+	+	+	+	++	+	++	+	+	+	+	+	+	<.05	0.403
Anterior horn of Lx	+	+	NA	+	-	-	NA	+	+	+	+++	++	++	+	+	++	++	+	++	++	++	+	+++	NA	+	+	+	++	+	<.05	0.412
Anterior horn of Sx	+	-	NA	-	-	-	NA	+	-	++	NA	++	+	+	+	+	+	+	+	NA	NA	+	+++	NA	-	+	+	NA	+	<.05	0.458
Aggregation of macrophages																															
Facial nuclei	++	-	+	-	+	-	-	-	-	++	NA	+	-	++	+	++	+	++	+	+	+	+	++	NA	+	++	++	+	+	<.01	0.518
Hypoglossal nuclei	+	-	-	-	-	-	-	-	-	+	NA	-	+	++	+	+	-	+++	+	++	+	+	+++	-	++	+++	+	++	-	<.01	0.634
Anterior horn of Cx	+	+	-	+	+	-	-	+	+	++	+	+	+	++	+	++	++	+	+	+	+	-	++	NA	+	+	+	-	-	0.78	0.073
Anterior horn of Tx	+	+	NA	-	-	-	NA	+	+	++	+	++	+	++	+	+	+	+	+	++	++	+	++	+	+	+	+	+	+	<.05	0.435
Anterior horn of Lx	-	++	NA	+	-	-	NA	-	-	+	-	-	+	+	+	++	+	+++	++	+++	++	+	+++	NA	+	+++	++	++	++	<.01	0.721
Anterior horn of Sx	-	+	NA	+	-	-	NA	-	-	+	NA	-	+	+	+	+	+	+++	++	NA	NA	+	++	NA	+	+++	++	NA	+	<.01	0.751
Bunina bodies	+	+	-	+	+	-	-	-	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+		
Brain TDP-43 disease type	A	A	A	A	A	A	C	C	C	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B		

Findings shown include the severity of neuronal loss, gliosis, phosphorylated TAR DNA-binding protein of 43 kDa (pTDP-43) pathological changes, and aggregations of macrophages and the presence of Bunina bodies in the lower motor neuron systems. The severity of each pathological change was graded as 0 (none [-, not colored]), 1 (mild [+, green]), 2 (moderate [++, yellow]), or 3 (severe [+++, red]). Neuropathological changes became increasingly severe in those in whom amyotrophic lateral sclerosis (ALS) symptoms/signs preceded those of frontotemporal lobar degeneration (FTLD; ALS-FTLD), as well as the FTLD-ALS (FTLD symptoms/signs preceding those of ALS) and FTLD without ALS groups (Spearman rank order). Cx indicates cervical cord; Lx, lumbar cord; NA, not assessed; Sx, sacral cord; TDP-43, TAR DNA-binding protein of 43 kDa; and Tx, thoracic cord.

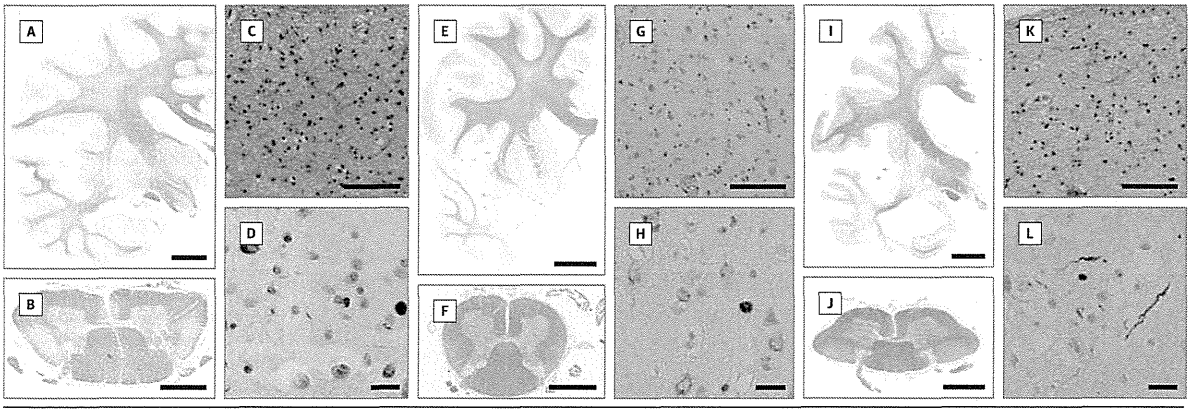
According to cortical TDP-43 pathological findings,[5] 29 patients were classified into 3 subtypes: A (n = 6), B (n = 20), or C (n = 3). Patients with FTLD without ALS showed type A or C disease, whereas those with FTLD-ALS or ALS-FTLD all showed type B disease (Figure 2 and **Figure 3**). For all the subtypes, the LMN system showed neuropathological changes that were indicative of ALS, including pTDP-43-positive neuronal and glial inclusions, neuronal loss, and gliosis. In patients with type A disease (**Figure 4**A-H), the severity of neuronal loss and gliosis in LMN systems ranged from none to moderate. Five patients (83%) in this group had pTDP-43-positive, skeinlike cytoplasmic and/or nuclear inclusions (Figure 4B and C), and 4 (67%) had Bunina bodies (Figure 4E and F) in the LMNs.

All 20 patients in the type B group (Figure 4I-L) showed neuronal loss, gliosis, and pTDP-43-positive skeinlike cytoplasmic inclusions in the LMN systems, and 18 (90%) had Bunina bodies. Among the 3 patients with type C disease (Figure 4M-P), 1 (33%) had mild loss of the LMNs (Figure 4M), and all 3 (100%) had pTDP-43-positive skeinlike cytoplasmic inclusions in the LMNs (Figure 4N). Unlike patients with the other subtypes, those with type C disease lacked Bunina bodies. Moreover, thick dystrophic neurites were prominent in the spinal anterior horn in patients with type A or C disease but rarely present in those with type B disease (Figure 4G and O). These dystrophic neurites were larger in diameter (8-12 μm) than those found in the cortices. In a double immunohistochemical analysis, pTDP-43-positive inclusions were found within the cytoplasm of ChAT-positive neurons in patients with type A, B, and C disease (Figure 4H, L, and P).

Pathological Evaluations of the Upper Motor Neuron System

In the primary motor cortex, neuronal loss and gliosis were evident in 5 patients with FTLD without ALS (56%), 2 with FTLD-ALS (25%), and 3 with ALS-FTLD (25%). Myelin pallor in the CST was evident in 6 patients with FTLD without ALS (67%), 1 with FTLD-ALS (12%), and 2 with ALS-FTLD (17%). Aggregations of macrophages in the CST were evident in 4 patients with FTLD without ALS (44%), 5 with FTLD-ALS (62%), and 6 with ALS-FTLD (50%).

Figure 3. Semimacroscopic Appearances and Brain Pathological Findings in Patients With Type A, B, and C Pathological Changes



Findings in patients with type A (A-D) , B (E-H), and C (I-L) pathological changes. In a patient with type A pathological change, cerebral coronal sections showed cortical atrophy of the parasylvian region (A). Transverse section of the cervical cord showed marked myelin pallor in the corticospinal tract (B). Microscopically, the frontal cortices showed marked neuronal loss (C) and phosphorylated TAR DNA-binding protein of 43 kDa (pTDP-43)-positive neuronal inclusions and short dystrophic neurites (D). In a patient with type B pathological change, the cerebral cortex showed severe temporal atrophy (E), neuronal loss (G), and pTDP-43-positive neuronal inclusions (H). The corticospinal tract showed mild myelin pallor (F). In a patient with type C patholgoical change, the frontal and temporal cortices showed severe atrophy (I), marked neuronal loss (K), and pTDP-43-positive long dystrophic neurites (L). The corticospinal tract showed marked myelin pallor (J). Klüver-Barrera staining (A, B, E, F, I, and J), hematoxylin-eosin staining (C, G, and K), and pTDP-43 immunohistochemistry (D, H, and L) were performed. Scale bars represent 1 cm (A, E, and I), 3 mm (B, F, and J), 100 μm (C, G, and K), and 20 μm (D, H, and L). Original magnifications are ×1 (A, B, E, F, I, and J), ×200 (C, G, and K), and ×400 (D, H, and L).

Anti-UBQLN-2 and Anti-p62N Immunohistochemistry

No patients showed any cerebellar UBQLN-2-positive or p62N-positive structures. In the temporal lobes, UBQLN-2-positive structures were occasionally observed in 8 patients, but abundant, thick, and aggregatelike structures, which are found in patients with C9ORF72 expansions, were not observed (eFigure 3 in Supplement). We presumed that our patients did not have C9ORF72 expansions.

Discussion

Our study demonstrated that pTDP-43-associated pathological changes were common in the spinal anterior horns of the FTLD without ALS, FTLD-ALS, and ALS-FTLD groups. Neuronal loss and gliosis were most severe among the ALS-FTLD group, followed by the FTLD-ALS and then the FTLD without ALS groups. Our results clearly demonstrated the pathological continuum among TDP-43-associated FTLD and ALS, even at the LMN level.

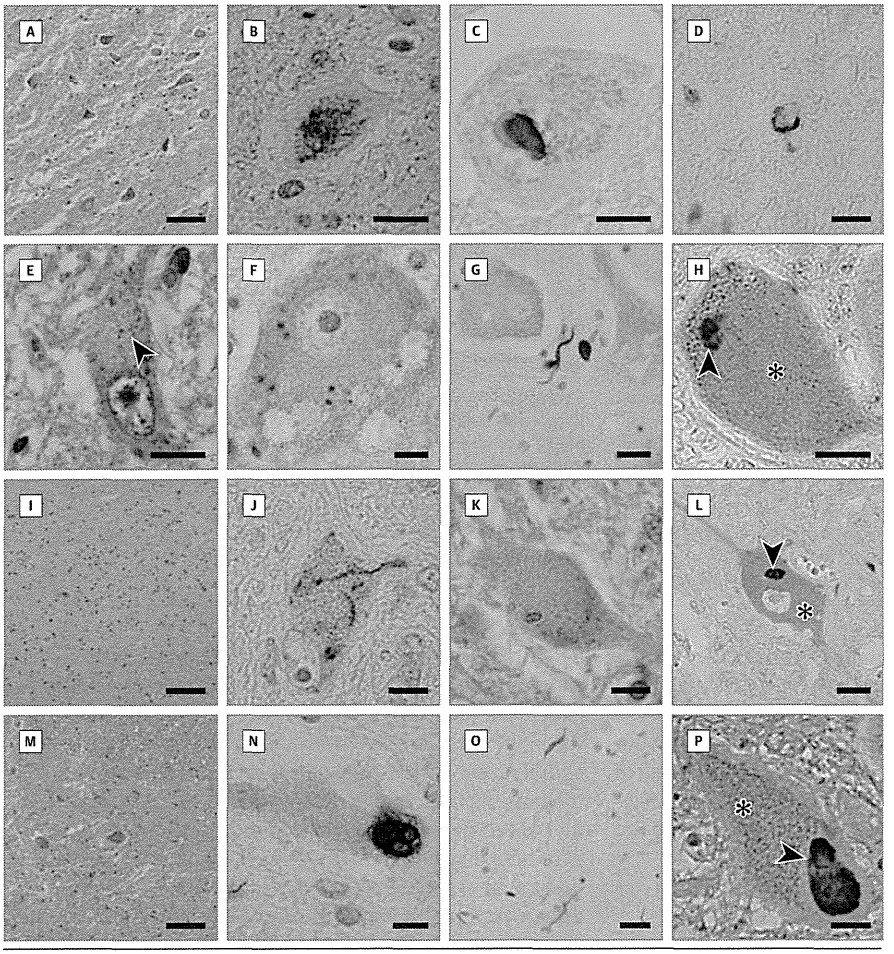
Although the FTLD without ALS group that lacked LMN symptoms showed a loss of LMNs, the degree of neuronal loss and TDP-43 disease were generally mild in this group. Experiment data using ALS mouse models revealed that symptoms developed when approximately 29% of spinal motor neurons were lost.[25] Further investigation will be needed to clarify whether LMN involvement occurs in a later stage of illness or progresses very slowly compared with cerebral involvement in FTLD without ALS.

Our results revealed that the FTLD-TDP types A, B, and C were associated with neuropathological changes corresponding to ALS in the spinal motor neurons. The severity of neuronal loss and pTDP-43 disease in the spinal motor neurons may differ quantitatively among these neuropathological subtypes. Based on cortical TDP-43 pathological findings, patients in the type B group had severe neuronal loss and diffuse pTDP-43-positive neuronal inclusions, which were entirely identical to ALS, whereas these changes were mild in the type C group. In type A, LMN pathological findings were diverse regardless of clinical duration; their severity and extension may be heterogeneous among patients with type A disease, unlike those with type B or C disease. Indeed, type A disease has also been identified in the FTLD with ALS phenotype in sporadic or familial (C9ORF72 expansion or progranulin gene mutations) form.[2,5,21,26] Dystrophic neurites were prominent in the spinal anterior horn of patients with type A or C disease. In our patient series, Bunina bodies were observed in most patients with type A or B disease but were absent in those with type C disease, findings consistent with those of previous studies.[3,17]

Several studies have demonstrated that some patients with FTLD-TDP, particularly type C, showed marked CST degeneration.[3,11,17,27] We also observed a marked myelin pallor in the CST in 67% of patients with FTLD without ALS, 12% with FTLD-ALS, and 16% with ALS-FTLD (50% for type A, 15% for type B, and 100% for type C). Some patients showed neuronal loss or gliosis in the primary motor cortex to varying extents. Furthermore, patients with FTLD without ALS often exhibited severe degenerative changes in broad areas of the frontal cortices. The broad involvement of the frontal lobes might also contribute to the CST degeneration because CST fibers arise not only from the primary motor cortex but also from the premotor cortex and supplementary motor areas.[28]

Two limitations of our study is that the evaluation of slight or very mild muscle weakness was not completed and that there were few patients with electromyographic data in

Figure 4. Pathological Findings of Spinal Motor Neuron in Subtypes of TAR DNA-Binding Protein of 43 kDa (TDP-43) Pathological Changes



Patients with type A (A-H), type B (I-L), and type C (M-P) pathological changes. A patient with type A pathological change showed mild neuronal loss (A), phosphorylated TDP-43 (pTDP-43)-positive skeinlike cytoplasmic inclusions (B), nuclear inclusions (C), and glial inclusions (D), Bunina bodies (E [arrow] and F) in the spinal anterior horn, and dystrophic neurites (G). In a patient with type B pathological change, neuronal loss (I), pTDP-43-positive skeinlike cytoplasmic inclusions (J), and Bunina bodies (K) were markedly observed. In a patient with type C pathological change, the spinal anterior horn showed mild neuronal loss (M), pTDP-43-positive skeinlike cytoplasmic inclusions (N), and dystrophic neurites (O). Double immunohistochemistry for choline acetyltransferase (ChAT) and pTDP-43 revealed cytoplasmic inclusions (violet [arrows]) present within the cytoplasm of a ChAT-positive spinal motor neuron (brown [asterisks]) of patients with type A (H), B (L), or C (P) pathological change. Hematoxylin-eosin staining (A, E, I, K, and M), pTDP-43 immunohistochemistry (B, C, D, G, J, N, and O), cystatin-C (F), and double immunohistochemical analysis for pTDP-43 and ChAT (H, L, and P) were performed. Scale bars represent 100 (A, I, and M), 20 (G, L, and O), and 10 (B-F, H, J, K, N, and P) μm. Original magnifications are ×100 (A, I, and M), ×400 (G, L, and O), and ×1000 (B-F, H, J, K, N, and P).

the FTLD without ALS group. However, our clinical data demonstrated that patients with FTLD without ALS had significantly longer survival times than those with FTLD-ALS or ALS-FTLD. These prognostic data correspond well to previous results.[29,30] In addition, the causes of death differed considerably between the FTLD without ALS group and the FTLD-ALS and ALS-FTLD groups. Respiratory failure was observed in patients with FTLD-ALS or ALS-FTLD but not in those with FTLD without ALS, and respiratory failure was strongly associated with severity of LMN loss. These results support the view that classification of FTLD based on the presence of LMN involvement was applicable in this study.

In conclusion, the LMN systems of FTLD-TDP generally show neuropathological changes that are indicative of ALS, although the severity of pathological changes differs among clinical phenotypes or subtypes of cortical TDP-43 disease. A pathological continuity between FTLD-TDP and ALS is supported by evidence of LMN involvement.

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Acquisition of data: Riku, Watanabe, Yoshida, Masuda, Senda, Sobue.
Analysis and interpretation of data: Riku, Watanabe, Yoshida, Tatsumi, Mimuro, Iwasaki, Katsuno, Iguchi, Ishigaki, Udagawa, Sobue.
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REFERENCES

1. Cairns NJ, Neumann M, Bigio EH, et al. TDP-43 in familial and sporadic frontotemporal lobar degeneration with ubiquitin inclusions. *Am J Pathol*. 2007;171(1):227-240.

2. Josephs KA, Whitwell JL, Murray ME, et al. Corticospinal tract degeneration associated with TDP-43 type C pathology and semantic dementia. *Brain*. 2013;136(pt 2):455-470.

3. Mackenzie IR, Neumann M, Bigio EH, et al. Nomenclature and nosology for neuropathologic subtypes of frontotemporal lobar degeneration: an update. *Acta Neuropathol*. 2010;119(1):1-4.

4. Whitwell JL, Jack CR Jr, Parisi JE, et al. Does TDP-43 type confer a distinct pattern of atrophy in frontotemporal lobar degeneration? *Neurology*. 2010;75(24):2212-2220.

5. Mackenzie IR, Neumann M, Baborie A, et al. A harmonized classification system for FTLD-TDP pathology. *Acta Neuropathol*. 2011;122(1):111-113.

6. Mackenzie IR, Baborie A, Pickering-Brown S, et al. Heterogeneity of ubiquitin pathology in frontotemporal lobar degeneration: classification and relation to clinical phenotype. *Acta Neuropathol*. 2006;112(5):539-549.

7. Sampathu DM, Neumann M, Kwong LK, et al. Pathological heterogeneity of frontotemporal lobar degeneration with ubiquitin-positive inclusions delineated by ubiquitin immunohistochemistry and novel monoclonal antibodies. *Am J Pathol*. 2006;169(4):1343-1352.

8. Neumann M, Sampathu DM, Kwong LK, et al. Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Science*. 2006;314(5796):130-133.

9. Josephs KA, Dickson DW. Frontotemporal lobar degeneration with upper motor neuron disease/primary lateral sclerosis. *Neurology*. 2007;69(18):1800-1801.

10. Geser F, Lee VM, Trojanowski JQ. Amyotrophic lateral sclerosis and frontotemporal lobar degeneration: a spectrum of TDP-43 proteinopathies. *Neuropathology*. 2010;30(2):103-112.

11. Snowden J, Neary D, Mann D. Frontotemporal lobar degeneration: clinical and pathological relationships. *Acta Neuropathol*. 2007;114(1):31-38.

12. Geser F, Martinez-Lage M, Robinson J, et al. Clinical and pathological continuum of multisystem TDP-43 proteinopathies. *Arch Neurol*. 2009;66(2):180-189.

13. Nishihira Y, Tan CF, Hoshi Y, et al. Sporadic amyotrophic lateral sclerosis of long duration is associated with relatively mild TDP-43 pathology. *Acta Neuropathol*. 2009;117(1):45-53.

14. Geser F, Stein B, Partain M, et al. Motor neuron disease clinically limited to the lower motor neuron is a diffuse TDP-43 proteinopathy. *Acta Neuropathol*. 2011;121(4):509-517.

15. Yoshida M. Amyotrophic lateral sclerosis with dementia: the clinicopathological spectrum. *Neuropathology*. 2004;24(1):87-102.

16. Kobayashi Z, Tsuchiya K, Arai T, et al. Clinicopathological characteristics of FTLD-TDP showing corticospinal tract degeneration but lacking lower motor neuron loss. *J Neurol Sci*. 2010;298(1-2):70-77.

17. Brooks BR, Miller RG, Swash M, Munsat TL; World Federation of Neurology Research Group on Motor Neuron Diseases. El Escorial revisited: revised criteria for the diagnosis of amyotrophic lateral sclerosis. *Amyotroph Lateral Scler Other Motor Neuron Disord*. 2000;1(5):293-299.

18. Neary D, Snowden JS, Gustafson L, et al. Frontotemporal lobar degeneration: a consensus on clinical diagnostic criteria. *Neurology*. 1998;51(6):1546-1554.

19. Braak H, Alafuzoff I, Arzberger T, Kretzschmar H, Del Tredici K. Staging of Alzheimer disease-associated neurofibrillary pathology using paraffin sections and immunocytochemistry. *Acta Neuropathol*. 2006;112(4):389-404.

20. Simón-Sánchez J, Dopper EG, Cohn-Hokke PE, et al. The clinical and pathological phenotype of C9ORF72 hexanucleotide repeat expansions. *Brain*. 2012;135(pt 3):723-735.

21. Murray ME, DeJesus-Hernandez M, Rutherford NJ, et al. Clinical and neuropathologic heterogeneity of c9FTD/ALS associated with hexanucleotide repeat expansion in C9ORF72. *Acta Neuropathol*. 2011;122(6):673-690.

22. Konno T, Shiga A, Tsujino A, et al. Japanese amyotrophic lateral sclerosis patients with GGGGCC hexanucleotide repeat expansion in C9ORF72. *J Neurol Neurosurg Psychiatry*. 2013;84(4):398-401.

23. Brettschneider J, Van Deerlin VM, Robinson JL, et al. Pattern of ubiquilin pathology in ALS and FTLD indicates presence of C9ORF72 hexanucleotide expansion. *Acta Neuropathol*. 2012;123(6):825-839.

24. Pringle CE, Hudson AJ, Munoz DG, Kiernan JA, Brown WF, Ebers GC. Primary lateral sclerosis. Clinical features, neuropathology and diagnostic criteria. *Brain*. 1992;115(pt 2):495-520.

25. Morrison BM, Janssen WG, Gordon JW, Morrison JH. Time course of neuropathology in the spinal cord of G86R superoxide dismutase transgenic mice. *J Comp Neurol*. 1998;391(1):64-77.

26. Baker M, Mackenzie IR, Pickering-Brown SM, et al. Mutations in progranulin cause tau-negative frontotemporal dementia linked to chromosome 17. *Nature*. 2006;442(7105):916-919.

27. Yokota O, Tsuchiya K, Arai T, et al. Clinicopathological characterization of Pick's disease versus frontotemporal lobar degeneration with ubiquitin/TDP-43-positive inclusions. *Acta Neuropathol*. 2009;117(4):429-444.

28. Standring S, ed. *Gray's Anatomy: The Anatomical Basis of Clinical Practice*. 39th edition. London, England: Churchill Livingstone; 2004.

29. Josephs KA, Knopman DS, Whitwell JL, et al. Survival in two variants of tau-negative frontotemporal lobar degeneration: FTLD-U vs FTLD-MND. *Neurology*. 2005;65(4):645-647.

30. Hodges JR, Davies R, Xuereb J, Kril J, Halliday G. Survival in frontotemporal dementia. *Neurology*. 2003;61(3):349-354.

A Case of α-*Synuclein* Gene Duplication Presenting With Head-Shaking Movements

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ABSTRACT

Background: *PARK4* is a candidate locus for familial Parkinson's disease (PD), combined with multiplication of the α-*synuclein* gene (*SNCA*). The eventual phenotype is dependent on the copy number of *SNCA*. Mutations in *leucine-rich repeat kinase 2* (*LRRK2*) are also causative of parkinsonism. This report describes a man who presented at our hospital complaining of a stagger after running and difficulty in handling the mouse of a personal computer, having suffered tremors since his twenties. Nine months after treatment and discharge, he developed titubation and began to drag his right foot.

Methods: We examined the patient's family pedigree for *SNCA* dosage, using quantitative polymerase chain reaction. We also screened this pedigree for mutations in *parkin* and *LRRK2*, using gene-sequencing techniques.

Results: We identified the proband, his sister, and his paternal uncle as carrying a duplication of *SNCA*. In addition, we found that the proband and his mother carried the G2385R variant of the *LRRK2*, a strong risk factor for PD in Asians and the rare V1450I variant, although only the proband showed symptoms of parkinsonism. No mutations were found in *parkin*.

Conclusions: The combination of *SNCA* gene duplication and *LRRK2* G2385R variant may explain the early onset of disease in this patient. ©2012 *Movement* Disorder Society

Key Words: head shaking; familial Parkinson's disease; alpha-synuclein gene duplication; leucine-rich repeat kinase 2

Parkinson's disease (PD) is a progressive disorder of the nervous system, with a significant number of patients having a family history of the disease. In 1900, Golbe et al. reported on a large kindred, where PD was inherited in an autosomal-dominant fashion.[1] Later, Polymeropoulos et al. identified a mutation in the α-*synuclein* gene (*SNCA*) of this family.[2] Alpha-synuclein is a major component of Lewy bodies and Lewy neurites. Abnormalities in *SNCA* include not only point mutations, but also variable copy numbers. In addition, *PARK4* has been reported on as a candidate locus for familial PD in cases with multiple copies of *SNCA*.[3] Reports show that patients with homozygous *SNCA* duplications suffer earlier ages of PD onset and death and present with more-severe cognitive impairment, compared with patients carrying heterozygous *SNCA* duplications.[4,5] These data suggest that the PD phenotype is dependent on the *SNCA* copy number.

Patients with heterozygous *SNCA* duplications present with various clinical features. Dementia has been observed in some families, although cognitive decline does not appear to be prominent in this cohort of patients.[6] Familial PD with heterozygous *SNCA* duplications shows 33% to 50% penetrance. It would seem likely that *SNCA* duplications alone are not enough to trigger PD, and that an additional risk factor is required. Mutations in *leucine-rich repeat kinase 2* (*LRRK2*) cause parkinsonism in *PARK8*-linked fami-

Additional Supporting Information may be found in the online version of this article.

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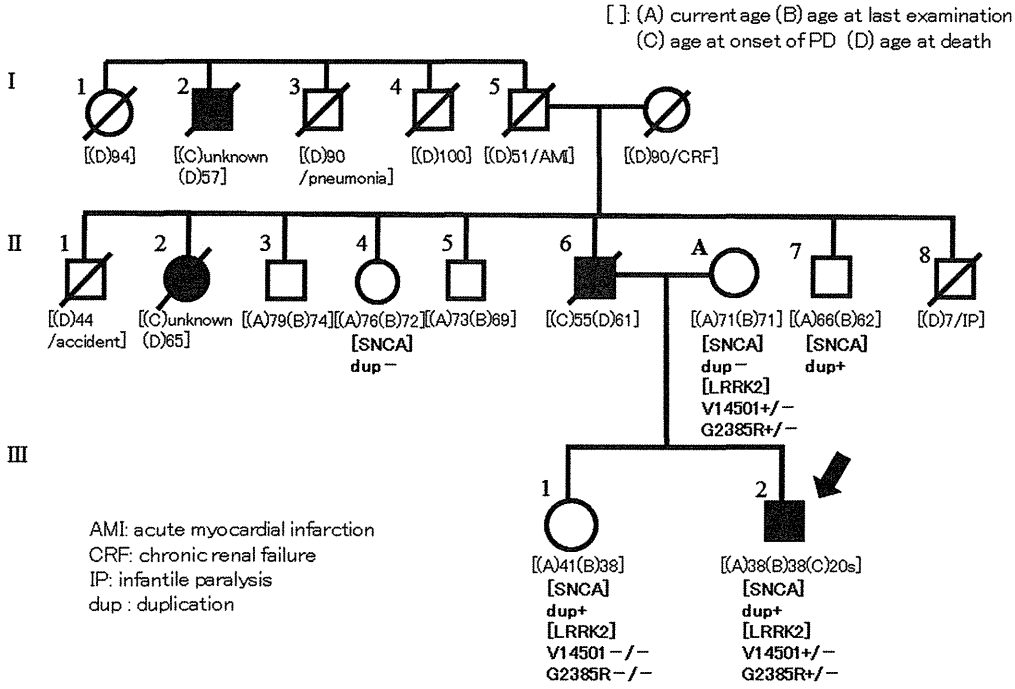

FIG. 1. Pedigree of patient III-2. Black boxes represent affected patients. Five members (II-4, II-A, II-7, III-1, and III-2) were examined clinically by a neurologist, and blood samples were collected. III-2 was affected, and II-7 and III-1 were carriers of the *SNCA* duplication.

lies as an autosomal-dominant form of familial PD.[7] The most common mutation in *LRRK2* is G2019S, found in Caucasian and North African patients.[8] Conversely, variants that represent risk factors for sporadic PD are distributed over a wide domain. Moreover, mutations in *SNCA* and *LRRK2* have been identified as strong risk factors for PD in two genome-wide association studies (GWAS).[9] The G2385R variant of *LRRK2* constitutes a specific risk factor in Asian sporadic PD, with a frequency of 10%.

It is probable that altered *SNCA* and *LRRK2* together could drive both the development and severity of PD symptoms. In fact, previous reports have observed genetic mutations of *LRRK2* in combination with other genes, such as *parkin* and the *GRB10-interacting GYF protein 2* (*GIGYF2*), in patients with PD.[10] However, there is discordance in the influence of multiple genetic variants on the clinical phenotype of PD. Both *SNCA* multiplication and *LRRK2* mutations are causative genetic events associated with autosomal-dominant familial PD at a higher frequency than any other genetic events.

Here, we detail the case of a patient with *SNCA* duplication and the *LRRK2* G2385R variant, presenting with head-shaking movements. The patient's pedigree shows familial PD inherited in an autosomal-dominant manner. Head tremors have not previously been reported on in cases with *SNCA* duplication.

Patients and Methods

Patients

The proband (III-2; Fig. 1) had experienced tremors in his right hand since his twenties. The tremor then appeared in his left hand a few years later. At the age of 33, he first visited our hospital, complaining of a stagger after running and difficulty in handling the mouse of a personal computer.

His father (II-6), paternal aunt (II-2), and paternal uncle (I-2) had developed parkinsonism in their fifties.

Genetic analysis

Each subject provided written informed consent. Genomic DNA was extracted from peripheral blood using a QIAamp DNA Blood Maxi Kit (QIAGEN, Valencia, CA). All participants were examined for gene dosage of *SNCA* by quantitative polymerase chain reaction, as described previously.[6] We also screened all the exons and exon/intron boundaries of *LRRK2* for mutations in the proband (III-2; Fig. 1), using the BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA). Screening of the proband's mother and sister was limited to exons 31, 41, and 48 of *LRRK2*. When

novel or rare variants of *LRRK2* were detected, direct sequencing of samples from 114 unrelated healthy subjects was performed (mean age at sampling: 56.0 ± 15.4 years; range, 23–98; female/male ratio: 1.05). This study was approved by the ethics committee at Juntendo University School of Medicine (Tokyo, Japan).

Results

Clinical Findings

Neurological examinations revealed cogwheel rigidity, bradykinesia, and tremors at rest. These signs were more pronounced in the right limbs. Scores of the Mini–Mental State Examination and frontal assessment battery were normal (30 and 18, respectively), but the times taken in the Trail-Making Test were 1 minute and 59 seconds in part A and 2 minutes and 20 seconds in part B, suggestive of a mild decline in attentiveness. Our patient displayed no orthostatic hypotension. Brain T2-weighted MRI yielded normal results. Voxel-based specific regional analysis for Alzheimer's disease (VSRAD), using T1-weighted three-dimensional (3D) MRI, showed atrophy of cerebral gray matter, localized to the left-side Brodmann area 10 and the left cerebellum (Fig. 2). The ratio of cardiac radiolabeled metaiodobenzylguanidine (MIBG) accumulation in the region of interest in the heart to that of the mediastinum (H/M ratio) was reduced (early, 1.58; delayed, 1.26).

Our patient was started on ropinirole hydrochloride at 0.75 mg/day, and this was increased over a period of 4 weeks to a maintenance dose of 3 mg/day. His bradykinesia and tremors improved slightly, and he was discharged from our hospital. Nine months after discharge, he developed "yes-yes" titubation at a frequency of 1 Hz and began to drag his right foot (see Video, Segment 1). His titubation stopped in the decubitus position with anteflexion of the neck. Gait disturbance improved when walking backward. Because his bradykinesia was progressive, the dose of ropinirole hydrochloride was increased to 6 mg/day, and levodopa/carbidopa was added at a dose of 400 mg (four times a day). The titubation and foot dragging showed some degree of improvement with L-dopa/carbidopa. Six months after starting L-dopa/carbidopa, horizontal head movement emerged along with the "yes-yes" titubation (see Video, Segment 2). At this time, we noted an "on-off" phenomenon, with an on-period of 2 hours. During the off-period, both bradykinesia and titubation were exacerbated. Entacapone was added at a dose of 400 mg/day, prolonging the on-period to 3 hours, but the patient was unable to go to the bathroom unaided during the night. Zonisamide was then started at a dose of 25 mg/day, and within 2 months, he was able to complete this task.

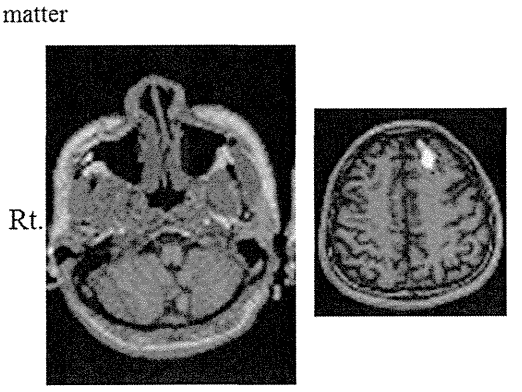

FIG. 2. VSRAD showed atrophy of cerebral gray matter localized to the left-side Brodmann area 10 and left cerebellum. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

An MIBG examination of the proband's sister (III-1) showed a normal H/M ratio.

Mutation Screening

The proband (III-2), his sister (III-1), and paternal uncle (II-7) were identified as having *SNCA* duplications. The G2385R variant of *LRRK2*, a strong risk factor for PD in Asians,[11] and the rare variant, V1450I[12] (which was absent from 114 healthy subjects), were present in the proband (III-2) and his mother (II-A), but within the family, only the proband showed symptoms or signs of parkinsonism.

We also screened the *parkin* gene for mutations, but found none. No analyses for other genes related to dystonia were performed.

Discussion and Conclusion

The unique features of this case were the early age at onset, compared to previously reported cases with *SNCA* duplication, and the marked dystonia in the neck and right leg. Dragging of the right foot was the result of dystonia, because this symptom disappeared when the patient walked backward. The titubation followed a stereotypic pattern, appearing with specific postures, and disappearing with resumption of a resting posture. Both symptoms mirrored each other in fluctuations, and both improved during the on-period. From these results, we concluded that the titubation also represented a form of dystonia. Dystonia is often present in familial PD, particularly in patients carrying *parkin* mutations, but is uncommon in families with *SNCA* duplications. The histopathological basis for primary dystonia remains unknown, although the disease is generally regarded as a disorder of basal ganglia and its efferent connections to the thalamus and brainstem, which, in turn, implies

functional abnormalities in the cerebellum.[13] Reductions in cerebellothalamic connectivity correlate with increased motor-activation responses.[14] In this case, VSRAD using T1-weighted 3D MRI[15] showed atrophy in the left Brodmann area 10 and the left cerebellum. These abnormalities may be responsible for dystonia.

Although triplication of *SNCA* leads to early-onset PD with dementia, the complication of dementia in patients with *SNCA* duplication remains controversial.[6,17–19]

The initial symptoms of *SNCA* duplication manifest 15 years after those in *SNCA* triplication families (50 years of age versus 35, respectively).[6,16,18,20] Onset occurred at a younger age in this study, compared with past reports. In addition, both *SNCA* duplication and the *LRRK2* G2385R variant were identified within our patient, together representing a strong risk for sporadic PD in Asian patients. *LRRK2* is involved in cytotoxicity and expression of *SNCA* in cellular and animal models of PD.[21,22] One possibility is that the *LRRK2* G2385R variant is related to this rare phenotype of *SNCA* duplication. Because our study included only a partial analysis of *LRRK2*, further investigation of this gene and all other modifying genes in cases of younger onset PD with head-shaking movement appears warranted. The lack of any apparent cognitive decline in this case may be the result of the younger age of this proband.

Legend to the Video

Segment 1. The patient showed "yes-yes" titubation at a frequency of 1 Hz. No tremors were present in the limbs at rest. While walking, he dragged his right leg.

Segment 2. Six months after the start of therapy with L-dopa/carbidopa, horizontal movement was added to the "yes-yes" titubation. Dragging of his right leg disappeared while walking backward. ■

References

1. Golbe LI, Di Iorio G, Bonavita V, Miller DC, Duvoisin RC. A large kindred with autosomal dominant Parkinson's disease. Ann Neurol 1990;27:276–282.
2. Polymeropoulos MH, Lavedan C, Leroy E, et al. Mutation in the α-synuclein gene identified in families with Parkinson's disease. Science 1997;276:2045–2047.
3. Singleton AB, Farrer M, Johnson J, et al. α-Synuclein locus triplication cause Parkinson's disease. Science 2003;302:841.
4. Ikeuchi T, Kakita A, Shiga A, et al. Patients homozygous and heterozygous for SNCA duplication in a family with parkinsonism and dementia. Arch Neurol 2008;65:514–519.
5. Muenter MD, Fomo LS, Homykiewicz O, et al. Hereditary form of parkinsonism-dementia. Ann Neurol 1998;43:768–781.
6. Nishioka K, Hayashi D, Farrer MJ, et al. Clinical heterogeneity of α-synuclein gene duplication in Parkinson's disease. Ann Neurol 2006;59:298–309.
7. Funayama M, Hasegawa K, Ohta E, et al. An LRRK2 mutation as a cause for the parkinsonism in the original Park8 family. Ann Neurol 2005;57:918–921.
8. Ishihara L, Gibson RA, Warren L, et al. Screening for Lrrk2 G2019S and clinical comparison of Tunisian and North American Caucasian Parkinson's disease families. Mov Disord 2007;22: 55–61.
9. Satake W, Nakabayashi Y, Mizuta I, et al. Genome-wide association study identifies common variants at four loci as genetic risk factors for Parkinson's disease. Nat Genet 2009;41: 1303–1307.
10. Dächsel JC, Mata IF, Ross OA, et al. Digenic parkinsonism: investigation of the synergistic effects of PRKN and LRRK2. Neurosci Lett 2006;410:80–84.
11. Funayama M, Li Y, Tomiyama H, et al. Leucine-rich repeat kinase 2 G2385R variant is a risk factor for Parkinson disease in Asian population. Neuroreport 2007;18:273–275.
12. Ross OA, Soto-Ortolaza AI, Heckman MG, et al. Genetic Epidemiology Of Parkinson's Disease (GEO-PD) Consortium. Association of LRRK2 exonic variants with susceptibility to Parkinson's disease: a case-control study. Lancet Neurol 2011; 10:898–908.
13. Neychev VK, Fan X, Mitev VI, et al. The basal ganglia and cerebellum interact in the expression of dystonic movement. Brain 2008;131:2499–2509.
14. Argyelan M, Carbon M, Niethammer M, et al. Cerebellothalamocortical connectivity regulates penetrance in dystonia. J Neurosci 2009;29:9740–9747.
15. Hirata Y, Matsuda H, Nemoto K, et al. Voxel-based morphometry to discriminate early Alzheimer's disease from controls. Neurosci Lett 2005;382:269–274.
16. Ibáñez P, Bonnet A-M, Débarges B, et al. Causal relation between besynuclein gene duplication and familial Parkinson's disease. Lancet 2004;364:1169–1171.
17. Chartier-Harlin MC, Kachergus J, Roumier C, et al. α-synuclein locus duplication as a cause of familial Parkinson's disease. Lancet 2004;364:1167–1169.
18. Uchiyama T, Ikeuchi T, Ouchi Y, et al. Prominent psychiatric symptoms and glucose hypometabolism in a family with a SNCA duplication. Neurology 2008;71:1289–1290.
19. Obi T, Nishioka K, Ross OA, et al. Clinicopathologic study of a SNCA gene duplication patient with Parkinson's disease and dementia. Neurology 2008;70:238–241.
20. Fuchs J, Nilsson C, Kachergus J, et al. Phenotypic variation in a large Swedish pedigree due to SNCA duplication and triplication. Neurology 2007;68:916–922.
21. Carballo-Carbajal I, Weber-Endress S, Rovelli G, et al. Leucine-rich repeat kinase 2 induces alpha-synuclein expression via the extracellular signal-regulated kinase pathway. Cell Signal 2010;22: 821–827.
22. Lin X, Parisiadou L, Gu XL, et al. Leucine-rich repeat kinase 2 regulates the progression of neuropathology induced by Parkinson's-disease-related mutant alpha-synuclein. Neuron 2009;64: 807–827.

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Analyses of the *MAPT*, *PGRN*, and *C9orf72* mutations in Japanese patients with FTLD, PSP, and CBS

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ABSTRACT

Background: Mutations in the microtubule associated protein tau (*MAPT*) and progranulin (*PGRN*) have been identified in several neurodegenerative disorders, such as frontotemporal lobar degeneration (FTLD), progressive supranuclear palsy (PSP), and corticobasal syndrome (CBS). Recently, *C9orf72* repeat expansion was reported to cause FTLD and amyotrophic lateral sclerosis (ALS). To date, no comprehensive analyses of mutations in these three genes have been performed in Asian populations. The aim of this study was to investigate the genetic and clinical features of Japanese patients with *MAPT*, *PGRN*, or *C9orf72* mutations.

Methods: *MAPT* and *PGRN* were analyzed by direct sequencing and gene dosage assays, and *C9orf72* repeat expansion was analyzed by repeat-primed PCR in 75 (48 familial, 27 sporadic) Japanese patients with FTLD, PSP, or CBS.

Results: We found four *MAPT* mutations in six families, one novel *PGRN* deletion/insertion, and no repeat expansion in *C9orf72*. Intriguingly, we identified a *de novo MAPT* p.S285R mutation. All six patients with early-onset PSP and the abnormal eye movements that are not typical of sporadic PSP had *MAPT* mutations. The gene dosages of *MAPT* and *PGRN* were normal.

Discussion: *MAPT* p.S285R is the first reported *de novo* mutation in a sporadic adult-onset patient. *MAPT* mutation analysis is recommended in both familial and sporadic patients, especially in early-onset PSP patients with these abnormal eye movements. Although *PGRN* and *C9orf72* mutations were rare in this study, the *PGRN* mutation was found in this Asian FTLD. These genes should be studied further to improve the clinicogenetic diagnoses of FTLD, PSP, and CBS.

1. Introduction

Mutations in the microtubule-associated protein tau (*MAPT*) and the progranulin (*PGRN*) genes have been identified in families with frontotemporal dementia and parkinsonism linked to chromosome 17 [1–3]. Recently, two studies reported that the expansion of a noncoding GGGGCC hexanucleotide repeat in the *C9orf72* gene is

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a major cause of both frontotemporal lobar degeneration (FTLD) and amyotrophic lateral sclerosis (ALS) [4,5].

Each of these genes can be associated with multiple clinical entities. Patients with *MAPT* mutations may receive diagnoses of frontotemporal dementia (FTD), primary progressive aphasia (PPA), or progressive supranuclear palsy (PSP). Rarely, corticobasal syndrome (CBS) or FTD with ALS (FTD-ALS) may be manifested in these patients [6]. The clinical diagnoses of patients with *PGRN* mutations include FTD, PPA, and CBS [6]. *C9orf72* repeat expansion causes FTD, ALS, FTD-ALS [4,5], PPA [5,7], and CBS [8] phenotypes. Thus, due to the complicated and often overlapping genetic and phenotypic variability in these patients, an accurate diagnosis of these clinical entities before autopsy is often difficult for clinicians.

To date, few comprehensive screening studies of these three genes have been performed in Asian populations. The aims of this study are to characterize the roles of known and, more importantly, novel disease-causing genes and to investigate the genetic and clinical features of FTLD, PSP, and CBS patients with *MAPT*, *PGRN*, and *C9orf72* mutations. In this study, we also describe the abnormal eye movements that are generally not observed in sporadic PSP but occur in early-onset PSP patients bearing *MAPT* mutations.

2. Methods

2.1. Subjects

We studied 75 Japanese patients who were diagnosed with FTLD, PSP, and CBS with or without a family history of disease. FTLD was divided into three subclasses: behavioral variant FTD (bvFTD), FTD-ALS, and PPA. The clinical diagnoses were established according to the consensus criteria for FTD [9], PPA [10], PSP [11], and CBS [12]. The characteristics of the 75 analyzed patients (69 index patients) are shown in Table 1. This study was approved by the ethics committee of the Juntendo University School of Medicine. Each subject provided written informed consent. All of the subjects in the control cohort were Japanese individuals and were evaluated by neurologists to ensure that no subjects exhibited any clinical manifestations of neurodegenerative diseases.

2.2. Genetic analyses

For direct sequence analysis, each exon was amplified by polymerase chain reaction (PCR) using published primers for *MAPT* [13] and *PGRN* [2] in a standard protocol. Dideoxy cycle sequencing was performed using Big Dye Terminator chemistry (Applied Biosystems, Foster City, CA). These products were loaded into ABI310 and 3130 automated DNA sequence analyzers and analyzed with DNA Sequence Analysis software (Applied Biosystems). To provide a qualitative assessment of the presence of an expanded $(GGGGCC)_n$ hexanucleotide repeat in the *C9orf72* gene, we performed repeat-primed PCR as previously described [4]. The normal repeat number of the GGGGCC hexanucleotide was determined in all of the patients using genotyping primers, as previously described [4]. The PCR products were analyzed on an ABI3130 DNA Analyzer and visualized using Gene Mapper software (Applied Biosystems).

Table 1
The clinical diagnoses and characteristics of 75 patients (69 index patients).

Clinical phenotype	No.	% of total	% of Male	Mean (SD) AAO (range, years)	Familial	Sporadic
FTLD	38	50.7	39.5	57.1 (±12.4), 36–78	21	17
bvFTD	29	38.7	34.5	54.5 (±12.6), 36–78	18	11
FTD-ALS	2	2.7	100	67.5 (±1.5), 66–69	1	1
PPA	7	9.3	42.9	65.0 (±7.4), 58–77	2	5
PSP	25	33.3	68.0	59.8 (±13.0), 40–76	18	7
CBS	12	16.0	33.3	58.4 (±9.52), 40–71	9	3
Total	75	100	48.0	58.2 (±12.3), 36–78	48	27
Index patients	69	92.0	46.4	58.9 (±12.4), 36–78	42	27
Relatives	6	8	66.7	50.3 (±6.6), 44–61	6	0

FTLD = frontotemporal lobar degeneration.
bvFTD = behavioral variant frontotemporal dementia.
FTD-ALS = frontotemporal dementia with amyotrophic lateral sclerosis.
PPA = primary progressive aphasia; PSP = progressive supranuclear palsy.
CBS = corticobasal syndrome; SD = standard deviation; AAO = age at onset.

2.3. Multiplex ligation-dependent probe amplification (MLPA)

To confirm the gene dosages of *MAPT* and *PGRN*, we performed MLPA using the SALSA MLPA P275-B1 MAPT-PGRN kit (MRC-Holland, Amsterdam, The Netherlands). The DNA detection/quantification protocol was provided by the manufacturer. The products were quantified using the ABI3130 Genetic Analyzer and Gene Mapper v3.7 (Applied Biosystems). The kit contains 32 probes, including 13 *MAPT* probes (located in exons 1–13) and 5 *PGRN* probes (located in exons 1, 3, 6, 10, and 12) located within other genes on chromosome 17q21. The MLPA data were analyzed as described previously [14].

2.4. Exon-trapping analysis

To determine whether a novel *MAPT* mutation was pathogenic, we performed an exon-trapping analysis. We used a wild-type construct and constructs containing the novel *MAPT* p.S285R or the IVS10+3 intronic mutation [15]. The *MAPT* sequences included exon 10, 34 nucleotides of the upstream intronic sequence and 85 nucleotides of the downstream intronic sequence. The PCR products were subcloned into the splicing vector pSPL3 (Invitrogen, Carlsbad, CA), and exon trapping was performed as described previously [15].

2.5. Paternity testing

Microsatellite analysis with 10 markers (D2S293, D3S3521, D4S2971, D5S495, D6S16171, D7S2459, D8S1705, D16S430, D18S450, and D20S842) was performed in Patient 1 and his parents to confirm paternity.

2.6. TA cloning

The novel *PGRN* heterozygous deletion/insertion found in this study, *PGRN* p.G338RfsX23 (c.1012_1013delGGinsC), was confirmed by cloning the PCR products into the pCR4-TOPO Vector using the TOPO TA Cloning kit (Invitrogen) and sequencing the two haplotypes of the heterozygote.

3. Results

3.1. Results of MAPT analysis

3.1.1. Genetic and molecular analyses of MAPT

In this study, we identified nine patients with *MAPT* mutations from six families. Four heterozygous missense mutations in *MAPT*, p.L266V, p.N279K, p.N296N, and the novel p.S285R (Supplementary Fig. 1), were identified by direct sequencing. None of the 182 normal Japanese controls included in this study had the *MAPT* p.S285R. In addition, we examined the amino acid sequences of the MAPT protein in other species and found that the site of the p.S285R mutation was highly conserved (see Supplementary Fig. 2). The novel p.S285R mutation in *MAPT* was detected in Patient 1 but not in his parents (Fig. 1A and Supplementary Fig. 1). The parentage of this patient and the DNA authenticity were confirmed using a microsatellite panel (see Supplementary Table 1). These results suggest that p.S285R is a *de novo* mutation. To investigate whether the p.S285R mutation is pathogenic, we performed an exon-trapping analysis. The p.S285R mutation produced a marked increase in the splicing of exon 10 (Fig. 1B) and resulted in the overproduction of tau isoforms that contain 4-repeat tau, such as IVS10+3 [15]. These results indicate that the p.S285R mutation is a novel, *de novo* pathogenic mutation. Previously, p.L266V, p.N279K, and p.N296N had been reported as pathogenic mutations [16–18].

Table 2 lists the clinical features of all of the *MAPT*- and *PGRN*-positive patients in this study, and Supplementary Fig. 3 shows Pedigrees C, D, E, F, and G. The average age at disease onset of patients with a single heterozygous *MAPT* mutation was 42.3 ± 2.9 (range: 37–46) years. MLPA analysis showed no gene dosage abnormalities (multiplications or deletions) in *MAPT* in this cohort.

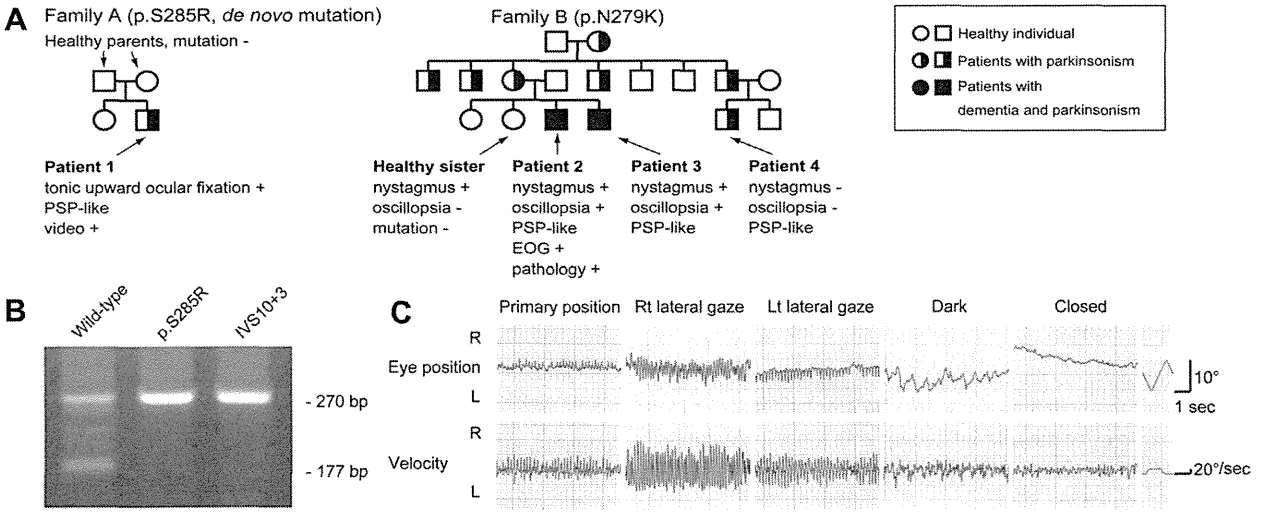

Fig. 1. (A) The pedigrees of families A and B. (B) Exon-trapping analysis for the effects of the *MAPT* p.S285R mutation on exon 10 splicing. (C) Horizontal electro-oculogram recordings in Patient 2.

3.1.2. Clinical presentations of MAPT-positive patients with the abnormal eye movements that are generally not observed in patients with sporadic PSP

3.1.2.1. Patient 1 (MAPT p.S285R). This patient was a 46-year-old man who presented with difficulty speaking and breathing. The patient had no family history of dementia or movement disorders (Fig. 1A). A physical examination revealed gait disturbance, limb bradykinesia, and frequent falling. At age 47, the patient exhibited palilalia and a mild obsession with eating. The patient's Mini-Mental State Examination (MMSE) score was 28/30, but his Frontal Assessment Battery score was 12/18. The patient exhibited a slowing of saccadic eye movements with a relative preservation of smooth pursuit, vertical supranuclear gaze palsy, and tonic upward ocular fixation (see Video Supplement); when the patient's eyes opened after closing, they remained fixated upward and could not be moved voluntarily to the primary position (i.e., Bell's phenomenon remained). To overcome this disability, the patient extended his neck, which resulted in a reflex downward movement of the eyes (the vestibulo-ocular reflex), and next he slightly flexed his neck to a neutral position with his eyes in the primary position. Later, the patient developed bradykinesia and postural instability with frequent falling. L-dopa/benserazide (up to 900 mg/day) was ineffective. The patient's condition gradually deteriorated, and he developed dementia, retrocollis, vertical and horizontal supranuclear palsy, and bradykinesia. At age 49, the patient died of suffocation from the aspiration of food material. No autopsy was performed. The clinical diagnosis was probable PSP.

3.1.2.2. Patient 2 (MAPT p.N279K). This patient was the older brother of Patient 3 (Fig. 1A). Patient 2 was a 42-year-old man who exhibited oscillopsia, micrographia, and a shuffling gait. This patient reported having had nystagmus without oscillopsia since childhood. A neurological examination revealed marked horizontal nystagmus. The patient's pupils were isocoric, and his visual acuity was normal. The patient presented with rigidity, bradykinesia, and postural tremor in the upper limbs. Electro-oculography revealed horizontal pendular nystagmus in the primary position and in all gaze directions (Fig. 1C). L-dopa/benserazide at 200 mg/day mildly alleviated his parkinsonism. Two years later, the patient developed prominent postural instability and became prone to falling. Upward and downward gaze palsy and apraxia of eyelid opening were also noted. At that time, the clinical diagnosis was possible PSP with a family history of dementia and parkinsonism. The patient's cognitive function deteriorated gradually. At age 52, he was bedridden and required a gastrostomy. The patient died of pneumonia at age 54. A postmortem pathological examination of the brain revealed mild atrophy of the frontal lobe and the tegmentum of the midbrain and pons. Microscopic analysis showed severe degenerative changes in the substantia nigra and the subcortical nuclei. Immunohistochemistry using anti-phosphorylated tau (p-tau) antibodies revealed numerous tau-positive neuronal and glial inclusions in the frontotemporal cortex, white matter, and the subcortical nuclei (see Supplementary Fig. 4). These p-tau deposits reacted with anti-4-repeat tau antibodies but not with anti-3-repeat tau antibodies.

3.1.2.3. Patient 3 (MAPT p.N279K). This patient was the younger brother of Patient 2 (Fig. 1A). At age 44, Patient 3 noticed clumsiness in his right hand and oscillopsia. The patient reported having nystagmus since childhood. A neurological examination revealed large, horizontal pendular nystagmus in the primary position and in all gaze directions. The patient's visual acuity, pupils, and light reflexes were all normal. Mild bradykinesia and rigidity in the neck and the right upper limb were noted. Postural tremor in both hands and the tongue and postural instability were observed. Treatment with 600 mg/day of L-dopa/carbidopa was not effective. The patient's oscillopsia gradually worsened, and eventually he was unable to read printed materials. At age 47, the patient developed upward and downward gaze palsy, slowing of saccades, and apraxia of eyelid opening. The patient had prominent postural instability and was prone to falling. The patient's first clinical diagnosis was possible PSP with a family history of dementia and parkinsonism. The patient died at age 56. An autopsy was not performed.

3.1.2.4. Patients 5, 6, and 7 (MAPT p.N279K). The clinical presentations of these three patients have been described previously [19]. All three patients had clinical diagnoses of possible PSP (Table 2) and visual grasping [19,20].

3.2. Results of PGRN analysis

3.2.1. Genetic Analyses of PGRN

We identified one patient with a *PGRN* mutation (Table 2, Supplementary Fig. 3). One novel heterozygous deletion/insertion

Table 2
Clinical features of patients with *MAPT* and *PGRN* mutations.

Family	A	B			C	D		E	F	G
Patient	1	2	3	4	5	6	7	8	9	10
Gene					*MAPT*					*PGRN*
Genotyping					Heterozygous					
Nucleotide change	c.853A > C	c.837T > G	c.837T > G	c.837T > G	c.837T > G	c.837T > G	c.837T > G	c.796C > G	c.888T > C	c.1012_1013delGGinsC
Amino acid change	p.S285R	p.N279K	p.N279K	p.N279K	p.N279K	p.N279K	p.N279K	p.L266V	p.N296N	p.G338RfsX23
Exon	10	10	10	10	10	10	10	9	10	9
Mode of inheritance	*de novo*	AD	AD	AD	NA	AD	AD	AD	AD	AD
Age at onset, years	46	42	44	46	41	42	43	37	44	59
Age at evaluation, years	47	47	45	50	44	44	45	38	49	61
Age at death, years	49	54	56	alive	51	54	51	alive	alive	alive
Sex	M	M	M	M	F	F	F	F	M	F
Clinical syndromes	PSP	PSP	PSP	PSP	PSP	PSP	PSP	bvFTD	PSP	PPA
Clinical features										
Initial symptoms	P	P	P	P	P	P	P	dementia	P	aphasia
Personality/behavior changes	−	+	−	−	−	−	−	+	+	−
Mini mental state examination score	28/30	NA	NA	28/30	NA	NA	NA	0	24/30	29/30
Hasegawa dementia scale-revised[a]	NA	18/30	NA	NA	21/30	28/30	30/30	0	21/30	29/30
Nonfluent spontaneous speech	−	−	−	−	−	−	−	−	−	+
Apraxia of eyelid opening	−	+	+	+	+	+	+	−	−	−
Abnormal eye movements										
Supranuclear gaze palsy	+	+	+	+	+	+	+	−	+	−
Tonic upward ocular fixation	+	−	−	−	−	−	−	−	−	−
Oscillopsia with CN	−	+	+	−	−	−	−	−	−	−
Visual grasping	−	−	−	−	+	+	+	−	−	−
Parkinsonism										
Bradykinesia	+	+	+	+	+	+	+	−	+	−
Rigidity	−	+	+	+	+	+	+	−	+	−
Tremor	−	+	+	−	−	−	−	−	−	−
Postural instability	+	+	+	+	+	+	+	−	+	−
Response to L-dopa	−	partial[b]	−	partial[b]	partial[b]	partial[b]	partial[b]	NA	+	NA
Pyramidal sign	+	−	NA	−	+	−	+	+	+	−
Features of motor neuron disease	−	−	−	−	−	−	−	−	−	−
Reference					[19]	[19]	[19]			

AD = autosomal dominant.
P = parkinsonism; NA = not available.
CN = congenital nystagmus; PSP = progressive supranuclear palsy.
bvFTD = behavioral variant frontotemporal dementia; PPA = primary progressive aphasia.
[a] The Hasegawa dementia scale-revised is a brief dementia screening scale. The maximum score of the Hasegawa dementia scale-revised is 30 points. There was a significant difference in the mean score between the demented and non-demented subjects when the cut-off point was set at 20/21 [31].
[b] A partial response to L-dopa indicates that L-dopa was effective only in the early stages.

mutation in *PGRN*, p.G338RfsX23 (c.1012_1013delGGinsC), was detected by direct sequencing and TOPO TA cloning sequencing (Supplementary Fig. 1). None of the 182 normal Japanese controls included in this study had the *PGRN* p.G338RfsX23 (c.1012_1013delGGinsC) mutations. The age at disease onset of the patient with the heterozygous *PGRN* deletion/insertion was 59 years. Novel *PGRN* variants with unknown significance, p.R18Q and p.N118del, are listed in Table 3. MLPA analysis showed no gene dosage abnormalities in *PGRN*.

3.2.2. A clinical presentation of a novel PGRN mutation

3.2.2.1. Patient 10 (PGRN p.G338RfsX23, c.1012_1013delGGinsC). This patient, a 59-year-old woman, developed word-finding difficulties and underwent surgical clipping at age 54 for an unruptured

Table 3
Novel variants with unknown significance.

Gene	Nucleotidechange	Amino acid change	Exon	Amino acid conservation	Mean AAO (years)	Frequency		*P* value	Clinical diagnosis
						Patients *N* (%)	Controls *N* (%)		
PGRN	c.56G > A	p.R19Q	1	not conserved	66	1/69 (1.4)	0/186 (0)	0.605	PSP ($n = 1$)
PGRN	c.352_354delAAC	p.N118del	4	not conserved	53	3/69 (4.3)	3/272 (1.1)	0.187	bvFTD ($n = 3$)

AAO = age at onset.
PSP = progressive supranuclear palsy.
bvFTD = behavioral variant frontotemporal dementia.