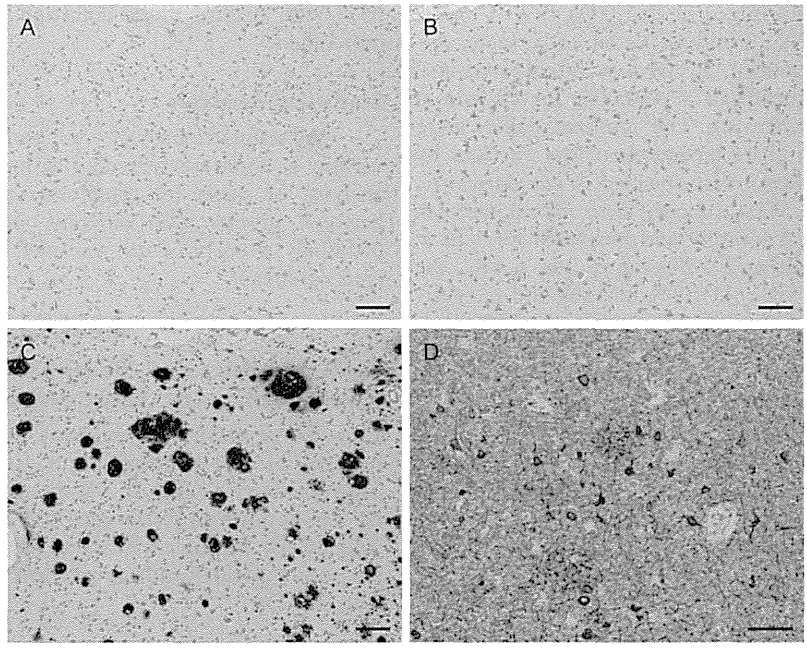

Fig. 1. Pathological profiles of the brains. Representative images of immunohistochemical analysis of the cortices of ε2/ε3 without amyloid (amyloid-free, AF; **A**), ε3/ε3 (AF; **B**), and ε3/ε3 with Alzheimer's disease pathology (AD; **C,D**) are shown. Immunohistochemical analysis was performed with an anti-Aβ 11–28 antibody (12B2; A–C) and antiphosphorylated tau antibody (AT8; D). Scale bars = 100 μm in A–C; 50 μm in D.

were extensively homogenized by sonication and the number of fractions for discontinuous sucrose-density-gradient centrifugation was increased (Fig. 2B). In comparison with the conventional detergent method using Triton X-100, in which the LDM marker protein and lipid (prion protein and GM1 ganglioside, respectively) were clearly separated from a non-LDM marker protein, the transferrin receptor (data not shown), the present detergent-free method provided satisfactorily pure LDMs; the levels of the LDM markers are relatively high in the upper phase (fractions 4–6) compared with a low level of the transferrin receptor (Fig. 2C).

Cholesterol Levels in SPMs and LDMs

We analyzed the cholesterol levels in SPMs and LDMs. The cholesterol levels in SPMs prepared from amyloid-free ε2/ε3 brains were significantly lower than those prepared from amyloid-free ε3/ε3 brains (Fig. 3A). The cholesterol levels in SPMs prepared from ε3/ε3 brains with AD pathology were significantly lower than those prepared from amyloid-free ε3/ε3 brains (Fig. 3A). Differences between amyloid-free ε2/ε3 brains and amyloid-free ε3/ε3 brains and between amyloid-free ε3/ε3 brains and ε3/ε3 brains with AD pathology were more pronounced in LDMs (Fig. 3B).

Ganglioside Levels in SPMs and LDMs

We analyzed ganglioside levels in SPMs and LDMs by quantitative LC-MS analysis. In SPMs, no changes in the levels of gangliosides were detected (Fig. 4A). In LDMs, the levels of some species of gangliosides, including GT1(d20:1–18:0) and OAc-GT1(d20:1–18:0), decreased in the ε3/ε3 brains with AD pathology in comparison with the amyloid-free ε3/ε3 brains (Fig. 4B). Previous studies showed a decrease in the levels of major gangliosides, including GM1, GD1, and GT1, in the cortices of AD brains (Op Den Velde and Hooghwinkel, 1975; Crino et al., 1989; Kracun et al., 1991). In this study, although the difference between amyloid-free ε3/ε3 brains and ε3/ε3 brains with AD pathology was not statistically significant, the levels of GD1(d20:0–18:0) tended to decrease in the presence of AD pathology. However, the levels of GM1 were apparently unchanged.

DISCUSSION

It remains to be clarified how the *APOE* genotype increases or decreases the risk of AD development. Despite intensive efforts, it is poorly understood how the *APOE* genotype is linked to the initiation of amyloid deposition, a fundamental core of AD pathology, in the brain. Previous studies suggested that, first, amyloidogenic proteins, including Aβ, likely assemble into fibrils through interaction with lipids (for review see Gorbenko and Kinnunen, 2006); second, microdomains or lipid rafts are deeply involved in the pathogenesis of AD, including Aβ assembly (for review see Rushworth and Hooper, 2010; Hicks et al., 2012); and, third, amyloid deposition in the brain starts at presynaptic terminals (Bugiani et al., 1990; Probst et al.,

1991). These lines of evidence prompted us to explore the pathological significance of the *APOE* genotype in amyloid deposition by directly examining lipids of synaptic membranes and synaptic membrane microdomains of autopsied brains. Our results suggest that the inhibitory effect of ε2 on amyloid deposition is attributed to keeping cholesterol in synaptic membranes and/or synaptic membrane microdomains under a certain level, which is prerequisite for the initiation of Aβ assembly into fibrils.

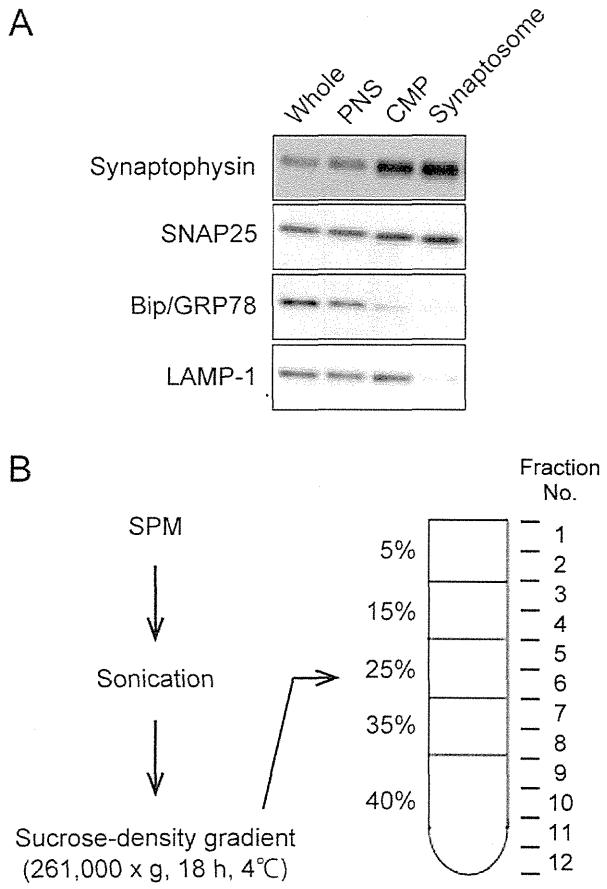

Fig. 2. Preparation and characterization of synaptosomes and low-density membrane microdomains (LDMs). **A:** Collected whole homogenates, postnuclear supernatant (PNS), crude mitochondrial pellet (CMP), and synaptosomes were analyzed by Western blotting using the antibodies to the following subcellular compartment markers: synaptophysin and SNAP25 for synapses, Bip/GRP78 for the endoplasmic reticulum, and LAMP-1 for lysosomes. **B:** Outline of LDM preparation from synaptic plasma membrane (SPM). After centrifugation, fractions (1 ml) were sequentially collected from top (fraction 1) to bottom (fraction 12). **C:** Collected fractions and a sonicated sample without fractionation (whole) were analyzed by Western blotting using antibodies to the following compartment markers: transferrin receptor (TfR) for non-LDMs and flotillin-1 (Flot-1), prion protein (PrP), and choleratoxin B subunit (CTB) for LDMs. Fractions 4–6 (indicated by an asterisk) were collected as LDM samples.

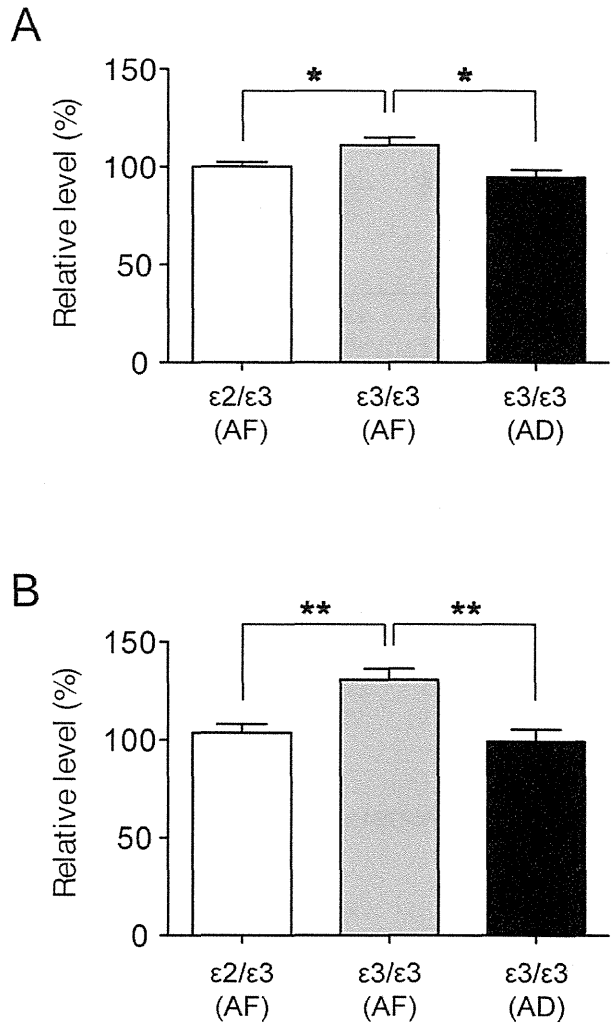

Fig. 3. Cholesterol levels in synaptic plasma membranes (SPMs) and low-density membrane microdomains (LDMs). SPMs (**A**) and LDMs (**B**) were prepared from the cortices of ε2/ε3 without amyloid (amyloid-free; AF), ε3/ε3 (AF), and ε3/ε3 with Alzheimer's disease pathology (AD). The levels of cholesterol were determined by using an Amplex-red cholesterol assay kit. Each column indicates mean ± SEM ($n = 8$, $*P < 0.05$, $**P < 0.01$).

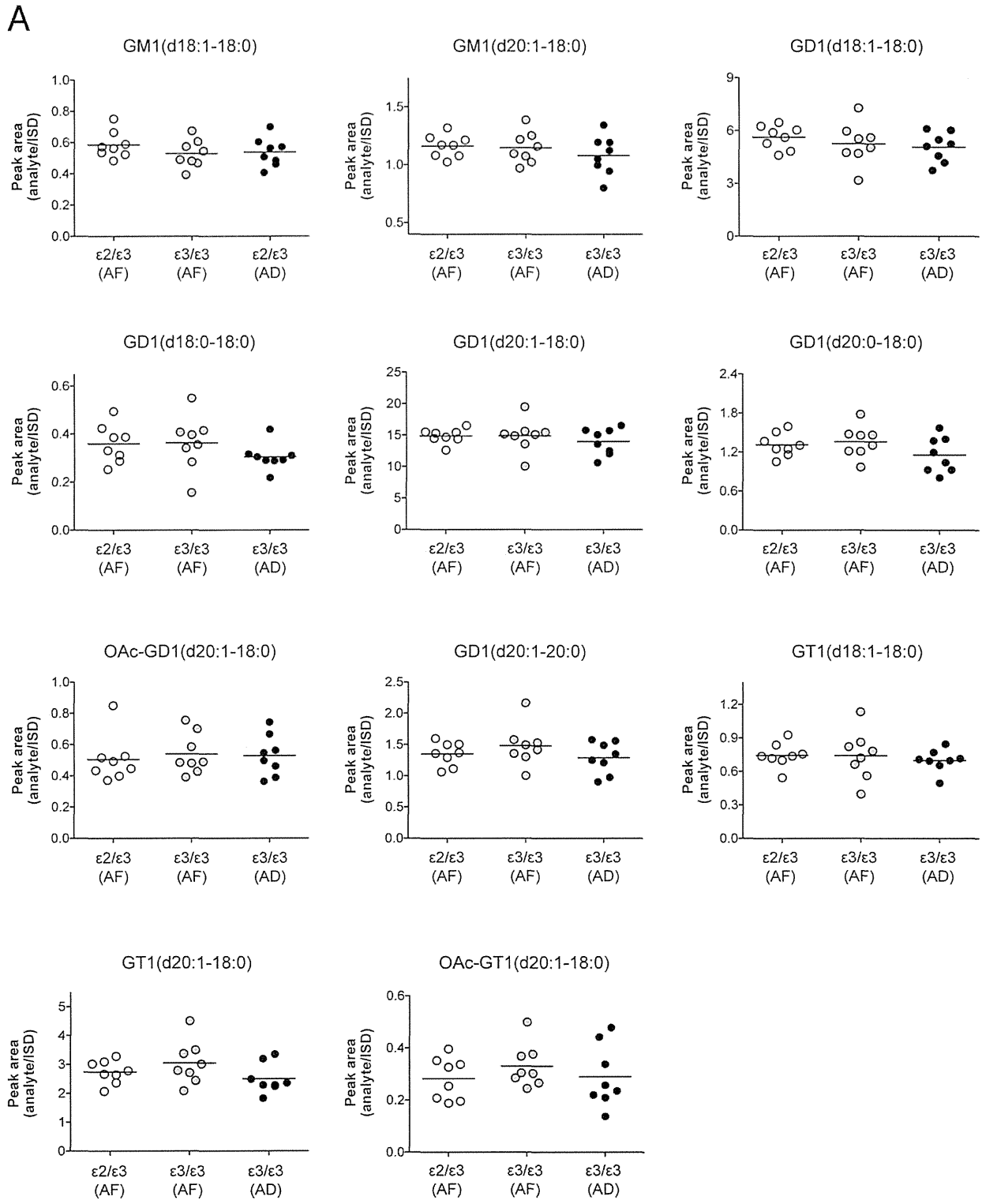

Fig. 4. Ganglioside levels in synaptic plasma membranes (SPMs) and low-density membrane microdomains (LDMs). SPMs (**A**) and LDMs (**B**) were prepared from the cortices of ε2/ε3 without amyloid (amyloid-free; AF), ε3/ε3 (AF), and ε3/ε3 with Alzheimer's disease pathology (AD). Gangliosides were analyzed by liquid chromatograpy-mass spectroscopy (LC-MS). The peak area of each ganglioside was determined with GM1(d18:1-^{13}C16:0; A) or GM3(d18:1–14:0; B) as an internal standard (ISD). Each bar represents the mean value in the group (n = 8).

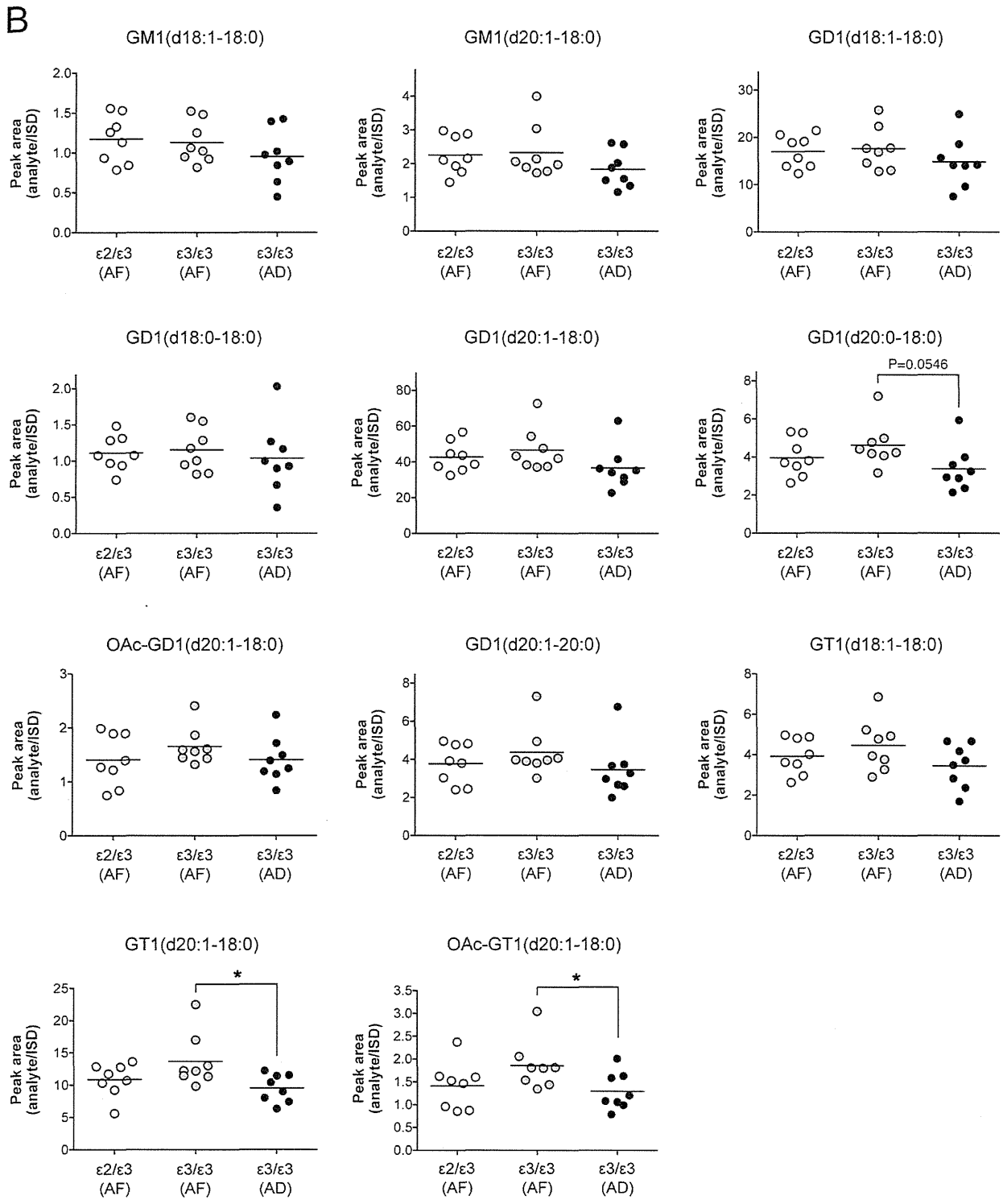

Fig. 4. Continued

In our early study, we quantitatively analyzed synaptic membrane lipids using human *APOE* knock-in mouse brains (ε3 knock-in and ε4 knock-in). In that study, the levels of cholesterol significantly increased in the exofacial leaflet of the synaptic membrane of ε4 knock-in mouse brains compared with ε3 knock-in and wild-type mouse brains (Hayashi et al., 2002), suggesting that the pathological significance of ε4 is linked to an increase in the levels of cholesterol in synaptic membranes. Although the scope of the current study is only the comparison between ε2 and ε3, note that ε2 decreases the levels of cholesterol in synaptic membranes and synaptic membrane microdomains. Overall, the *APOE* genotype likely has an impact on the regulation of cholesterol levels at presynaptic terminals and thereby modulates amyloid deposition in the brain.

Although it remains to be clarified how ε2 decreases the level of cholesterol in synaptic membranes and synaptic membrane microdomains, apolipoprotein E-dependent cholesterol regulation in neurons has been studied in vitro by our group and other groups (Michikawa et al., 2000; Rapp et al., 2006). Notably, cholesterol efflux from neurons was found to be regulated by apolipoprotein in an isoform-dependent manner; the order of potency was E2 > E3 > E4, i.e., apolipoproteins encoded by ε2, ε3, and ε4, respectively (Michikawa et al., 2000). On the other hand, apparently there was no isoform-dependent difference between E2 and E3 in the extent of cholesterol supply to neurons (Rapp et al., 2006). Thus, although further studies are needed, it may be possible to conclude that ε2 provides a negative balance of cholesterol dynamics in neuronal membranes compared with ε3 and ε4.

In this study, the presence of AD pathology decreased the level of cholesterol and some species of gangliosides in synaptic membranes and/or synaptic membrane microdomains. Elucidation of the mechanisms underlying alterations in neuronal lipid levels under various pathological conditions will be a challenging task. Many studies of AD have been carried out concerning on this subject (Molander-Melin et al., 2005; Gylys et al., 2007); however, results reported to date are not consistent but rather varied. The discrepancy probably is due to differences in the method of preparing neuronal membranes and, to a greater extent, to differences in the pathological stages of brains or brain regions examined. In addition, as shown in this study and another study (Bandaru et al., 2009), the *APOE* genotype substantially affects the levels of lipids, or at least cholesterol, in neuronal membranes. Thus, in lipid-chemical studies of autopsied brains, attention should be paid to the *APOE* genotype of the individuals from whom specimens are obtained.

There are several implications of our findings from the viewpoint of the roles of cholesterol in AD development. First, cholesterol in membranes accelerates the formation of GM1 ganglioside-bound Aβ (GAβ), an endogenous seed for Alzheimer's amyloid (Yanagisawa et al., 1995; Hayashi et al., 2004), through facilitation of the formation of GM1 ganglioside clusters, which are required for GAβ generation (Kakio et al., 2001), and through tuning of GM1 ganglioside conformation (Fantini et al., 2013). Second, cholesterol increases the activities of secretases, which are involved in Aβ production in membrane microdomains or lipid rafts (for review see Di Paolo and Kim, 2011). Third, cholesterol enhances internalization of the amyloid precursor protein, leading to increased Aβ secretion (Cossec et al., 2010b) in association with the enlargement of early endosomes (Cossec et al., 2010a), which is the earliest cellular pathologic feature of AD (Cataldo et al., 2000). These findings strongly suggested that the decrease in cholesterol level by ε2 suppresses amyloid deposition. In addition, the decrease in cholesterol levels can also be beneficial in prevention of the clinical onset of AD, because pathological processes of AD, such as amyloid pore formation by Aβ oligomers, result in cognitive dysfunction of AD (Esparza et al., 2013), likely dependenjt on cholesterol levels in membranes (Di Scala et al., 2013). Alternatively, membrane microdomains or lipid rafts are critical for the function and even survival of neurons; thus, alterations in the levels of lipids in the presence of AD pathology, as observed in this study, likely lead to the perturbation of microdomains or lipid raft integrity, seriously and detrimentally affecting neurons in AD (for review see Hicks et al., 2012). In summary, this study, together with our previous study of human *APOE* knock-in mouse brains (Hayashi et al., 2002), suggests that the *APOE*-genotype-linked modulation of AD development is attributed to the regulation of cholesterol levels in synaptic membranes and/or synaptic membrane microdomains.

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The authors have no conflicts of interest to disclose.

REFERENCES

Adachi T, Saito Y, Hatsuta H, Funabe S, Tokumaru AM, Ishii K, Arai T, Sawabe M, Kanemaru K, Miyashita A, Kuwano R, Nakashima K, Murayama S. 2010. Neuropathological asymmetry in argyrophilic grain disease. J Neuropathol Exp Neurol 69:737–744.

Ariga T, McDonald MP, Yu RK. 2008. Role of ganglioside metabolism in the pathogenesis of Alzheimer's disease—a review. J Lipid Res 49: 1157–1175.

Bandaru VV, Troncoso J, Wheeler D, Pletnikova O, Wang J, Conant K, Haughey NJ. 2009. ApoE4 disrupts sterol and sphingolipid metabolism in Alzheimer's but not normal brain. Neurobiol Aging 30:591–599.

Benjamin R, Leake A, McArthur FK, Ince PG, Candy JM, Edwardson JA, Morris CM, Bjertness E. 1994. Protective effect of apoE epsilon 2 in Alzheimer's disease. Lancet 344:473.

Braak H, Braak E. 1991. Neuropathological stageing of Alzheimer-related changes. Acta Neuropathol 82:239–259.

Bugiani O, Giaccone G, Verga L, Pollo B, Ghetti B, Frangione B, Tagliavini F. 1990. Alzheimer patients and Down patients: abnormal presynaptic terminals are related to cerebral preamyloid deposits. Neurosci Lett 119:56–59.

Cataldo AM, Peterhoff CM, Troncoso JC, Gomez-Isla T, Hyman BT, Nixon RA. 2000. Endocytic pathway abnormalities precede amyloid beta deposition in sporadic Alzheimer's disease and Down syndrome:

differential effects of APOE genotype and presenilin mutations. Am J Pathol 157:277–286.

Cossec JC, Marquer C, Panchal M, Lazar AN, Duyckaerts C, Potier MC. 2010a. Cholesterol changes in Alzheimer's disease: methods of analysis and impact on the formation of enlarged endosomes. Biochim Biophys Acta 1801:839–845.

Cossec JC, Simon A, Marquer C, Moldrich RX, Leterrier C, Rossier J, Duyckaerts C, Lenkei Z, Potier MC. 2010b. Clathrin-dependent APP endocytosis and Abeta secretion are highly sensitive to the level of plasma membrane cholesterol. Biochim Biophys Acta 1801:846–852.

Cotman CW, Matthews DA. 1971. Synaptic plasma membranes from rat brain synaptosomes: isolation and partial characterization. Biochim Biophys Acta 249:380–394.

Crino PB, Ullman MD, Vogt BA, Bird ED, Volicer L. 1989. Brain gangliosides in dementia of the Alzheimer type. Arch Neurol 46: 398–401.

Di Paolo G, Kim TW. 2011. Linking lipids to Alzheimer's disease: cholesterol and beyond. Nat Rev Neurosci 12:284–296.

Di Scala C, Troadec JD, Lelièvre C, Garmy N, Fantini J, Chahinian H. 2013. Mechanism of cholesterol-assisted oligomeric channel formation by a short Alzheimer β-amyloid peptide. J Neurochem (in press).

Esparza TJ, Zhao H, Cirrito JR, Cairns NJ, Bateman RJ, Holtzman DM, Brody DL. 2013. Amyloid-β oligomerization in Alzheimer dementia versus high-pathology controls. Ann Neurol 73:104–119.

Fantini J, Yahi N, Garmy N. 2013. Cholesterol accelerates the binding of Alzheimer's β-amyloid peptide to ganglioside GM1 through a universal hydrogen-bond-dependent sterol tuning of glycolipid conformation. Front Physiol 4:120.

Folch J, Lees M, Sloane Stanley GH. 1957. A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem 226:497–509.

Fontaine RN, Harris RA, Schroeder F. 1980. Aminophospholipid asymmetry in murine synaptosomal plasma membrane. J Neurochem 34: 269–277.

Gorbenko GP, Kinnunen PK. 2006. The role of lipid–protein interactions in amyloid-type protein fibril formation. Chem Phys Lipids 141: 72–82.

Gylys KH, Fein JA, Yang F, Miller CA, Cole GM. 2007. Increased cholesterol in Abeta-positive nerve terminals from Alzheimer's disease cortex. Neurobiol Aging 28:8–17.

Hayashi H, Igbavboa U, Hamanaka H, Kobayashi M, Fujita SC, Wood WG, Yanagisawa K. 2002. Cholesterol is increased in the exofacial leaflet of synaptic plasma membranes of human apolipoprotein E4 knock-in mice. Neuroreport 13:383–386.

Hayashi H, Kimura N, Yamaguchi H, Hasegawa K, Yokoseki T, Shibata M, Yamamoto N, Michikawa M, Yoshikawa Y, Terao K, Matsuzaki K, Lemere CA, Selkoe DJ, Naiki H, Yanagisawa K. 2004. A seed for Alzheimer amyloid in the brain. J Neurosci 24:4894–4902.

Hicks DA, Nalivaeva NN, Turner AJ. 2012. Lipid rafts and Alzheimer's disease: protein–lipid interactions and perturbation of signaling. Front Physiol 3:189.

Holtzman DM, Herz J, Bu G. 2012. Apolipoprotein E and apolipoprotein E receptors: normal biology and roles in Alzheimer disease. Cold Spring Harbor Perspect Med 2:a006312.

Igbavboa U, Avdulov NA, Schroeder F, Wood WG. 1996. Increasing age alters transbilayer fluidity and cholesterol asymmetry in synaptic plasma membranes of mice. J Neurochem 66:1717–1725.

Kakio A, Nishimoto SI, Yanagisawa K, Kozutsumi Y, Matsuzaki K. 2001. Cholesterol-dependent formation of GM1 ganglioside-bound amyloid beta-protein, an endogenous seed for Alzheimer amyloid. J Biol Chem 276:24985–24990.

Kracun I, Rosner H, Drnovsek V, Heffer-Lauc M, Cosovič C, Lauc G. 1991. Human brain gangliosides in development, aging and disease. Int J Dev Biol 35:289–295.

Lichtenberg D, Goñi FM, Heerklotz H. 2005. Detergent-resistant membranes should not be identified with membrane rafts. Trends Biochem Sci 30:430–436.

Lingwood D, Simons K. 2007. Detergent resistance as a tool in membrane research. Nat Protoc 2:2159–2165.

Lippa CF, Smith TW, Saunders AM, Hulette C, Pulaski-Salo D, Roses AD. 1997. Apolipoprotein E-epsilon 2 and Alzheimer's disease: genotype influences pathologic phenotype. Neurology 48:515–519.

Liu CC, Kanekiyo T, Xu H, Bu G. 2013. Apolipoprotein E and Alzheimer disease: risk, mechanisms and therapy. Nat Rev Neurol 9:106–118.

Matsuzaki K, Kato K, Yanagisawa K. 2010. Abeta polymerization through interaction with membrane gangliosides. Biochim Biophys Acta 1801: 868–877.

Michikawa M, Fan QW, Isobe I, Yanagisawa K. 2000. Apolipoprotein E exhibits isoform-specific promotion of lipid efflux from astrocytes and neurons in culture. J Neurochem 74:1008–1016.

Molander-Melin M, Blennow K, Bogdanovic N, Dellheden B, Månsson JE, Fredman P. 2005. Structural membrane alterations in Alzheimer brains found to be associated with regional disease development; increased density of gangliosides GM1 and GM2 and loss of cholesterol in detergent-resistant membrane domains. J Neurochem 92:171–182.

Nagafuku M, Okuyama K, Onimaru Y, Suzuki A, Odagiri Y, Yamashita T, Iwasaki K, Fujiwara M, Takayanagi M, Ohno I, Inokuchi J. 2012. CD4 and CD8 T cells require different membrane gangliosides for activation. Proc Natl Acad Sci U S A 109:E336–E342.

Nagy Z, Esiri MM, Jobst KA, Johnston C, Litchfield S, Sim E, Smith AD. 1995. Influence of the apolipoprotein E genotype on amyloid deposition and neurofibrillary tangle formation in Alzheimer's disease. Neuroscience 69:757–761.

Oikawa N, Goto M, Ikeda K, Taguchi R, Yanagisawa K. 2012. The γ-secretase inhibitor DAPT increases the levels of gangliosides at neuritic terminals of differentiating PC12 cells. Neurosci Lett 525:49–53.

Op Den Velde W, Hooghwinkel GJ. 1975. The brain ganglioside pattern in presenile and senile dementia. J Am Geriatr Soc 23:301–303.

Oyama F, Shimada H, Oyama R, Ihara Y. 1995. Apolipoprotein E genotype, Alzheimer's pathologies and related gene expression in the aged population. Brain Res Mol Brain Res 29:92–98.

Persaud-Sawin DA, Lightcap S, Harry GJ. 2009. Isolation of rafts from mouse brain tissue by a detergent-free method. J Lipid Res 50:759–767.

Polvikoski T, Sulkava R, Haltia M, Kainulainen K, Vuorio A, Verkkoniemi A, Niinistö L, Halonen P, Kontula K. 1995. Apolipoprotein E, dementia, and cortical deposition of beta-amyloid protein. N Engl J Med 333:1242–1247.

Probst A, Langui D, Ipsen S, Robakis N, Ulrich J. 1991. Deposition of beta/A4 protein along neuronal plasma membranes in diffuse senile plaques. Acta Neuropathol 83:21–29.

Rapp A, Gmeiner B, Hüttinger M. 2006. Implication of apoE isoforms in cholesterol metabolism by primary rat hippocampal neurons and astrocytes. Biochimie 88:473–483.

Reiman EM, Chen K, Liu X, Bandy D, Yu M, Lee W, Ayutyanont N, Keppler J, Reeder SA, Langbaum JB, Alexander GE, Klunk WE, Mathis CA, Price JC, Aizenstein HJ, DeKosky ST, Caselli RJ. 2009. Fibrillar amyloid-beta burden in cognitively normal people at 3 levels of genetic risk for Alzheimer's disease. Proc Natl Acad Sci U S A 106: 6820–6825.

Rushworth JV, Hooper NM. 2010. Lipid rafts: linking Alzheimer's amyloid-β production, aggregation, and toxicity at neuronal membranes. Int J Alzheimers Dis 2011:603052.

Schmechel DE, Saunders AM, Strittmatter WJ, Crain BJ, Hulette CM, Joo SH, Pericak-Vance MA, Goldgaber D, Roses AD. 1993. Increased amyloid beta-peptide deposition in cerebral cortex as a consequence of apolipoprotein E genotype in late-onset Alzheimer disease. Proc Natl Acad Sci U S A 90:9649–9653.

Tiraboschi P, Hansen LA, Masliah E, Alford M, Thal LJ, Corey-Bloom J. 2004. Impact of APOE genotype on neuropathologic and neurochemical markers of Alzheimer disease. Neurology 62:1977–1983.

Yamamoto N, Igbabvoa U, Shimada Y, Ohno-Iwashita Y, Kobayashi M, Wood WG, Fujita SC, Yanagisawa K. 2004. Accelerated Abeta aggregation in the presence of GM1-ganglioside-accumulated synaptosomes of aged apoE4-knock-in mouse brain. FEBS Lett 569:135–139.

Yamamoto N, Matsubara T, Sato T, Yanagisawa K. 2008. Age-dependent high-density clustering of GM1 ganglioside at presynaptic neuritic terminals promotes amyloid beta-protein fibrillogenesis. Biochim Biophys Acta 1778:2717–2726.

Yanagisawa K. 2007. Role of gangliosides in Alzheimer's disease. Biochim Biophys Acta 1768:1943–1951.

Yanagisawa K, Odaka A, Suzuki N, Ihara Y. 1995. GM1 ganglioside-bound amyloid beta-protein (A beta): a possible form of preamyloid in Alzheimer's disease. Nat Med 1:1062–1066.

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Clinical features of genetic Creutzfeldt-Jakob disease with V180I mutation in the prion protein gene

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ABSTRACT

Objectives: Genetic Creutzfeldt-Jakob disease (CJD) due to V180I mutation in the prion protein gene (*PRNP*) is of great interest because of the differences from sporadic CJD and other genetic prion diseases in terms of clinical features, as well as pathological and biochemical findings. However, few systematic observations about the clinical features in patients with this unique mutation have been published. Therefore, the goal of this study was to relate this mutation to other forms of CJD from a clinical perspective.

Design: We analysed clinical symptoms, prion protein genetics, biomarkers in cerebrospinal fluid (CSF) and MRI of patients.

Participants: 186 Japanese patients with the V180I mutation in *PRNP*.

Results: Our results indicate that the V180I mutation caused CJD at an older age, with a slower progression and a lower possibility of developing myoclonus, cerebellar, pyramidal signs and visual disturbance compared with classical sporadic CJD with methionine homozygosity at codon 129 of *PRNP*. Cognitive impairment was the major symptom. Diffuse hyperintensity of the cerebral cortex in diffusion-weighted MRI might be helpful for diagnosis. Owing to the low positivity of PrP^{Sc} in the CSF, genetic analysis was often required for a differential diagnosis from slowly progressive dementia.

Conclusions: We conclude that the V180I mutation in *PRNP* produces a late-developing and slow-developing, less severe form of CJD, whose lesions are uniquely distributed compared with sporadic and other genetic forms of CJD.

Strengths and limitations of this study

- The study used the largest V180I prion protein mutation cohort yet to be published to improve statistical power.
- The study compared the V180I variant of CJD to other genetic and sporadic variants of CJD, not just to non-CJD controls, allowing comparisons to be made across the spectrum of prion diseases.
- The study compared the V180I variant with regard to other mutations (the 129 and 219 codon polymorphisms) known to alter disease progression in other variants.
- The study was limited by focusing primarily on clinical features and retrospective data, making interpretation of the potential mechanisms differentiating disease progression in V180I and other CJD variants difficult or impossible without future studies.

INTRODUCTION

Prion diseases are transmissible and lethal neurodegenerative diseases that affect humans and animals.[1] In humans, prion disease can be categorised into sporadic, acquired and genetic forms.[2] The genetic form of prion disease (gPrD) that is caused by mutations in the prion protein gene (*PRNP*) accounts for 10.2% of cases in Europe and 16.7% in Japan.[3 4]

The epidemiological distributions of patients with gPrD were reported to be different between European countries and Japan. While the E200K mutation occurs most frequently in Europe,[3] the V180I mutation is the most frequent mutation in Japan.[5] Currently, several reports indicate that the V180I mutation in *PRNP* accounts for specific clinical and pathological findings.[5–8] Because patients with V180I rarely have a family history of the disease, the question of whether this mutation causes prion disease persists. On the other hand, patients with V180I show several specific clinical features different from those of sporadic Creutzfeldt-Jakob disease (sCJD) or other gPrDs.[9] We have previously reviewed clinical symptoms and cerebrospinal fluid (CSF) markers of several *PRNP* mutations, including V180I.[5] Patients with V180I are readily

distinguishable from patients with other dementia because they show specific hyperintensity in the cerebral cortex in diffusion-weighted MRI. We present some clinical features in genetic CJD (gCJD) with V180I and Alzheimer's disease in table 1.

In the current study, in order to better elucidate the clinical characteristics of the V180I mutation, we analysed the surveillance data of 186 patients with V180I, including the occurrence rate of neurological symptoms, the period of time between disease onset and the occurrence of these symptoms, biomarkers in the CSF, MRI and EEG data, and codon 129 polymorphism in *PRNP*. Our study indicates that myoclonus and periodic sharp wave complexes (PSWCs) in the EEG, which are included in the diagnostic criteria of CJD, occur less frequently in patients with V180I.

METHODS

Patients

The Prion Disease Surveillance Committee in Japan diagnosed gPrDs in accordance with the WHO Case Definition Criteria for epidemiological surveillance. Information on each patient was collected between April 1999 and September 2013, after the current Prion Disease Surveillance Committee of Japan began the comprehensive surveillance on prion diseases in Japan. In the current study, we analysed the surveillance data of 186 patients with definite or probable gPrD with a V180I mutation. In order to differentiate clinical features of patients with V180I from sCJD, we compared the V180I patient group with patients having sCJD with type-1 PrP^{Sc} and methionine homozygosity at codon 129 (sCJD-MM1) of *PRNP*, a classical type of prion disease. In this study, 59 patients with sCJD-MM1 with definitive diagnosis were included as a control.

Clinical analysis

We collected information on age of onset, sex, family history, clinical duration of each sign or symptom (duration from onset to death, or to the point when we confirmed the condition of the patient if he or she was alive and to the point when clinical signs were observed) and the clinical signs themselves (first symptom, dementia, psychological disturbance, cerebellar disturbance, visual disturbance, pyramidal or extrapyramidal signs, myoclonus and akinetic mutism). The appearance of PSWCs in the EEG and hyperintensities in the MRI was examined as previously described[4] The open reading frame and polymorphisms of codons 129 and 219 of the *PRNP* gene were analysed after genomic DNA was extracted from the patients' blood, as previously described.[10]

Table 1 Comparison of clinical characteristics between gPrD-V180I and Alzheimer's disease (AD)

	gPrD-V180I	AD
Age at onset (years)	Late 70s	Early 70s
Period from onset to death (years)	2–3	4–8
Myoclonus	+	Late stage
PSWCs on EEG	+−	−
MRI findings	Cortical hyperintensity	Hippocampal atrophy
CSF findings	Total τ↑↑↑, PrP^{Sc} (+)	Aβ42↓

CSF, cerebrospinal fluid; gPrD, genetic form of prion disease; PSWCs, periodic sharp wave complexes.

CSF biomarkers

CSF analysis of all patients was performed at Nagasaki University.[11] We evaluated 14-3-3 and total τ (t-τ) protein levels in the CSF by western blotting as previously described.[5] PrP^{Sc} in the CSF was detected by real-time quaking induced conversion (RT-QUIC), as previously described.[12] Briefly, CSF was incubated with recombinant human prion protein (residues 23–231 of human PrP with methionine at codon 129) at 37°C with intermittent shaking. Four wells were tested twice for each CSF sample and the sample was decided as positive when two or more of the four wells showed more than 1000 reactive fluorescence units (thioflavin T) within 48 h. The kinetics of fibril formation was monitored by reading the fluorescence intensity every 10 min.

Statistical analysis

The Mann-Whitney U test was used for the statistical comparisons of age of onset, disease duration and the level of τ protein in the CSF. Fisher's exact probability test was used for the comparisons of sex, the rate of occurrence of each clinical sign, presence of PSWCs in the EEG, presence of hyperintensity in the MRI and rate of positive detection of 14-3-3 and PrP^{Sc} proteins. For analysis of the correlation between CSF markers and each clinical parameter, analysis of variance or multiple comparison tests (χ^2 and Kruskal-Wallis) were used. Significance was defined as $p<0.05$. Analyses were performed using GraphPad Prism 5 software (GraphPad Software, La Jolla, California, USA) and IBM SPSS Statistics (IBM, New York, New York, USA).

Ethical issues

Informed consent from the family of each patient was obtained for the current study. The study was performed in accordance with the ethical standards laid down by the 2013 Declaration of Helsinki.

RESULTS

Comparison of clinical features between patients with V180I-MM and sCJD-MM1

Since gCJD with V180I mutation contains codon 129 methionine homozygosity (129MM) and methionine/valine heterozygosity (129MV), we compared patients with V180I and 129MM (V180I-MM) and sCJD-MM1 or MM2 (table 2), and V180I-MV and sCJD-MV (there are no pathologically defined MV1 cases in Japan; table 3).

Table 2 Clinical features of codon 129 homozygosity of methionine among V180I, sCJD-MM1 and sCJD-MM2

	V180I-MM n=139	sCJD-MM1 n=59	p Value (vs V180I-MM)	sCJD-MM2 n=8	p Value (vs V180I-MM)
Male/female	58/81	25/34		5/3	0.53
Codon 219	135 EE; 4 NA	54 EE; 5 NA		8 EE	
Age at onset (years)*	77.3±6.8 (78, 44–93, n=139)	68.9±9.1 (70, 40–89, n=59)	<0.001	60.3±11.9 (63, 43–74, n=8)	<0.001
Period from onset to death (months)*	23.1±15.1 (19, 5–70, n=75)	17.2±12.5 (15, 1–60, n=57)	0.032	22.3±12.0 (20, 10–50, n=8)	0.98
Myoclonus†	46/130 (35.4%)	52/59 (88.1%)		4/8 (50%)	<0.001
Period from onset to myoclonus (months)*	6.4±6.1 (5, 0–36, n=38)	2.0±2.4 (1, 0–13, n=49)	<0.001	7.3±4.0 (8, 3–11, n=3)	0.92
Cognitive impairment†	138/138 (100%)	59/59 (100%)		8/8 (100%)	1
Period from onset to cognitive impairment (months)*‡	0.5±1.4 (0, 0–7, n=121)	0.6±1.0 (0, 0–6, n=55)	1	15.6±40.3 (0, 0–115, n=8)	<0.001
Pyramidal signs†	66/132 (50%)	40/54 (74.1%)		2/7 (28.6%)	0.004
Period from onset to pyramidal sign (months)*	3.9±5.8 (2.5, 0–36, n=58)	2.9±4.4 (2, 0–24, n=38)	0.53	12 (n=1)	
Extrapyramidal signs†	71/133 (53.4%)	30/52 (57.7%)		2/8 (25.0%)	0.23
Period from onset to extrapyramidal signs (months)*	3.8±3.5 (3, 0–19, n=58)	2.2±4.3 (1, 0–24, n=29)	0.13	13.0±1.4 (13, 12–14, n=2)	0.002
Cerebellar dysfunction†	40/119 (33.6%)	32/45 (71.1%)		3/7 (42.9%)	<0.001
Period from onset to cerebellar dysfunction (months)*	2.9±2.7 (3, 0–9, n=33)	0.7±0.9 (1, 0–3, n=31)	<0.001	12.7±1.2 (12, 12–14, n=3)	<0.001
Visual disturbance†	10/109 (9.2%)	28/49 (57.1%)		2/7 (28.6%)	<0.001
Period from onset to visual disturbance (months)*	2.2±1.5 (2, 0–4, n=10)	0.7±1.7 (0, 0–7, n=26)	0.036	0 (n=2)	0.15
Psychiatric symptoms†	68/130 (52.3%)	32/51 (62.7%)		5/7 (71.4%)	0.36
Period from onset to psychiatric symptoms (months)*	1.6±3.0 (0, 0–19, n=62)	0.8±0.9 (1, 0–3, n=29)	0.32	5.3±7.1 (3, 0–15, n=4)	0.024
Akinetic mutism†	74/137 (54.0%)	44/57 (77.2%)		2/8 (25.0%)	0.001
Period from onset to akinetic mutism (months)*	9.8±6.6 (8, 1–27, n=64)	3.6±4.3 (2, 0–23, n=42)	<0.001	18 (n=2)	
PSWCs on EEG†	10/131 (7.6%)	55/59 (93.2%)		2/7 (28.6%)	<0.001
Hyperintensities on MRI	135/136 (99.3%)	57/57 (100%)	<0.001	5/8 (62.5%)	0.092
Positive rate of 14-3-3 protein in CSF†	46/53 (86.8%)	27/31 (87.1%)	1	NA	
Positive rate of t-τ protein in CSF†	48/53 (90.6%)	27/31 (87.1%)	0.72	NA	
Amount of t-τ protein in CSF (pg/mL) *	2965±1712 (2400, 146.0–9940.0, n=53)	7950±8423 (5450, 150.0–40120.0, n=29)	<0.001	NA	
Positive rate of PrP^{Sc} in CSF†	36/53 (67.9%)	27/30 (90.0%)	0.032	NA	

Codon 219 is presented with total cases of that polymorphism type. EE means glutamic acid homozygous. NA means data not available.
Medians are compared using analysis of variance with Dunnett's post hoc test for age of onset, the period from disease onset to death or the appearance of each symptom and sign, the two-tailed Mann-Whitney U test for the period from onset to akinetic mutism and the CSF biomarker level. Frequencies of positive cases are compared using the two-tailed Fisher's exact test.
*Age of onset, period of time from disease onset to death or the appearance of each symptom and sign and CSF biomarker level are presented as mean±SD (median, range, cases).
†Frequencies of positive cases are presented as positive cases/total cases (percentage).
‡These were zero-inflated.
CSF, cerebrospinal fluid; PSWCs, periodic sharp wave complexes; sCJD, sporadic Creutzfeldt-Jakob disease; t-τ, total τ.