showed saccadic eye movement, ataxic speech, truncal and limb ataxia, mild rigidity and bilateral hearing disturbances. He showed no bulbar palsy, pyramidal sign, muscle atrophy or sensory disturbances. His total score on the international cooperative ataxia rating scale[9] was 64. He showed no orthostatic hypotension or dysuria. His score on the mini-mental state examination was 24/30, and his total IQ score on the Wechsler adult intelligence scale was 78. He showed amnesia but no disturbances of daily living according to amnesia. Brain MRI showed accentuated cerebellar atrophy in the anterior lobe (Fig. 1A) and small ischemic changes in the cerebral white matter. Single photon emission computed tomography (SPECT) showed relatively normal cerebellar perfusion and mild diffuse hypoperfusion in the cerebrum, but well-preserved in the precuneus and posterior cingulate. ^{123}I-meta-iodobenzylguanidine scintigraphy indicated normal cardiac uptake. Cerebrospinal fluid examination revealed largely normal measurements, with the exception of values for two indicators. The cerebrospinal fluid biomarker amyloid beta 1–42 was reduced (212 pg/mL; cutoff at our institute, >400), and phosphoryrated tau was elevated (54.6 pg/mL; cutoff at our institute, <50). Genetic analysis revealed a mutation of −16 G > T q.22 and insertion of a pentanucleotide repeat expansion (3.3 kb). The patient's father (Fig. 2, I.1) and older brother (Fig. 2, II.4) showed the same mutation and repeat expansion of SCA31. APOE4 genotype showed ε3/ε3. The patient was diagnosed as having SCA31 with mild cognitive impairment due to Alzheimer's disease (AD) and administered taltirelin hydrate without effect. After discharge from our hospital, he lived in a nursing home. His cognitive function showed a rapid decline and his daily living activities gradually declined; for example, he showed agitation and took off his clothing inappropriately.

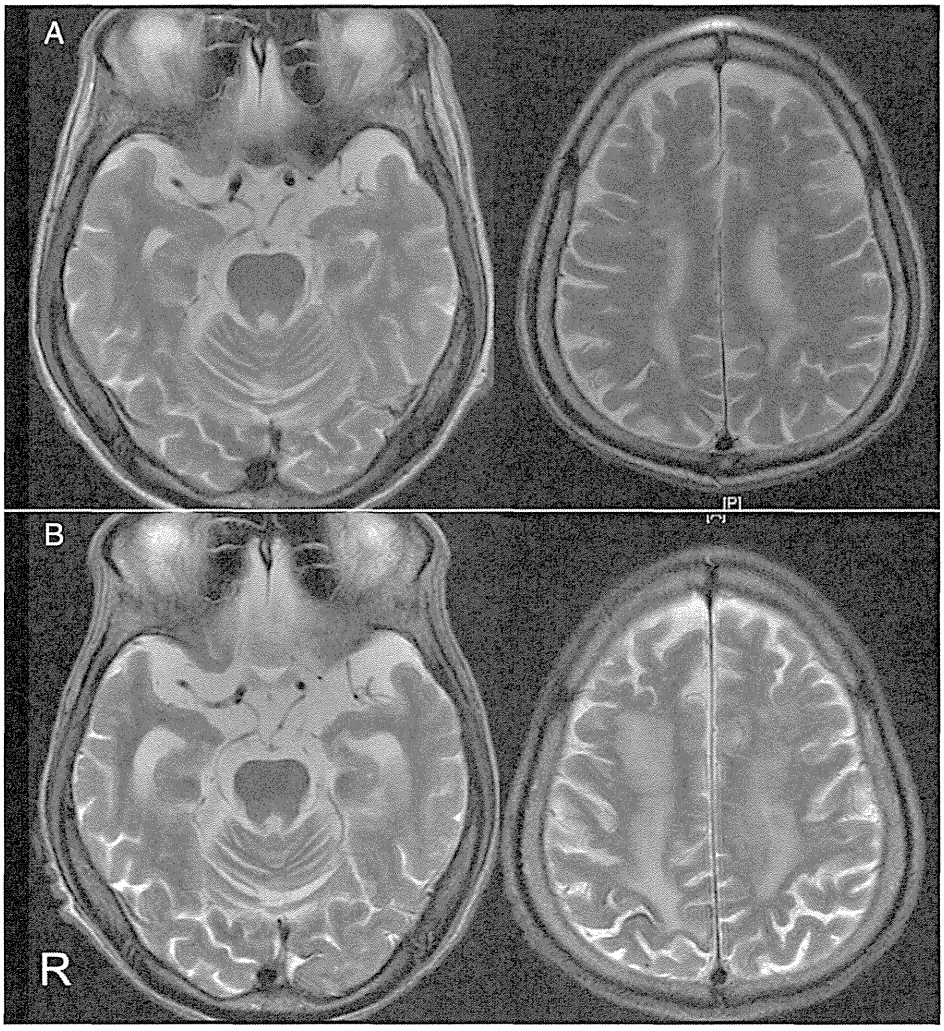


Fig. 1 T2-weighted axial MRI of the patient at ages 73 (A) and 75 (B). Cerebellar anterior lobe shows atrophy. Medial temporal lobe shows progressive atrophy with white matter hyperintensities.

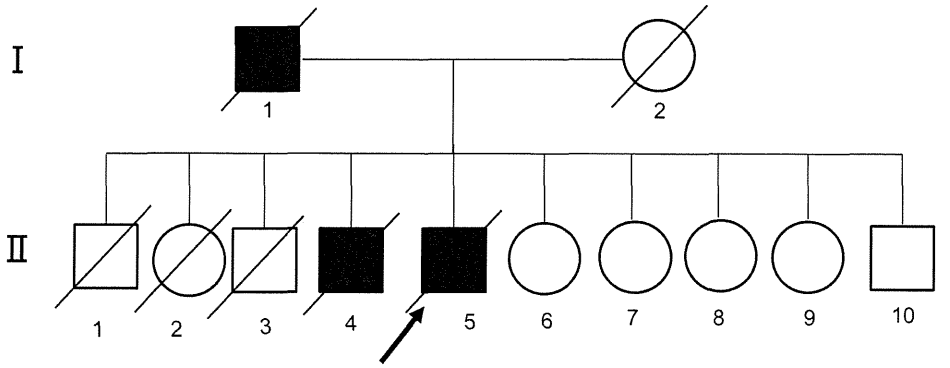


Fig. 2 Pedigree of patient in our case study. Black arrow shows the proband. Black boxes indicate patients who showed cerebellar ataxia.

Sometimes he wandered around at midnight. At age 75, he could not communicate with others (including his caregivers) and often showed delirium. Furthermore, he could neither eat nor get dressed on his own. At this point, he was readmitted to our hospital. We could not assess the cognitive decline because of severe dementia. Brain MRI showed progressive ventricular dilatation and cortical atrophy, especially in the medial temporal lobe, along with changes in white matter (Fig. 1B). We could not perform SPECT or cerebrospinal fluid examination. The patient was diagnosed with concomitant AD dementia. After discharge from our hospital, he died of respiratory failure at the age of 76. The total clinical course was about 11 years. His father (Fig. 2, I.1) showed dysarthria and ataxia, and died at the age of 70 with no dementia. His older brother (Fig. 2, II.4) showed dysarthria and ataxia at the age of 46, dysphagia at the age of 62, could not walk at the age of 78, and also showed dementia at the age of 79.

METHODS

Neuropathological examination was conducted according to the following methods. Six-micrometer-thick serial sections of formalin-fixed, paraffin-embedded, representative areas were stained with HE and by the KB method. Selected sections were stained with Gallyas–Braak silver staining to test for senile changes. Immunohistochemistry was performed with the following antibodies: anti-phosphorylated tau (AT8, monoclonal; Innogenetics, Temse, Belgium), anti-beta amyloid 11–28 (12B2, monoclonal; IBL, Maebashi, Japan), anti-phosphorylated α-synuclein (pSyn#64, monoclonal; DAKO, Glostrup, Denmark), anti-polyglutamine (1C2, monoclonal; Millipore, Billerica, MA, USA), anti-calbindin D28 K (KD-15, monoclonal; Sigma, St. Louis, MO, USA), anti-neurofilament (2F11, monoclonal; DAKO, Glostrup, Denmark), anti-glial fibrillary acidic protein (polyclonal; DAKO, Glostrup, Denmark) and anti-phosphorylated TDP43 (PSer409/410, monoclonal; Cosmobio, Tokyo, Japan). Immunoreactions were visualized by 3,3′-diaminobenzidine tetrahydrochloride. Sections were counterstained with hematoxylin.

RESULTS

Neuropathology

The brain weighed 1400 g after fixation. Gross examination confirmed mild temporal lobe atrophy and accentuated cerebellar atrophy in the anterior lobe (Fig. 3A), grayish discoloration of cerebral white matter and dilatation of lateral ventricles (Fig. 4A). The substantia nigra and locus coeruleus were well pigmented. The dentate nucleus of the cerebellum showed grayish discoloration. The hippocampus and amygdala were relatively well preserved.

Microscopic examination revealed severe Purkinje cell loss that was accentuated in the anterior lobe. The molecular layer showed moderate gliosis and moderate cell loss in the granular cell layer (Fig. 3B). The surviving Purkinje cells were often atrophic and surrounded by a characteristic amorphous material, the so-called halo-like amorphous material reported by Ishikawa *et al.*[10] (Fig. 3C,D). The dentate nucleus of the cerebellum showed mild grumose degeneration. The inferior olivary nucleus showed mild gliosis. The red nucleus, globus pallidus and subthalamic nucleus were well preserved. There was no corticospinal degeneration. Cerebral white matter showed marked myelin loss and small infarcts were present (Fig. 4B). The auditory system, including the cochlear nuclei, trapezoid body, lateral meniscus, superior olivary nucleus and inferior colliculus in the brainstem were well preserved. Cerebellar white matter showed moderate gliosis. The spinal cord was not examined.

Immunohistochemical analysis with an anti-calbindin D antibody showed amorphous Purkinje cell dendrites (Fig. 3E). Small ubiquitin-positive granules were occasionally present in surviving Purkinje cells (Fig. 3F) and cerebel-

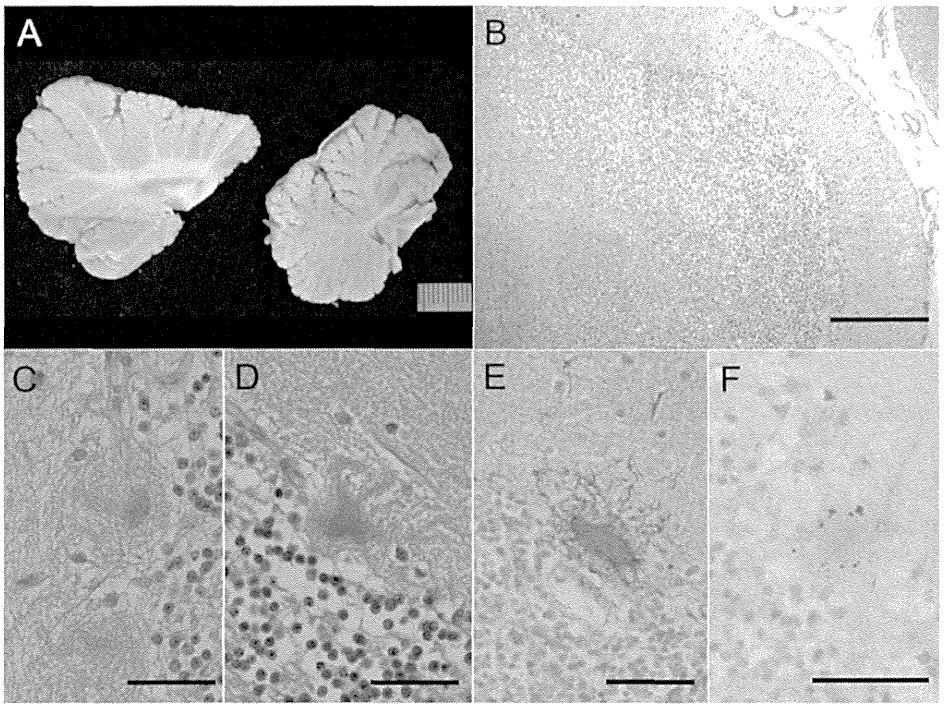


Fig. 3 Neuropathologic findings in spinocerebellar ataxia type 31. (A) Gross examination confirmed cerebellar atrophy and the dentate nucleus showed grayish discoloration. Bar = 1 cm. (B) Low-magnification image of the anterior lobe of the cerebellum. Severe Purkinje cell loss, moderate gliosis of the molecular layer and moderate cell loss of the granular cell layer were observed. Bar = 300 μm. (C) Purkinje cell atrophy and halo-like amorphous material were evident. Bar = 30 μm. (D) A small number of Purkinje cells showed sprouting. Bar = 30 μm. (E) Anti-calbindin immunohistochemistry showed granular deposition of Purkinje cell processes. Bar = 50 μm. (F) Anti-ubiquitin immunohistochemistry showed granular deposition in Purkinje cells. Bar = 50 μm.

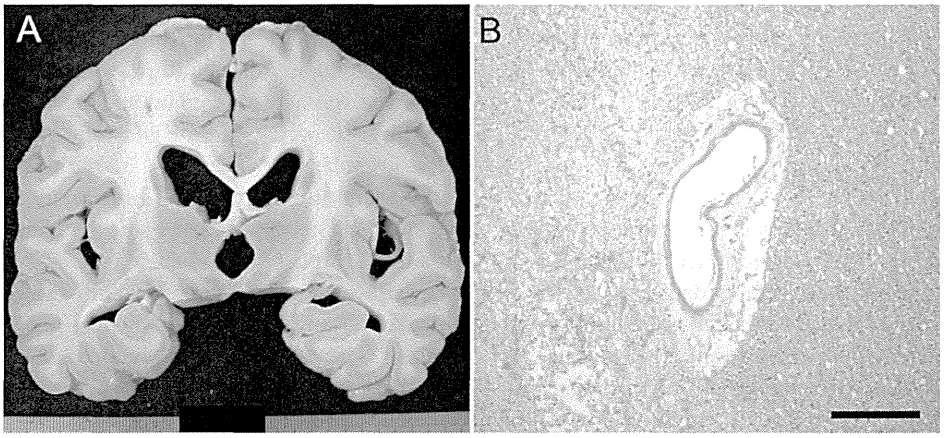


Fig. 4 Neuropathologic findings of cerebral white matter. (A) Gross examination confirmed grayish discoloration of cerebral white matter and dilatation of lateral ventricles. (B) Cerebral white matter shows rarefaction of myelin, arteriolosclerosis and small infarction. HE, bar = 200 μm.

lar white matter. Analysis with an anti-polyglutamine antibody showed no aggregation of polyglutamine in Purkinje cells. Senile plaques were widely distributed throughout the neocortex, hippocampus, precentral gyrus and thalamus (Fig. 5A; Braak amyloid stage C[11], Thal Phase 3[12]). There was extensive amyloid angiopathy in the neocortex and cerebellar leptomeningeal vessels (Fig. 5B). Alzheimer-type NFTs involved the entorhinal and transentorhinal cortex, with only a few scattered in the hippocampus (Fig. 5C; Braak NFT stage II[11]). In contrast,

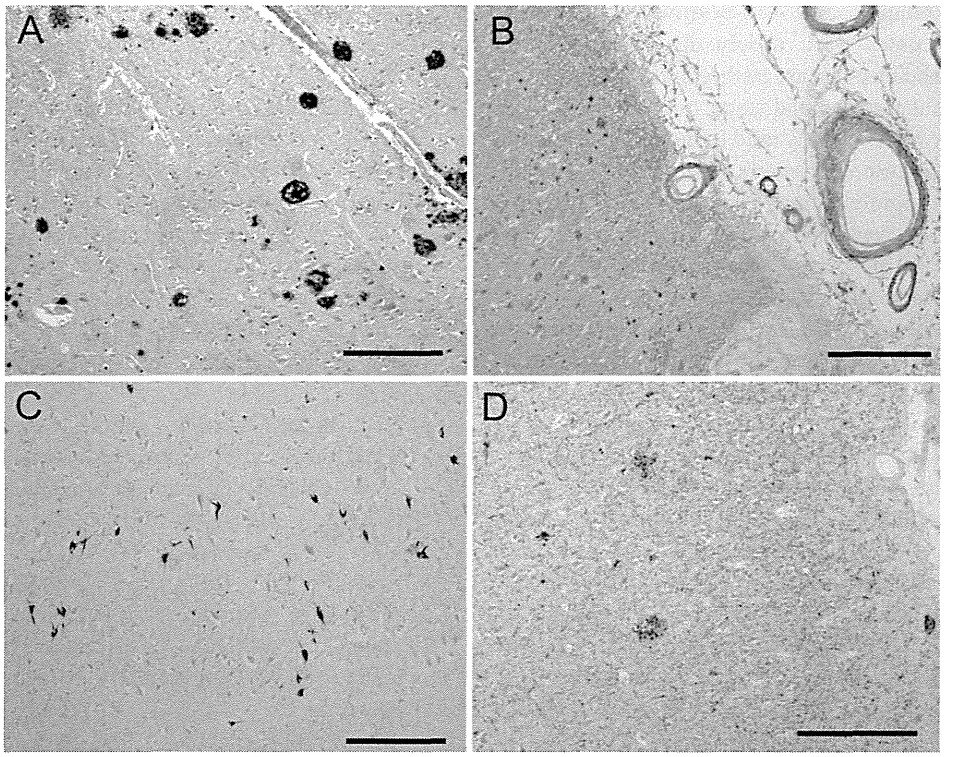


Fig. 5 Senile changes in the patient of our case study. (A) Anti-amyloid beta 11–28 immunohistochemistry demonstrated (A) classic and diffuse plaques in the precentral gyrus, as well as (B) abundant amyloid angiopathy with splitting of the arterial wall. (C) The Gallyas–Braak silver method revealed mild to moderate neurofibrillary tangles in the entorhinal cortex. (D) Anti-phosphorylated tau immunohistochemistry demonstrated tau-positive degenerative processes of senile plaques and tau-positive (Gallyas-negative) grains and neuritis in the frontal cortex. Bar = 200 μm.

AT8-immunoreactive neurites and processes of neuritic plaques were widely distributed in the neocortex (Fig. 5D; AT8 stage IV,[13] CERAD B[14]). According to the newly defined scoring system of the National Institute on Aging-Alzheimer's Association,[15] the A, B and C scores were 2, 2 and 2, respectively, and the patient was categorized as having moderate Alzheimer pathology. AT8-immunoreactive argyrophilic grains were widely distributed, although few ballooned neurons or coiled bodies were present. These grains could not be detected with the Gallyas–Braak method (BBAR stage 0.5[16]). There were no phosphorylated α-synuclein-immunoreactive structures in the amygdala, medulla oblongata, substantia nigra, locus coeruleus or sympathetic ganglia. There were no phosphorylated TDP43-immunoreactive structures in the hippocampus, medulla oblongata or amygdala.

DISCUSSION

This is the first report of SCA31 with severe dementia at the terminal stage. In Japan, where it was initially described, SCA31 represents the fourth most common form of autosomal dominant cerebellar ataxia.[3,17] Clinically, SCA31 presents with a relatively pure cerebellar phenotype that includes ataxia, dysarthria, oculomotor impairments and variable hearing loss.

There are some reports of neuropathologic examinations of SCA31. Clinically, the patient in our case study exhibited a short clinical course and showed dementia at the terminal stage. Niimi *et al.* reported an autopsy case of SCA31 with a clinical course of 4 years.[6] Although their study encompassed only a short period, pathological changes and hallmarks of SCA31 were present. In other case studies, the clinical course was more than 15 years.[5,18] These reports showed severe Purkinje cell loss in the anterior lobe of the cerebellum and dendritic changes, reminiscent of the "somatic sprouting" seen in kinky hair disease.[19] In our case study, the cerebellar pathology, especially in relation to Purkinje cells, was largely consistent with these reports. However, the halo-like amorphous materials in our case study showed mild dendritic changes, and cerebellar Purkinje cells were relatively preserved. These observations might relate to the shorter clinical course of the disease in our patient.

Our patient's brain showed the underlying pathology of dementia at the terminal stage. The frontal cortex showed

moderate senile plaques, and AT8-positive neurites and degenerative processes were widely distributed in the neuropil. This Alzheimer disease pathology contributed to cognitive decline at the terminal stage, but the Alzheimer disease pathology was relatively mild to moderate, whereas the clinical presentation was severe. In this case, cognitive decline worsened rapidly within 2 years from mild cognitive impairment to severe dementia. Myotonic tonic dystrophy, the same as SCA31 in RNA mediated disorder, shows tauopathy with tau mis-splicing.[20] SCA31 may also contribute to accentuate AD pathology at the terminal stage. White matter changes, as well as small cortical and subcortical infarctions, could have influenced the cognitive decline and mild rigidity. According to arteriolosclerosis, white matter changes were due to vascular changes. Schmahmann *et al.*, who established the concept of cerebellar cognitive affective syndrome,[21] showed that lesions of the anterior lobe of the cerebellum produced only minor changes in executive and visual-spatial functions, so the severe dementia observed in this study cannot be explained solely in terms of cerebellar cognitive affective syndrome. Alzheimer pathologies in our case may be coincidental findings, but further studies are needed to clear the cognitive impairment in SCA31.

ACKNOWLEDGMENTS

The authors thank Ms. Sachi Kurimasa for her technical support. The authors also thank all the members of the Department of Neurology, Tottori University. This work was supported by Grants-in Aid from the Research Committee for Ataxic Disease, the Ministry of Health, Labour, and Welfare of Japan (K.N.).

REFERENCES

1. Sato N, Amino T, Kobayashi K *et al.* Spinocerebellar ataxia type 31 is associated with "inserted" pentanucleotide repeats containing (TGGAA)n. *Am J Hum Genet* 2009; **85**: 544–557.
2. Ishikawa K, Durr A, Klopstock T *et al.* Pentanucleotide repeats at the spinocerebellar ataxia type 31 (SCA31) locus in Caucasians. *Neurology* 2011; **77**: 1853–1855.
3. Nozaki H, Ikeuchi T, Kawakami A *et al.* Clinical and genetic characterizations of 16q-linked autosomal dominant spinocerebellar ataxia (AD-SCA) and frequency analysis of AD-SCA in the Japanese population. *Mov Disord* 2007; **22**: 857–862.
4. Ishikawa K, Toru S, Tsunemi T *et al.* An autosomal dominant cerebellar ataxia linked to chromosome 16q22.1 is associated with a single-nucleotide substitution in the 5′ untranslated region of the gene encoding a protein with spectrin repeat and Rho guanine-nucleotide exchange-factor domains. *Am J Hum Genet* 2005; **77**: 280–296.
5. Shintaku M, Kaneda D. Chromosome 16q22.1-linked autosomal dominant cerebellar ataxia: an autopsy case report with some new observations on cerebellar pathology. *Neuropathology* 2009; **29**: 285–292.
6. Niimi Y, Takahashi M, Sugawara E *et al.* Abnormal RNA structures (RNA foci) containing a pentanucleotide repeat (UGGAA) in the Purkinje cell nucleus is associated with spinocerebellar ataxia type 31 pathogenesis. *Neuropathology* 2013; **33**: 600–611.
7. Hayashi M, Adachi Y, Mori M, Nakano T, Nakashima K. Clinical and genetic epidemiological study of 16q22.1-linked autosomal dominant cerebellar ataxia in western Japan. *Acta Neurol Scand* 2007; **116**: 123–127.
8. Basri R, Yabe I, Soma H, Sasaki H. Spectrum and prevalence of autosomal dominant spinocerebellar ataxia in Hokkaido, the northern island of Japan: a study of 113 Japanese families. *J Hum Genet* 2007; **52**: 848–855.
9. Trouillas P, Takayanagi T, Hallett M *et al.* International Cooperative Ataxia Rating Scale for pharmacological assessment of the cerebellar syndrome. The Ataxia Neuropharmacology Committee of the World Federation of Neurology. *J Neurol Sci* 1997; **145**: 205–211.
10. Ishikawa K, Mizusawa H. The chromosome 16q-linked autosomal dominant cerebellar ataxia (16q-ADCA*): a newly identified degenerative ataxia in Japan showing peculiar morphological changes of the Purkinje cell. *Neuropathology* 2010. [Epub ahead of print]
11. Braak H, Braak E. Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathol* 1991; **82**: 239–259.
12. Thal DR, Rub U, Orantes M, Braak H. Phases of A beta-deposition in the human brain and its relevance for the development of AD. *Neurology* 2002; **58**: 1791–1800.
13. Alafuzoff I, Arzberger T, Al-Sarraj S *et al.* Staging of neurofibrillary pathology in Alzheimer's disease: a study of the BrainNet Europe Consortium. *Brain Pathol* 2008; **18**: 484–496.
14. Mirra SS, Heyman A, McKeel D *et al.* The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part II. Standardization of the neuropathologic assessment of Alzheimer's disease. *Neurology* 1991; **41**: 479–486.
15. Montine TJ, Phelps CH, Beach TG *et al.* National Institute on Aging-Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's

disease: a practical approach. *Acta Neuropathol* 2012; **123**: 1–11.
16. Saito Y, Ruberu NN, Sawabe M *et al.* Staging of argyrophilic grains: an age-associated tauopathy. *J Neuropathol Exp Neurol* 2004; **63**: 911–918.
17. Fujioka S, Sundal C, Wszolek ZK. Autosomal dominant cerebellar ataxia type III: a review of the phenotypic and genotypic characteristics. *Orphanet J Rare Dis* 2013; **8**: 14.
18. Owada K, Ishikawa K, Toru S *et al.* A clinical, genetic, and neuropathologic study in a family with 16q-linked ADCA type III. *Neurology* 2005; **65**: 629–632.
19. Hirano A, Llena JF, French JH, Ghatak NR. Fine structural changes in the cerebellar cortex in kinky hair disease. *Trans Am Neurol Assoc* 1976; **101**: 87–88.
20. Caillet-Boudin ML, Fernandez-Gomez FJ, Tran H, Dhaenens CM, Buee L, Sergeant N. Brain pathology in myotonic dystrophy: when tauopathy meets spliceopathy and RNAopathy. *Front Mol Neurosci* 2014; **6**: 57.
21. Schmahmann JD. Disorders of the cerebellum: ataxia, dysmetria of thought, and the cerebellar cognitive affective syndrome. *J Neuropsychiatry Clin Neurosci* 2004; **16**: 367–378.

Brain and Behavior

Open Access

Clinical evaluation of fatigue in Japanese patients with Parkinson's disease

Kenichiro Tanaka, Kenji Wada-Isoe, Mikie Yamamoto, Shugo Tagashira, Yuki Tajiri, Satoko Nakashita & Kenji Nakashima

Division of Neurology, Department of Brain and Neurosciences, Faculty of Medicine, Tottori University, 36-1 Nishi-cho, Yonago 683-8504, Japan

Keywords
Frequency of fatigue, gait disorder, Parkinson Fatigue Scale, relative factors of fatigue, the portable gait rhythmogram

Correspondence
Kenichiro Tanaka, Division of Neurology, Department of Brain and Neurosciences, Faculty of Medicine, Tottori University, 36-1 Nishi-cho, Yonago, 683-8504, Japan.
Tel: +81 859 38 6757;
Fax: +81 859 38 6759;
E-mail: ken0815@med.tottori-u.ac.jp

Funding Information
This work was supported by Grants-in-Aid from the Research Committee of CNS Degenerative Disease, the Ministry of Health, Labour and Welfare of Japan.

Received: 4 March 2014; Revised: 6 May 2014; Accepted: 9 June 2014

Brain and Behavior 2014; 4(5):643–649

doi: 10.1002/brb3.247

Abstract

Background: Fatigue is a common nonmotor symptom of Parkinson's disease (PD). Although the causes of fatigue were estimated in the previous reports, fatigue is not fully understood. To determine the frequency of and factors related to fatigue in patients with PD, we carried out clinical assessments in our university hospital. **Methods:** We used the Japanese version of the Parkinson Fatigue Scale (J-PFS). The J-PFS was administered to 110 patients with PD, and a cutoff point of 3.3 was used for the diagnosis of fatigue. Subsequently, demographic characteristics, clinical features, and medications utilized were evaluated to elucidate the factors related to fatigue. In particular, we focused on the relationship between fatigue and gait disorder assessed via the portable gait rhythmogram. **Results:** The frequency of fatigue in patients with PD was 52.7%. Univariate analysis revealed that factors significantly associated with fatigue were many motor symptoms and nonmotor symptoms. In addition, multivariate analysis revealed that gait disorder and constipation were independent factors related to fatigue. Furthermore, short-step walking and bradykinesia in gait disorder had especially a relationship with fatigue. **Conclusions:** More than half of our patients were judged having fatigue. Several factors, including motor and nonmotor symptoms, might be related to fatigue in patients with PD.

Introduction

Background

Recently, nonmotor symptoms in patients with Parkinson's disease (PD) have attracted attention. These nonmotor symptoms affect quality of life, institutionalization, and health care costs. Fatigue is one of the nonmotor symptoms of PD, and it might be critical for patient's quality of life. More than half of the patients with PD think that fatigue is one of the three most difficult symptoms (Friedman and Friedman 1993). Nevertheless, fatigue in patients with PD is not commonly identified in the clinical setting. Although the causes of fatigue were estimated in the previous reports, fatigue is not fully understood. In the past, fatigue pathophysiology was discussed, but it was not well characterized. Therefore, the causes of fatigue are divided into several symptoms (Kluger et al. 2013).

The taxonomies of fatigue

The taxonomies of fatigue vary; in one, fatigue is divided into peripheral fatigue and central fatigue (Chanudhuri and Behan 2000). According to this taxonomy, the fatigue of PD is included in central fatigue. Central fatigue is defined as the failure to initiate and/or sustain attentional tasks and physical activities requiring self motivation. In contrast, peripheral fatigue is fatigue from exercise or physical activity, and is defined as the inability in sustaining a specific force or work rate. In another system, fatigue is divided into mental fatigue and physical fatigue (Okuma 2012). With this classification, it was

reported that both mental fatigue and physical fatigue were related to fatigue in patients with PD (Lou et al. 2001).

Purpose

In this study, we analyzed fatigue in patients with PD, and to determine the frequency of fatigue and factors related to fatigue in PD, we evaluated clinical features, such as demographic characteristics, motor symptoms, nonmotor symptoms, and medications. In particular, we focused on the relationship between fatigue and gait disorder. Gait disorder is one of the most well-known motor symptoms of PD, and it decreases patient's quality of life, but it has not been studied in relation to fatigue fully. In this study, we assessed gait via the portable gait rhythmogram (Mitoma et al. 2010), we evaluated gait from several points of view, and we investigated its relationship with fatigue.

Methods

Subjects

The subjects for this study comprised of 110 patients diagnosed with idiopathic PD in the Department of Neurology at Tottori University Hospital between April 2011 and December 2011. The clinical diagnosis of PD was based on the UK PD Society Brain Bank criteria (Gibb and Lees 1988). PD patients diagnosed with dementia according to PD dementia criteria of Movement Disorder Society were excluded beforehand (Poewe et al. 2008).

Diagnosis of fatigue

We used the Japanese version of the Parkinson Fatigue Scale (J-PFS), which was derived from the Parkinson Fatigue Scale (PFS) in the UK (Okuma et al. 2009). The PFS was the first questionnaire developed for evaluating fatigue in patients with PD, and it consists of 16 items (Brown et al. 2005). Response options were "strongly disagree," "disagree," "do not agree or disagree," "agree," "strongly agree," which were scored 1 to 5, respectively. The overall PFS score was calculated as the mean response across all items (range, 1.0–5.0) and the cutoff point of 3.3 was used for the diagnosis of fatigue (an average score of 3.3 or greater indicates the existence of fatigue) (Okuma et al. 2009). The PFS is available in several languages and is used in other countries (Kummer et al. 2011; Hagell et al. 2012). The utility of the J-PFS for fatigue was reported in patients with PD (Okuma 2012).

Demographic characteristics, clinical features, and medications utilized

We analyzed the demographic and clinical characteristics of each group, including sex, age, age of disease onset defined from medical interviews, PD duration, degree of severity of motor symptoms, nonmotor symptoms (depression, apathy, sleep disturbance, excessive daytime sleepiness, REM sleep behavior disorder [RBD], restless leg syndrome [RLS], orthostatic hypotension, constipation, visual hallucinations, and olfactory dysfunction), medications (total levodopa equivalent dose [LEDs], levodopa, pramipexole, ropinirole, selegiline, and amantadine), and the heart-mediastinum (H/M) ratio of ^{123}I-meta-iodobezylguanidine (MIBG) myocardial scintigraphy (Wada-Isoe et al. 2007). We estimated motor symptoms by using the Hoehn–Yahr stage. Nonmotor symptoms were evaluated via questionnaires, including the Geriatric Depression Scale-15 (GDS-15) (Niino et al. 1991), the Apathy Scale (AS) (Okada et al. 1998), the Pittsburgh Sleep Quality Index (PSQI) (Buysse et al. 2003), the Japanese version of the Epworth Sleepiness Scale (JESS) (Takegami et al. 2009), and the REM Sleep Behavior Disorder Screening Questionnaire (RBDSQ) (Stiasny-Kolster et al. 2007). The GDS-15 has been validated for the diagnosis of depression, the AS for apathy, the PSQI for poor sleep, the JESS for excessive daytime sleepiness, and the RBDSQ for RBD. RLS was evaluated using the diagnostic criteria of the International RLS Study Group (Allem et al. 2003). Patients were assessed from medical interviews as positive or negative for orthostatic hypotension, constipation, visual hallucinations, and olfactory dysfunction.

We assessed gait as motor symptoms via the portable gait rhythmogram. The portable gait rhythmogram is a small device that measures the acceleration in trunk movements caused by step-in and kickoff in three dimensions, using accelerometers (Mitoma et al. 2010). It is attached to the patient's waist to evaluate gait for 10 m. The results do not include freezing of gait, since patients actually walk another 2 m before and after the 10 m. We focused on gait acceleration, gait speed, and step length in PD patients. Gait acceleration is the index proportional to floor reaction forces whose unit was measured via G/100 (G: gravity = 9.8 m/s^2) and the most important end point.

Statistical analysis

Data analysis was conducted with SPSS for Windows, version 18 (Chicago, IL). The results are presented as medians ± interquartiles range. In univariate analyses, intergroup differences were analyzed with a Mann–Whitney *U*-test, and categorical variances were examined with a χ^2

test. Correlation analyses were conducted with the Spearman's correlation test. Multivariate analyses were performed via logistic regression analysis. We used the stepwise forward method, and the variables which had statistical significance in univariate analyses were chosen. Regarding gait, we selected gait acceleration because it was the most important end point, and we did not include gait speed and step length because of multicollinearity problems. The goodness-of-fit of the final model was tested by the Hosmer–Lemeshow method. We used a level of 95% ($P < 0.05$) as the criterion for statistical significance.

This study was planned and conducted in accordance with the Declaration of Helsinki. The Ethics Committee of the Tottori University Faculty of Medicine approved the study prior to its implementation.

Results

Frequency of fatigue

We were able to administer the J-PFS and to determine the demographic characteristics, clinical features (except for gait), and medications utilized for all 110 PD patients.

The frequency of fatigue was 52.7% (males: 55.1% PD duration 10.0 ± 5.0 years, females: 50.8% PD duration 8.0 ± 3.0 years).

Table 1. Comparison of patients with PD with and without fatigue diagnosed via the J-PFS.

Variables	Overall $n = 110$	Without fatigue $n = 52$	With fatigue $n = 58$	*P*-value
J-PFS score	3.2 ± 1.0	2.3 ± 0.5	4.0 ± 0.5	
Demographic factors				
Male:Female	49:61	22:30	27:31	0.703*
Age (years)	70.0 ± 6.5	69.5 ± 6.0	72.0 ± 7.0	0.256
Age of disease onset (years)	61.5 ± 7.0	61.0 ± 6.5	63.0 ± 8.0	0.592
PD duration (year)	8.0 ± 4.5	7.0 ± 4.5	8.5 ± 4.5	0.147
Motor symptoms				
Hoehn–Yahr stage	2.0 ± 0.5	2.0 ± 0.5	3.0 ± 1.0	0.001
Acceleration (gait:G/100)[1]	24.0 ± 7.0	28.0 ± 5.5	20.0 ± 5.0	0.002
Speed (gait:m/min)[1]	56.5 ± 16.0	65.0 ± 16.5	45.0 ± 16.5	<0.001
Step length (gait:cm)[1]	48.5 ± 14.5	55.0 ± 14.0	38.0 ± 14.5	0.001
Nonmotor symptoms				
GDS-15	5.0 ± 2.5	5.0 ± 1.5	7.0 ± 3.0	0.002
AS	17.0 ± 4.0	16.0 ± 3.5	18.0 ± 5.5	0.026
PSQI	6.0 ± 2.0	5.5 ± 2.5	7.0 ± 2.5	0.057
JESS	6.0 ± 4.0	5.0 ± 2.5	8.0 ± 4.0	0.002
RBDSQ	4.0 ± 2.0	3.0 ± 2.0	4.0 ± 2.0	0.040
Restless leg syndrome (*n*)	14 (13%)	4 (8%)	10 (17%)	0.243*
Orthostatic hypotension (*n*)	50 (45%)	19 (37%)	31 (53%)	0.087*
Constipation (*n*)	84 (76%)	34 (65%)	50 (86%)	0.013*
Visual hallucinations (*n*)	33 (30%)	11 (21%)	22 (38%)	0.063*
Olfactory dysfunction (*n*)	40 (36%)	16 (31%)	24 (41%)	0.321*
Early H/M ratio (MIBG)	1.58 ± 0.21	1.68 ± 0.31	1.57 ± 0.15	0.330
Delayed H/M ratio (MIBG)	1.42 ± 0.28	1.60 ± 0.47	1.35 ± 0.16	0.192
Medication				
Total LEDs (mg)	464 ± 173	469 ± 189	462 ± 157	0.297
Dosage of levodopa (mg)	300 ± 100	300 ± 100	350 ± 100	0.003
Dosage of pramipexole (mg)	1.5 ± 1.2	1.0 ± 1.0	1.5 ± 1.2	0.574
Dosage of ropinirole (mg)	4.5 ± 3.5	5.5 ± 4.5	3.0 ± 2.0	0.356
Dosage of selegiline (mg)	2.5 ± 1.5	2.5 ± 1.5	3.8 ± 1.5	0.843
Dosage of amantadine (mg)	150 ± 69	200 ± 125	100 ± 38	0.210

J-PFS, Japanese version of the Parkinson Fatigue Scale; GDS-15, Geriatric Depression Scale-15; AS, Apathy Scale; PSQI, Pittsburgh Sleep Quality Index; JESS, Japanese version of the Epworth Sleepiness Scale; RBDSQ, REM Sleep Behavior Disorder Screening Questionnaire; total LEDs, total levodopa equivalent dose.

Medians ± interquartile range.

Mann–Whitney *U*-test.

*χ^2 test.

[1]75 patients were able to be assessed (without fatigue:with fatigue = 40:35).

Table 2. Correlation between the J-PFS and other continuous variables

	Spearman's ρ	*P*-value
Demographic factors		
Age (years)	0.182	0.057
Age of disease onset (years)	0.074	0.442
PD duration (year)	0.227	0.017
Motor, nonmotor symptoms		
Hoehn–Yahr stage	0.325	0.001
Acceleration (gait:G/100)[1]	−0.281	0.009
Speed (gait:m/min)[1]	−0.409	<0.001
Step length (gait:cm)[1]	−0.355	0.001
Early H/M ratio (MIBG)	−0.024	0.822
Delayed H/M ratio (MIBG)	−0.101	0.353
GDS-15	0.370	<0.001
AS	0.288	0.002
PSQI	0.251	0.008
JESS	0.269	0.004
RBDSQ	0.233	0.014
Medication		
Total LEDs (mg)	0.155	0.105
Dosage of levodopa (mg)	0.368	<0.001
Dosage of pramipexole (mg)	0.067	0.708
Dosage of ropinirole (mg)	−0.144	0.464
Dosage of selegiline (mg)	0.106	0.511
Dosage of amantadine (mg)	−0.244	0.364

J-PFS, Japanese version of the Parkinson Fatigue Scale; GDS-15, Geriatric Depression Scale-15; AS, Apathy Scale; PSQI, Pittsburgh Sleep Quality Index; JESS, Japanese version of the Epworth Sleepiness Scale; RBDSQ, REM Sleep Behavior Disorder Screening Questionnaire; Total LEDs, total levodopa equivalent dose.
[1]75 patients were able to be assessed (without fatigue: with fatigue = 40:35)

Demographic features related to fatigue

In univariate analyses, none of the demographic factors, (gender, age, age of disease onset or PD duration) was related to fatigue (Table 1). In analyses calculated via Spearman's correlation test, PD duration was only correlated with the J-PFS score (Table 2).

Motor symptoms related to fatigue

In univariate analyses, the severity of Hoehn–Yahr stage, decreased gait acceleration, reduced gait speed, and short-step length were all related to fatigue (Table 1). Of the 110 patients with PD, 75 were able to be assessed for gait via the portable gait rhythmogram. We could not assess 35 patients either because they could not walk by themselves or because we could not contact some of them. The 35 patients who could not be assessed for gait were older and have worse motor function than the 75 patients who could be assessed for gait (Table 3). In analyses calculated via Spearman's correlation test, the severity of Hoehn–Yahr stage, decreased gait acceleration, reduced gait speed, and short-step length were also correlated with the J-PFS score (Table 2).

Table 3. Comparison of patients who were assessed for gait and were not.

	Assessed for gait, n = 75	Not assessed for gait, n = 35	*P*-value
Male:Female	33:42	16:19	1.000[1]
Age (years)	69.5 ± 5.5	75.0 ± 7.0	0.007
Age of disease onset (years)	60.0 ± 6.5	66.0 ± 7.0	0.019
PD duration (year)	8.0 ± 4.0	8.5 ± 5.5	0.608
Hoehn–Yahr stage	2.0 ± 0.5	3.0 ± 1.0	0.029

Medians ± interquartile range.
Mann–Whitney *U*-test.
[1]χ^2 test.

Nonmotor symptoms related to fatigue

In univariate analyses, depression, apathy, excessive daytime sleepiness, REM sleep behavior disorder, and constipation were related to fatigue. The frequencies of sleep disturbance, orthostatic hypotension, and visual hallucinations in the patients with fatigue were higher than those patients without fatigue, but the differences did not reach the statistical significance (Table 1). In analyses calculated via Spearman's correlation test, depression, apathy, sleep disturbance, excessive daytime sleepiness, and REM sleep behavior disorder, were correlated with the J-PFS score (Table 2).

Relationship factors of dopamine replacement therapy with fatigue

In univariate analyses, only the dosage of levodopa was related to fatigue. Dosages of other medications (LEDs, pramipexole, ropinirole, selegiline, and amantadine) were not related to fatigue (Table 1). In analyses calculated via Spearman's correlation test, the dosage of levodopa was also correlated with the J-PFS score, but other medications were not correlated with fatigue (Table 2).

Results of multivariate analyses

In multivariate analyses of the patients we could assess for gait, gait acceleration and constipation were found to be independent factors related to fatigue (Table 4).

Discussion

Frequency of fatigue

We calculated the frequency of fatigue in Japanese patients with PD, and more than half had fatigue. Previously, the

Table 4. Logistic regression model predicting patients with fatigue via the J-PFS ($n = 75$).

	β	Odds ratio	95% of CI for odds ratio	*P*-value
Acceleration (gait:G/100)	−0.106	0.899	0.836–0.968	0.005
Constipation	1.408	4.088	1.174–14.235	0.042

J-PFS, Japanese version of the Parkinson Fatigue Scale; CI, confidence interval; GDS-15, Geriatric Depression Scale-15; AS, Apathy Scale; JESS, Japanese version of the Epworth Sleepiness Scale; RBDSQ, REM Sleep Behavior Disorder Screening Questionnaire.

Nagelkerke R2 0.257, Hosmer–Lemeshow test: χ^2 test = 4.949, $P = 0.763$.

Model contained Hoehn–Yahr stage, gait acceleration, GDS-15, AS, JESS, RBDSQ, constipation, and dosage of levodopa.

frequency of fatigue in patients with PD was reported in various areas, and in many, it ranged from 37% to 56% (Fabbrini et al. 2013), so the frequency of fatigue in our patients is similar to that reported in various studies. Although both physical and mental fatigue are involved in PD, the PFS takes mainly physical fatigue into account. It does not account for mental fatigue, so the fatigue of patients with PD in our study placed a disproportionate emphasis on physical fatigue (Falup-Pecurariu 2013). Nevertheless, several nonmotor symptoms were related to fatigue and evaluated via the J-PFS in this study. From the results, it is possible that nonmotor symptoms also contribute to fatigue assessed via the J-PFS.

Relative factors of fatigue

Past studies have indicated that many factors, including depression, excessive daytime sleepiness, sleep disturbance, motor symptoms, PD duration, and female gender are related to fatigue in PD (Karlsen et al. 1999; Alves et al. 2004; Havlikova et al. 2008; Okuma et al. 2009; Beiske et al. 2010; Valko et al. 2010; Kummer et al. 2011; Metta et al. 2011; Van Dijk et al. 2013). In our study, demographic features, such as gender, age, age of disease onset, and PD duration had little influence on fatigue. These results indicate that fatigue does not have the relation with demographic features. Motor symptoms, such as Hoehn–Yahr stage, gait acceleration, gait speed, and step length were related to fatigue. Gait acceleration was independently related to fatigue in multivariate analysis. In addition, gait speed and step length were also independent relative factors in the multivariate analysis including gait speed or step length instead of gait acceleration (Supporting information). These results indicated that there is a close relationship between gait disorder and fatigue. Although there are some reports describing a relationship between gait disorder and fatigue in PD patients (Garber and Friedman 2003; Rochester et al. 2004, 2006; Rahman et al. 2008), they were not enough because they were not evaluated as to what kind of gait disorder was related to fatigue. Gait disorder in patients with PD has several varieties, such as freezing of gait, short-step walking, festination, bradykinesia, etc. Decreased gait acceleration (floor reaction forces) was related to these gait disorders and was the most important end point. In our study, reduced gait speed and short-step length, as well as decreased gait acceleration, were related to fatigue, so short-step walking and bradykinesia as slow gait cycle walking might be more related to fatigue than festination as fast gait cycle walking. Nonmotor symptoms, such as depression, apathy, excessive daytime sleepiness, RBD, and constipation, were related to fatigue. Among them, constipation was an independent factor related to fatigue. There are some reports that indicate the relationship between other nonmotor symptoms and fatigue, but the relationship between constipation and fatigue has not been reported previously to the best of our knowledge. Therefore, this may be a new insight about fatigue for PD patients. Autonomic nervous systems might be relative factors with fatigue, so we investigated the relationship between fatigue and MIBG myocardial scintigraphy. MIBG myocardial scintigraphy was reported to have a relationship with other nonmotor symptoms, such as olfactory dysfunction and orthostatic hypotension (Oka et al. 2010; Manabe et al. 2011), but it was not related to fatigue in this study. Regarding dopamine replacement therapies, only the dosage of levodopa was related to fatigue, whereas total LEDs was not related. This may be because we could not use other medications, such as dopamine agonists, easily for patient with nonmotor symptoms as the medication that made these symptoms worse (judging from our study, patients with fatigue were likely to have other nonmotor symptoms which got worse by other medications, such as excessive daytime sleepiness, visual hallucination, etc.). In the previous study, the relationship between fatigue and levodopa was reported (Schifitto et al. 2008), but there is not another. These results indicated that medication has little influence on fatigue in PD patients.

Limitations

There are a few limitations of this study. First, the definition of fatigue in the patients in our study placed a disproportionate emphasis on physical fatigue, because we assessed fatigue via the J-PFS. Next, regarding motor symptoms, the evaluation of patients who could not walk by themselves was not sufficient, since we assessed the motor symptoms, except for Hoehn–Yahr stage, via portable gait rhythmography. In the gait assessment, we

could not evaluate freezing of gait. Finally, we did not study a true random sample of patients with PD, since our study population consisted of only those patients who visited our hospital.

Conclusion

Many PD patients had fatigue as reported in the past, and various factors, such as motor symptoms and non-motor symptoms, were related to fatigue, but it was not clear which of these symptoms related to fatigue caused fatigue or if these symptoms occurred from fatigue. However, these symptoms had no small effect on fatigue, so we should pay attention to fatigue in clinical settings.

Acknowledgments

We thank the staff at the Department of Neurology at Tottori University for their help in recruiting patients. This work was supported by Grants-in-Aid from the Research Committee of CNS Degenerative Disease, the Ministry of Health, Labour and Welfare of Japan.

Conflict of Interest

We have no conflicts of interest.

References

Allem, R. P., D. Picchietti, W. A. Hening, C. Trenkwalder, A. S. Walters, J. Montplaisi, et al. 2003. Restless legs syndrome: diagnostic criteria, special considerations, and epidemiology. A report from the restless legs syndrome diagnosis and epidemiology workshop at the National Institutes of Health. Sleep Med. 4:101–119.

Alves, G., T. Wentzel-Larsen, and J. P. Larsen. 2004. Is fatigue an independent and persistent symptom in patients with Parkinson disease. Neurology 63:1908–1911.

Beiske, A. G., J. H. Loge, M. J. Hjermstad, and E. Svensson. 2010. Fatigue in Parkinson's disease: frequency and associated factors. Mov. Disord. 25:2456–2460.

Brown, R. G., A. Dittner, L. Findley, and S. C. Wessely. 2005. The Parkinson fatigue scale. Parkinsonism Relat. Disord. 11:49–55.

Buysse, D. J., III. C. F. Reynold, T. H. Monk, S. R. Berman, and D. J. Kupfer. 2003. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatry 18:498–505.

Chanudhuri, A., and P. O. Behan. 2000. Fatigue and basal ganglia. J. Neurol. Sci. 179:34–42.

Fabbrini, G., A. Latorre, A. Suppa, M. Bloise, M. Frontoni, and A. Berardelli. 2013. Fatigue in Parkinson's disease: motor or non-motor symptoms. Parkinsonism Relat. Disord. 19:148–152.

Falup-Pecurariu, C. 2013. Fatigue assessment of Parkinson's disease patient in clinic: specific versus holistic. J. Neural. Transm. 120:577–581.

Friedman, J., and H. Friedman. 1993. Fatigue in Parkinson's disease. Neurology 43:2016–2018.

Garber, C. E., and J. F. Friedman. 2003. Effect of fatigue on physical activity and function in patients with Parkinson's disease. Neurology 60:1119–1124.

Gibb, W. R., and A. J. Lees. 1988. The relevance of the Lewy body to the pathogenesis of idiopathic Parkinson's disease. J. Neurol. Neurosurg. Psychiatry 51:745–752.

Hagell, P., T. Rosblom, and S. Palhagen. 2012. A Swedish version of the 16-item Parkinson fatigue scale (PFS-16). Act. Neurol. Scand. 125:288–292.

Havlikova, E., J. Rosenberger, I. Nagyova, B. Middel, T. Dubayova, Z. Gdovinova, et al. 2008. Clinical and psychosocial factors associated with fatigue in patients with Parkinson's disease. Parkinsonism Relat. Disord. 14:187–192.

Karlsen, K., J. P. Larsen, E. Tandberg, and K. Jørgensen. 1999. Fatigue in patients with Parkinson's disease. Mov. Disord. 14:237–241.

Kluger, B. M., L. B. Krupp, and R. M. Enoka. 2013. Fatigue and fatigability in neurologic illnesses. Neurology 80:409–416.

Kummer, A., P. Scalzo, F. Cardoso, and A. Teixeira. 2011. Evaluation of fatigue in Parkinson's disease using the Brazilian version of Parkinson's fatigue scale. Acta Neurol. Scand. 123:130–136.

Lou, J.-S., G. Kearns, B. Oken, G. Sexton, and J. Nutt. 2001. Exacerbated physical fatigue and mental fatigue in Parkinson's disease. Mov. Disord. 16:190–196.

Manabe, Y., D. Fujii, S. Kono, Y. Sakai, T. Tanaka, H. Narai, et al. 2011. Systemic blood pressure profile correlates with cardiac ^{123}I-MIBG uptake in patients with Parkinson's disease. J. Neurol. Sci. 18:922–925.

Metta, V., K. Logishetty, P. Martinez-Martin, H. M. Gage, P. E. Schartau, T. K. Kaluarachchi, et al. 2011. The possible clinical predictors of fatigue in Parkinson's disease: a study of 135 patients as part of International Nonmotor Scale Validation Project. Parkinsons Dis. 2011:1–7.

Mitoma, H., M. Yoneyama, and S. Orima. 2010. 24-hour recording of parkinsonian gait using a portable gait rhythmogram. Intern. Med. 49:2401–2408.

Niino, N., T. Imaizumi, and N. Kawakami. 1991. A Japanese translation of the Geriatric Depression Scale. Clin. Gerontol. 10:85–86.

Oka, H., C. Toyoda, M. Yogo, and S. Mochio. 2010. Olfactory dysfunction and cardiovascular dysautonomia in Parkinson's disease. J. Neurol. 257:969–976.

Okada, K., S. Kobayashi, K. Aoki, N. Suyama, and S. Yamaguchi. 1998. Assessment of motivational loss in poststroke patients using the Japanese version of Starkstein's Apathy scale. Jpn. J. Stroke 20:318–323.

Okuma, Y. 2012. Fatigue and weight loss in Parkinson's disease. Brain Nerve 64:384–393.

Okuma, Y., S. Kamei, A. Morita, F. Yoshii, T. Yamamoto, S. Hashimoto, et al. 2009. Fatigue in Japanese Patients with Parkinson's disease: A Study Using Parkinson Fatigue Scale. Mov. Disord. 24:1977–1983.

Poewe, W., S. Gauthier, D. Aarsland, J. B. Leverentz, P. Barone, D. Weintraub, et al. 2008. Diagnosis and management of Parkinson's disease dementia. Int. J. Clin. Pract. 62:1581–1587.

Rahman, S., H. J. Griffin, N. P. Quinn, and M. Jahanshahi. 2008. The factors that induce or overcome freezing of gait in Parkinson's disease. Behav. Neurol. 19:127–136.

Rochester, L., V. Hetherington, D. Jones, A. Nieuwboer, G. Kwakkel, and E. Van Wegen. 2004. Attending to the task: interference effects of functional tasks on walking in Parkinson's disease and the roles of cognition, depression, fatigue, and balance. Arch. Phys. Med. Rehabil. 85:1578–1585.

Rochester, L., D. Jones, V. Hetherington, A. Nieuwboer, A. Willems, G. Kwakkel, et al. 2006. Gait and gait-related activities and fatigue in Parkinson's disease: What is the relationship? Disabil. Rehabil. 28:1365–1371.

Schifitto, G., J. H. Friedman, D. Oakes, L. Shilman, C. L. Comella, K. Marek, et al. 2008. Fatigue in levodopa-naïve subjects with Parkinson disease. Neurology 71:481–485.

Stiasny-Kolster, K., G. Mayer, S. Schäfer, J. C. Möller, M. Heinzel-Gutenbrunner, and W. H. Oertel. 2007. The REM sleep behavior disorder screening questionnaire - a new diagnostic instrument. Mov. Disord. 22:2386–2393.

Takegami, M., Y. Sugimoto, T. Wakita, H. Noguchi, K. Chin, H. Kadotani, et al. 2009. Development of a Japanese version of the Epworth Sleepiness Scale (JESS) based on item response theory. Sleep Med. 10: 556–565.

Valko, P. O., D. Waldvogel, M. Weller, C. L. Bassetti, U. Held, and C. R. Baumann. 2010. Fatigue and excessive daytime sleepiness in idiopathic Parkinson's disease differently correlate with motor symptoms, depression, and dopaminergic treatment. Eur. J. Neurol. 17:1428–1436.

Van Dijk, J. P., E. Havlikova, J. Rosenberger, I. Nagyova, M. Skorvanek, and Z. Gdovinova. 2013. Influence of disease severity on fatigue in patients with Parkinson's disease is mainly mediated by symptoms of depression. Eur. Neurol. 70:201–209.

Wada-Isoe, K., M. Kitayama, K. Nakaso, and K. Nakashima. 2007. Diagnostic markers for diagnosing dementia with Lewy bodies: CSF and MIBG cardiac scintigraphy study. J. Neurol. Sci. 260:33–37.

Supporting Information

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

Table S1. Logistic regression model predicting patients with fatigue via the J-PFS (n = 75).

INTERNAL MEDICINE

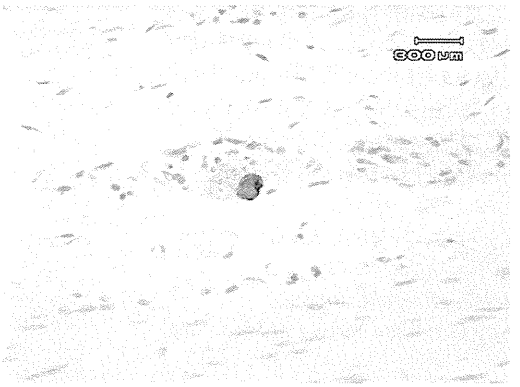
□ PICTURES IN CLINICAL MEDICINE □

Alpha-synuclein Accumulation in a Patient with Auerbach's Plexus of Pure Autonomic Failure

Taeko Kurashiki-Osaka[1], Tadashi Adachi[1,2], Hiroyuki Nakayasu[1] and Kenji Nakashima[2]

Key words: Lewy body, alpha-synuclein, pure autonomic failure, Auerbach's plexus, gastrointestinal tract, peripheral nerve

(Intern Med 53: 2261, 2014)
(DOI: 10.2169/internalmedicine.53.2294)



Picture.

A 76-year-old man complained of orthostatic hypotension and a disturbance of micturition. Six months later, gastric carcinoma was diagnosed and partial gastrectomy was performed. Two months after the operation, the patient was referred to our neurology department due to repeated syncope. A neurological examination revealed urinary retention and orthostatic hypotension; however, no gastrointestinal symptoms, cardiac dysfunction or extrapyramidal signs were observed. A head-up tilt test showed severe hypotension (baseline, 160/105 mmHg; at 60°, 112/77 mmHg), and the level of plasma catecholamine secretion was decreased. Furthermore, a decreased heart/mediastinum ratio (1.58) was evident on ^{123}I-metaiodobenzylguanidine scintigraphy. Pure autonomic failure (PAF) was diagnosed according to the diagnostic criteria of the American Autonomic Society and American Academy of Neurology (1). Alpha-synuclein immunostaining demonstrated intraneuritic alpha-synuclein deposits in Auerbach's plexus of the resected stomach (Picture). These findings support the concept that PAF represents an alpha-synucleinopathy (2).

The authors state that they have no Conflict of Interest (COI).

Acknowledgement

The authors wish to thank Dr. Shu Nakamoto for his kind preparation of the pathological samples.

References

1. Consensus statement on the definition of orthostatic hypotension, pure autonomic failure, and multiple system atrophy. The Consensus Committee of the American Autonomic Society and the American Academy of Neurology. Neurology **46**: 1470, 1996.
2. Shishido T, Ikemura M, Obi T, et al. Alpha-synuclein accumulation in skin nerve fibers revealed by skin biopsy in pure autonomic failure. Neurology **74**: 608-610, 2010.

[1]Department of Neurology, Tottori Prefectural Central Hospital, Japan and [2]Department of Brain and Neurosciences, Division of Neurology, Faculty of Medicine, Tottori University, Japan
Received for publication December 10, 2013; Accepted for publication April 29, 2014
Correspondence to Dr. Hiroyuki Nakayasu, nakayasuhi@gmail.com
 Journal Website: http://www.naika.or.jp/imonline/index.html

Journal of Alzheimer's Disease 41 (2014) 1031–1038
DOI 10.3233/JAD-140225
IOS Press

Short Communication

Lack of Genetic Association Between *TREM2* and Late-Onset Alzheimer's Disease in a Japanese Population

Akinori Miyashita[a,*], Yanan Wen[a], Nobutaka Kitamura[b], Etsuro Matsubara[c,1], Takeshi Kawarabayashi[c], Mikio Shoji[c], Naoki Tomita[d], Katsutoshi Furukawa[d], Hiroyuki Arai[d], Takashi Asada[e], Yasuo Harigaya[f], Masaki Ikeda[g], Masakuni Amari[g], Haruo Hanyu[h], Susumu Higuchi[i], Masatoyo Nishizawa[j], Masaichi Suga[k], Yasuhiro Kawase[l], Hiroyasu Akatsu[m,2], Masaki Imagawa[n], Tsuyoshi Hamaguchi[o], Masahito Yamada[o], Takashi Morihara[p], Masatoshi Takeda[p], Takeo Takao[q], Kenji Nakata[r], Ken Sasaki[r], Ken Watanabe[s], Kenji Nakashima[t], Katsuya Urakami[u], Terumi Ooya[v], Mitsuo Takahashi[w], Takefumi Yuzuriha[x], Kayoko Serikawa[y], Seishi Yoshimoto[y], Ryuji Nakagawa[y], Yuko Saito[z], Hiroyuki Hatsuta[aa], Shigeo Murayama[aa], Akiyoshi Kakita[bb], Hitoshi Takahashi[bb], Haruyasu Yamaguchi[cc], Kohei Akazawa[b], Ichiro Kanazawa[dd], Yasuo Ihara[ee], Takeshi Ikeuchi[a] and Ryozo Kuwano[a,*]

[a]*Department of Molecular Genetics, Brain Research Institute, Niigata University, Niigata, Japan*
[b]*Department of Medical Informatics, Niigata University, Niigata, Japan*
[c]*Department of Neurology, Hirosaki University Graduate School of Medicine, Hirosaki, Japan*
[d]*Department of Geriatric and Complementary Medicine, Tohoku University Graduate School of Medicine, Sendai, Japan*
[e]*Department of Psychiatry, University of Tsukuba, Tsukuba, Japan*
[f]*Department of Neurology, Maebashi Red Cross Hospital, Maebashi, Japan*
[g]*Department of Neurology, Gunma University Graduate School of Medicine, Maebashi, Japan*
[h]*Department of Geriatric Medicine, Tokyo Medical University, Tokyo, Japan*

Accepted 7 March 2014

[1]Present address: Department of Neurology, Oita University Faculty of Medicine, Yufu, Japan.

[2]Present address: Department of Medicine for Aging Place Community Health Care/Community-Based Medical Education, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan.

*Correspondence to: Ryozo Kuwano, 1-757 Asahimachi, Chuo-ku, Niigata, Niigata 951-8585, Japan. Tel.: +81 25 227 2274; Fax: +81 25 227 0793; E-mail: ryosun@bri.niigata-u.ac.jp (R. Kuwano); and Akinori Miyashita, 1-757 Asahimachi, Chuo-ku, Niigata 951-8585, Japan. Tel.: +81 25 227 2344; Fax: +81 25 227 0793; E-mail: miyashi@bri.niigata-u.ac.jp.

ISSN 1387-2877/14/$27.50

[i]Division of Clinical Research, Kurihama Alcoholism Center, Yokosuka, Japan
[j]Department of Neurology, Brain Research Institute, Niigata University, Niigata, Japan
[k]Higashi Niigata Hospital, Niigata, Japan
[l]Kawase Neurology Clinic, Sanjo, Japan
[m]Choju Medical Institute, Fukushimura Hospital, Toyohashi, Japan
[n]Imagawa Clinic, Osaka, Japan
[o]Department of Neurology and Neurobiology of Aging, Kanazawa University Graduate School of Medical Science, Kanazawa, Japan
[p]Department of Psychiatry, Osaka University Graduate School of Medicine, Suita, Japan
[q]Kurashiki Heisei Hospital, Kurashiki, Japan
[r]Kinoko Espoir Hospital, Kasaoka, Japan
[s]Watanabe Hospital, Tottori, Japan
[t]Department of Neurology, Tottori University, Yonago, Japan
[u]Department of Biological Regulation, Section of Environment and Health Science, Tottori University, Yonago, Japan
[v]Town Office, Onan, Japan
[w]Department of Clinical Pharmacology, Fukuoka University, Fukuoka, Japan
[x]Department of Psychiatry, National Hospital Organization, Hizen Psychiatric Center, Saga, Japan
[y]Ureshino-Onsen Hospital, Saga, Japan
[z]Department of Pathology, National Center Hospital of Neurology and Psychiatry, Tokyo, Japan
[aa]Department of Neuropathology, Tokyo Metropolitan Geriatric Hospital and Institute of Gerontology, Tokyo, Japan
[bb]Department of Pathology, Brain Research Institute, Niigata University, Niigata, Japan
[cc]Graduate School of Health Sciences, Gunma University, Maebashi, Japan
[dd]National Center Hospital of Neurology and Psychiatry, Tokyo, Japan
[ee]Department of Neuropathology, Doshisha University, Kizugawa, Japan

Abstract. Rare non-synonymous variants of *TREM2* have recently been shown to be associated with Alzheimer's disease (AD) in Caucasians. We here conducted a replication study using a well-characterized Japanese sample set, comprising 2,190 late-onset AD (LOAD) cases and 2,498 controls. We genotyped 10 non-synonymous variants (Q33X, Y38C, R47H, T66M, N68K, D87N, T96K, R98W, H157Y, and L211P) of *TREM2* reported by Guerreiro *et al.* (2013) by means of the TaqMan and dideoxy sequencing methods. Only three variants, R47H, H157Y, and L211P, were polymorphic (range of minor allele frequency [MAF], 0.0002–0.0059); however, no significant association with LOAD was observed in these variants. Considering low MAF of variants examined and our study sample size, further genetic analysis with a larger sample set is needed to firmly evaluate whether or not *TREM2* is associated with LOAD in Japanese.

Keywords: Alzheimer's disease, Japanese, rare variants, SNP, *TREM2*

INTRODUCTION

Alzheimer's disease (AD) is the main cause of dementia in the elderly. AD is thought to be caused by complex interactions between genetic and environmental factors. A twin study demonstrated that the heritability of late-onset AD (LOAD) is approximately 60~80% [1]. It is also assumed that multiple genes/loci contribute to LOAD development [2]. Rare non-synonymous mutations of *APP*, *PSEN1*, and *PSEN2* are well known to cause familial cases of early-onset AD (EOAD) [3], which accounts for several percent of AD. Concerning LOAD, genome-wide association studies with large numbers of subjects have been conducted, based on the common diseases-common variants hypothesis. As a result, over a dozen genes other than *APOE* have been to be associated with the susceptibility to LOAD [4–10].

TREM2 was recently identified as a novel susceptibility gene for LOAD in Caucasians by two independent study groups [11, 12], both studies being performed on the basis of the common diseases-rare variants hypothesis. A noteworthy fact is that the most significant non-synonymous variant, R47H

(rs75932628: CGC⟶CAC; and minor allele frequency [MAF] < about 1%), located within exon 2 of *TREM2*, shows an odds ratio (OR) range of 2.0–5.0 [11, 12], which is almost equal to the risk magnitude for the *APOE*-ε4 allele [13, 14]. The association of this variant with LOAD [15–19] as well as EOAD [20] has been reproducibly confirmed in multiple Caucasian populations. As to Asians, at present there has only been one genetic association study on *TREM2* variants and LOAD, a northern Han Chinese population being involved [21]. In that study, it was demonstrated that no *TREM2* variants, including R47H, examined show significant association with LOAD [21]. It is assumed that *TREM2* may be a Caucasian-specific susceptibility gene for AD. Therefore, in this study we attempted to replicate the association of *TREM2* with LOAD utilizing a Japanese sample set, comprising 4,688 subjects in total.

SUBJECTS AND METHODS

Subjects

This study was approved by the Institutional Review Board of Niigata University and by all participating institutes. All subjects were Japanese and anonymously genotyped.

We prepared a Japanese sample set, comprising 2,190 LOAD cases (clinically-verified, $n = 1{,}977$; and neuropathologically-characterized, $n = 213$) and 2,498 controls (clinically-verified, $n = 2{,}128$; and neuropathologically-characterized, $n = 370$) (Table 1). From power analysis on the basis of Guerreiro et al.'s study with Caucasians [11], this sample set was estimated to be large enough to detect risk alleles with an OR of 1.1–2.5 (range of risk allele frequency = 0.01–0.99, $\alpha = 0.05$, power = 80%) [29]. A large proportion of the clinically-verified subjects were the same (74.8%) as those in the overall sample set used in our previous genetic study on *GAB2* [22]. The LOAD patients met the criteria of the National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association for a diagnosis of probable AD [23]. Non-dementia controls were recruited from among elderly people living in an unassisted manner in the local community. Mini-Mental State Examination [24], Clinical Dementia Rating [25], and/or Function Assessment Staging [26] were applied to assess the severity of the cognitive impairment. All neuropathologically-characterized subjects were utilized in our recent genetic study on *SORL1* [27].

Extraction and quantification of genomic DNA, and *APOE* genotyping are described elsewhere [27, 28]. The *APOE* alleles exhibited strong association with LOAD, as expected: $p_{\text{allele}} = 6.71\text{E-}171$ with χ^2 test (χ^2 value = 783.7, degree of freedom = 2), and $OR_{\varepsilon 4/\varepsilon 3}$ (95% confidence interval [CI]) = 4.81 (4.26–5.42) and $OR_{\varepsilon 2/\varepsilon 3}$ (95% CI) = 0.59 (0.46–0.76).

TREM2 variants and genotyping

To determine whether or not *TREM2* is associated with LOAD in Japanese, we focused on 12 non-synonymous variants of this gene, which were examined in Guerreiro *et al.*'s study with Caucasians [11]: Q33X (rs104894002), Y38C (rs ID, not available), R47H (rs75932628), R62H (rs143332484), T66M (rs201258663), N68K (rs ID, not available), D87N (rs142232675), T96K (rs2234253), R98W (rs147564421), R136Q (rs149622783), H157Y (rs2234255), and L211P (rs2234256). However, two variants, R62H and R136Q, were excluded since one (R62H) did not satisfy the design criteria for the TaqMan® genotyping assay and the other (R136Q) did not work well on TaqMan® genotyping. Consequently, we determined the genotypes of the remaining ten *TREM2* variants using the TaqMan® method (Table 2, Supplementary Table 1). Heterozygotes were further evaluated by means of dideoxy DNA sequencing. Information on sequencing primers is available on request.

Statistical analysis

To detect genotyping errors, a Hardy-Weinberg equilibrium (HWE) test based on Fisher's exact test was conducted. From a 2×2 contingency table (case-control status and genotype [MM and Mm]), we computed genotypic p (p_{genotype}) based on Fisher's exact test and OR with 95% CI as the relative risk of disease for each polymorphic variant. We further performed multiple variant analysis as one of gene-based case-control association studies: distribution of minor-allele carriers (Mm) and non-carriers (MM) as to three polymorphic variants, R47H, H157Y and L211P, was compared between cases and controls on the basis of χ^2 test from a 2×2 contingency table. Subjects with undetermined genotype data in these variants were omitted for this analysis, with 4,582 subjects remaining. We used SNPAlyze software (DYNACOM, Japan; http://www.dynacom.co.jp/) for these statistical analyses, as described in detail elsewhere [35].

The statistical significance was set at $p < 0.05$.

Table 1
Demographics of the study sample set

	No. of subjects	Age		*APOE* allele frequency		
	(Female %)	Mean (SD)	Range	ε2	ε3	ε4
Cases	2,190 (70.1)	75.2 (6.2)	57–102	0.02	0.67	0.31
Controls	2,498 (54.9)	76.3 (6.6)	65–105	0.05	0.87	0.08

SD, standard deviation.

RESULTS AND DISCUSSION

We attempted to replicate the association of *TREM2* with LOAD in a Japanese sample set, comprising 4,688 subjects in total: cases, $n=2,190$; and controls, $n=2,498$ (Table 1). Three variants, R47H, H157Y, and L211P, were found to be polymorphic; however, the remaining seven, Q33X, Y38C, T66M, N68K, D87N, T96K, and R98W, did not show polymorphisms (Table 2, Supplementary Table 1). The MAF of the variants, R47H, H157Y, and L211P, were less than 0.01 (Supplementary Table 1). Concerning variant R47H [11, 12], three heterozygous subjects were observed: one clinically-verified case (female, age at onset of 76 years old, and *APOE*-ε3*3) and two neuropathologically-characterized controls (one female, age at death of 99 years old, and *APOE*-ε3*3; and one male, age at death of 79 years old, and *APOE*-ε3*3). Variant L211P exhibited the highest MAF among them: 0.0041 in cases and 0.0059 in controls (Supplementary Table 1). Variants R47H, H157Y, and L211P were all in HWE (Supplementary Table 1). In both single and multiple variant analyses, we observed no significant association of *TREM2* with LOAD (Table 2).

TREM2 is mainly expressed in microglia in the brain [30]. This protein directly interacts with a type I transmembrane adapter protein, DAP12 [30]. Recent whole transcriptome analysis of microglia, purified from mouse brains by means of flow cytometry, revealed that *TREM2* belongs to a DAP12-centered protein network, in which multiple microglial marker proteins such as Cd68 are included [31]. A *TREM2*-DAP12 signaling pathway is involved in innate immune responses as well as the differentiation of myeloid progenitor cells into mature microglia [30, 32]. Microglia play an important role in the clearance of amyloid-β protein in the brain [33]. Thus, it is likely that genomic variants of not only *TREM2* but also other genes involved in the *TREM2*-DAP12 signaling pathway may accelerate amyloid plaque deposition through microglial dysfunction [34]. Although none of the rare non-synonymous *TREM2* variants investigated here exhibited association with LOAD in our sample sets (Table 2), we could not rule out the possibility that *TREM2* is one of the crucial proteins for AD from the point of view of biological functions of this protein.

In conclusion, we were not able to detect the significant association of *TREM2* variants examined with LOAD in Japanese, which is consistent with a recent study involving Chinese [21]. On the other hand, *TREM2* has been reproducibly shown to be strongly associated with both LOAD [15–19] and EOAD [20] in multiple Caucasian sample sets. Given these data, *TREM2* may contribute to the susceptibility of LOAD only in Caucasians, i.e., not or only weakly in Asians. However, considering the very low MAF of variants investigated (Table 2, Supplementary Table 1) and our study sample size (Table 1), a large-scale meta-analysis is further needed to comprehensively evaluate whether or not *TREM2* is associated with LOAD in Asians.

ACKNOWLEDGMENTS

We wish to thank all participants for taking part in this study. This study was supported by: (1) the 'Japanese Alzheimer's Disease Neuroimaging Initiative' of the New Energy and Industrial Technology Development Organization (NEDO) (Grant number, P12009) in Japan (R.K.); (2) MEXT/JSPS KAKENHI, Grant-in-Aid for Scientific Research on Priority Areas and (B), from the Ministry of Education, Culture, Sports, Science and Technology of Japan (Grant numbers, 24310144 and 22129004) (R.K.); (3) MEXT KAKENHI, Grant-in-Aid for Scientific Research (C) (Grant numbers, 22790331 and 24510275), from the Ministry of Education, Culture, Sports, Science and Technology of Japan (A.M.); (4) Grant for Promotion of Niigata University Research Projects, Japan (24C076) (A.M.); (5) MEXT KAKENHI, Grant-in-Aid for Scientific Research (C) (Grant number, 24700371), from the Ministry of Education, Culture, Sports, Science and Technology of Japan (H.H.); (6) The Comprehensive Brain Science Network (Grant number, 221S003), the Ministry of Education, Cul-

Table 2
Genotypic distribution of three polymorphic variants, R47H, H157Y, and L211P, on *TREM2* in Japanese

Single variant analysis		Allele		Cases (frequency)			Controls (frequency)			$P_{genotype}$[a]	OR_{Mm} (95% CI)[b]
Variant	dbSNP	M	m	MM	Mm	mm	MM	Mm	mm		
R47H	rs75932628	G	a	2,171 (0.9995)	1 (0.0005)	0 (0.0)	2,477 (0.9992)	2 (0.0008)	0 (0.0)	1.00E+00	0.57 (0.05–6.30)
H157Y	rs2234255	C	t	2,147 (0.9972)	6 (0.0028)	0 (0.0)	2,474 (0.9984)	4 (0.0016)	0 (0.0)	5.29E-01	1.73 (0.49–6.13)
L211P	rs2234256	T	c	2,161 (0.9917)	18 (0.0083)	0 (0.0)	2,461 (0.9884)	29 (0.0116)	0 (0.0)	3.04E-01	0.71 (0.39–1.28)
Multiple variant analysis		Combind genotype		Cases (frequency)			Controls (frequency)			$P_{genotype}$[c]	OR_{CG-2} (95% CI)[d]
Combind variant	Combind dbSNP	CG-1	CG-2	CG-1	CG-2	others	CG-1	CG-2	others		
R47H-H157Y-L211P	rs75932628-rs2234255-rs2234256	GG-CC-TT	Ga-CC-TT, GG-Ct-TT, GG-CC-Tc	2,104 (0.9883)	25 (0.0117)	0 (0.0)	2,419 (0.9861)	34 (0.0139)	0 (0.0)	5.26E-01	0.85 (0.50–1.42)

In single variant analysis, only three variants, L211P, H157Y, and R47H, are shown here since heterozygotes (Mm) were observed. M, major allele; m, minor allele; MM, major genotype; Mm, heterozygous genotype; mm, minor genotype; CG, combined genotype. [a]Fisher's exact test; [b]OR_{Mm} (95% CI) for the heterozygote (Mm); [c]chi-squared test (degree of freedom = 1); [d]OR_{CG-2} (95% CI) for CG-2 (Ga-CC-TT, GG-Ct-TT, and GG-CC-Tc).

ture, Sports, Science and Technology of Japan (S.M.); and (7) National Center for Geriatrics and Gerontology Funds (Grant number, 23–42), Obu, Japan (S.M.).

Authors' disclosures available online (http://www.j-alz.com/disclosures/view.php?id=2196).

SUPPLEMENTARY MATERIAL

The supplementary table is available in the electronic version of this article: http://dx.doi.org/10.3233/JAD-140225.

REFERENCES

[1] Gatz M, Reynolds CA, Fratiglioni L, Johansson B, Mortimer JA, Berg S, Fiske A, Pedersen NL (2006) Role of genes and environments for explaining Alzheimer disease. *Arch Gen Psychiatry* 63, 168-174.

[2] Daw EW, Payami H, Nemens EJ, Nochlin D, Bird TD, Schellenberg GD, Wijsman EM (2000) The number of trait loci in late-onset Alzheimer disease. *Am J Hum Genet* 66, 196-204.

[3] Cruts M, Theuns J, Van Broeckhoven C (2012) Locus-specific mutation databases for neurodegenerative brain diseases. *Hum Mutat* 33, 1340-1344.

[4] Harold D, Abraham R, Hollingworth P, Sims R, Gerrish A, Hamshere ML, Pahwa JS, Moskvina V, Dowzell K, Williams A, Jones N, Thomas C, Stretton A, Morgan AR, Lovestone S, Powell J, Proitsi P, Lupton MK, Brayne C, Rubinsztein DC, Gill M, Lawlor B, Lynch A, Morgan K, Brown KS, Passmore PA, Craig D, McGuinness B, Todd S, Holmes C, Mann D, Smith AD, Love S, Kehoe PG, Hardy J, Mead S, Fox N, Rossor M, Collinge J, Maier W, Jessen F, Schürmann B, Heun R, van den Bussche H, Heuser I, Kornhuber J, Wiltfang J, Dichgans M, Frölich L, Hampel H, Hüll M, Rujescu D, Goate AM, Kauwe JS, Cruchaga C, Nowotny P, Morris JC, Mayo K, Sleegers K, Bettens K, Engelborghs S, De Deyn PP, Van Broeckhoven C, Livingston G, Bass NJ, Gurling H, McQuillin A, Gwilliam R, Deloukas P, Al-Chalabi A, Shaw CE, Tsolaki M, Singleton AB, Guerreiro R, Mühleisen TW, Nöthen MM, Moebus S, Jöckel KH, Klopp N, Wichmann HE, Carrasquillo MM, Pankratz VS, Younkin SG, Holmans PA, O'Donovan M, Owen MJ, Williams J (2009) Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease. *Nat Genet* 41, 1088-1093.

[5] Lambert JC, Heath S, Even G, Campion D, Sleegers K, Hiltunen M, Combarros O, Zelenika D, Bullido MJ, Tavernier B, Letenneur L, Bettens K, Berr C, Pasquier F, Fiévet N, Barberger-Gateau P, Engelborghs S, De Deyn P, Mateo I, Franck A, Helisalmi S, Porcellini E, Hanon O, European Alzheimer's Disease Initiative Investigators, de Pancorbo MM, Lendon C, Dufouil C, Jaillard C, Leveillard T, Alvarez V, Bosco P, Mancuso M, Panza F, Nacmias B, Bossù P, Piccardi P, Annoni G, Seripa D, Galimberti D, Hannequin D, Licastro F, Soininen H, Ritchie K, Blanché H, Dartigues JF, Tzourio C, Gut I, Van Broeckhoven C, Alpérovitch A, Lathrop M, Amouyel P (2009) Genome-wide association study identifies variants at CLU and CR1 associated with Alzheimer's disease. *Nat Genet* 41, 1094-1099.

[6] Seshadri S, Fitzpatrick AL, Ikram MA, DeStefano AL, Gudnason V, Boada M, Bis JC, Smith AV, Carassquillo MM, Lambert JC, Harold D, Schrijvers EM, Ramirez-Lorca R, Debette S, Longstreth WT Jr, Janssens AC, Pankratz VS, Dartigues JF, Hollingworth P, Aspelund T, Hernandez I, Beiser A, Kuller LH, Koudstaal PJ, Dickson DW, Tzourio C, Abraham R, Antunez C, Du Y, Rotter JI, Aulchenko YS, Harris TB, Petersen RC, Berr C, Owen MJ, Lopez-Arrieta J, Varadarajan BN, Becker JT, Rivadeneira F, Nalls MA, Graff-Radford NR, Campion D, Auerbach S, Rice K, Hofman A, Jonsson PV, Schmidt H, Lathrop M, Mosley TH, Au R, Psaty BM, Uitterlinden AG, Farrer LA, Lumley T, Ruiz A, Williams J, Amouyel P, Younkin SG, Wolf PA, Launer LJ, Lopez OL, van Duijn CM, Breteler MM; CHARGE Consortium; GERAD1 Consortium; EADI1 Consortium (2010) Genome-wide analysis of genetic loci associated with Alzheimer disease. *JAMA* 303, 1832-1840.

[7] Hollingworth P, Harold D, Sims R, Gerrish A, Lambert JC, Carrasquillo MM, Abraham R, Hamshere ML, Pahwa JS, Moskvina V, Dowzell K, Jones N, Stretton A, Thomas C, Richards A, Ivanov D, Widdowson C, Chapman J, Lovestone S, Powell J, Proitsi P, Lupton MK, Brayne C, Rubinsztein DC, Gill M, Lawlor B, Lynch A, Brown KS, Passmore PA, Craig D, McGuinness B, Todd S, Holmes C, Mann D, Smith AD, Beaumont H, Warden D, Wilcock G, Love S, Kehoe PG, Hooper NM, Vardy ER, Hardy J, Mead S, Fox NC, Rossor M, Collinge J, Maier W, Jessen F, Rüther E, Schürmann B, Heun R, Kölsch H, van den Bussche H, Heuser I, Kornhuber J, Wiltfang J, Dichgans M, Frölich L, Hampel H, Gallacher J, Hüll M, Rujescu D, Giegling I, Goate AM, Kauwe JS, Cruchaga C, Nowotny P, Morris JC, Mayo K, Sleegers K, Bettens K, Engelborghs S, De Deyn PP, Van Broeckhoven C, Livingston G, Bass NJ, Gurling H, McQuillin A, Gwilliam R, Deloukas P, Al-Chalabi A, Shaw CE, Tsolaki M, Singleton AB, Guerreiro R, Mühleisen TW, Nöthen MM, Moebus S, Jöckel KH, Klopp N, Wichmann HE, Pankratz VS, Sando SB, Aasly JO, Barcikowska M, Wszolek ZK, Dickson DW, Graff-Radford NR, Petersen RC; Alzheimer's Disease Neuroimaging Initiative, van Duijn CM, Breteler MM, Ikram MA, DeStefano AL, Fitzpatrick AL, Lopez O, Launer LJ, Seshadri S; CHARGE consortium, Berr C, Campion D, Epelbaum J, Dartigues JF, Tzourio C, Alpérovitch A, Lathrop M; EADI1 consortium, Feulner TM, Friedrich P, Riehle C, Krawczak M, Schreiber S, Mayhaus M, Nicolhaus S, Wagenpfeil S, Steinberg S, Stefansson H, Stefansson K, Snaedal J, Björnsson S, Jonsson PV, Chouraki V, Genier-Boley B, Hiltunen M, Soininen H, Combarros O, Zelenika D, Delepine M, Bullido MJ, Pasquier F, Mateo I, Frank-Garcia A, Porcellini E, Hanon O, Coto E, Alvarez V, Bosco P, Siciliano G, Mancuso M, Panza F, Solfrizzi V, Nacmias B, Sorbi S, Bossù P, Piccardi P, Arosio B, Annoni G, Seripa D, Pilotto A, Scarpini E, Galimberti D, Brice A, Hannequin D, Licastro F, Jones L, Holmans PA, Jonsson T, Riemenschneider M, Morgan K, Younkin SG, Owen MJ, O'Donovan M, Amouyel P, Williams J (2011) Common variants at ABCA7, MS4A6A/MS4A4E, EPHA1, CD33 and CD2AP are associated with Alzheimer's disease. *Nat Genet* 43, 429-435.

[8] Naj AC, Jun G, Beecham GW, Wang LS, Vardarajan BN, Buros J, Gallins PJ, Buxbaum JD, Jarvik GP, Crane PK, Larson EB, Bird TD, Boeve BF, Graff-Radford NR, De Jager PL, Evans D, Schneider JA, Carrasquillo MM, Ertekin-Taner N, Younkin SG, Cruchaga C, Kauwe JS, Nowotny P, Kramer P, Hardy J, Huentelman MJ, Myers AJ, Barmada MM, Demirci FY, Baldwin CT, Green RC, Rogaeva E, St George-Hyslop P, Arnold SE, Barber R, Beach T, Bigio EH, Bowen JD, Boxer A, Burke JR, Cairns NJ, Carlson CS, Carney RM, Carroll SL, Chui HC, Clark DG, Corneveaux J, Cotman CW, Cummings JL, DeCarli C, DeKosky ST, Diaz-Arrastia R,