(2) Sense of self-efficacy. Sense of self-efficacy was measured using the Japanese version of the General Self-Efficacy Scale (SE).[13] General self-efficacy is the belief in one's competence to cope with variable stressful or challenging demands, whereas specific self-efficacy is restricted to a specific demand. SE is designed to assess optimistic self-belief to cope with a variety of difficult demands in life. The mean ± SD SE was 77.30 ± 14.13 in men aged 65–74 years, 75.68 ± 13.96 in women aged 65–74 years, 71.86 ± 15.24 in men aged 75 years and over, and 72.37 ± 14.87 in women aged 75 and over.[14]

(3) Personality traits. Personality traits were assessed using the Big Five scale of personality traits.[15] The Big Five factors are extraversion, neuroticism, openness to experience, conscientiousness, and agreeableness. As the personality traits have sociocultural implication, we used the scale that was developed and validated in Japan.

Functional capacity for independent living

Functional capacity for independent living was assessed by the Tokyo Metropolitan Institute of Gerontology Index of Competence (TMIG-IC), which was designed to measure higher-level functional capacities in community-dwelling elderly residents who could not be adequately assessed by existing basic or instrumental activities of daily living scales.[16,17] TMIG-IC consists of 13 items on three subscales: instrumental self-maintenance, intellectual activity, and social role. The mean ± SD TMIG-IC was 10.8 ± 3.0 in individuals with a mean age of 72.5 years (n = 1809).[18]

Social factors

(1) Social network size. Social network size was assessed using the Japanese version of the abbreviated Lubben Social Network Scale,[19] which evaluates the size of a social network that is attributable to family ties and a parallel set attributable to friendship ties. The scores range from 0 to 30, and higher scores indicate larger social networks. In Japanese samples, the mean ± SD Lubben Social Network Scale score was 16.2 ± 5.1 in individuals aged 67.0 ± 6.8 years (n = 232).[20]

(2) Range of activity. The Life-Space Assessment (LSA) assessed a subject's range of activities based on how far and how often a person moves to each of the defined levels and any assistance needed to get to each level. LSA can assess the full range of mobility, ranging from mobility dependent on assistance from another person and limited to the room where a person sleeps daily, to travel out of the person's town independently during the month preceding the assessment.[21] The mean ± SD LSA was 91.6 ± 13.8 in individuals aged 74.0 ± 5.5 years (n = 321).[22]

Cognitive function

Cognitive function was measured by MMSE, and two subtests of the 5-Cog test were analyzed in the present study:[23] memory test (category-cued delayed recall test consisting of 32 words in eight categories) and executive function test (dual-task test requiring alternating attention). The mean ± SD scores in cognitively normal subjects (age range: 65–80 years; n = 800) were 12.0 ± 5.8 on the memory test and 20.1 ± 9.1 on the executive function test.

Physical factors

Aged individuals generally suffer from multiple diseases in various stages. Thus, it is difficult to obtain comprehensive information from a health questionnaire. Therefore, we did not include physical factors as variables. Instead, we established inclusion criteria, and participants were limited to those who could live independently in the community.

Analysis

We compared the SDL scores between the NC and MCI groups using a two-sample t-test. Pearson's correlation coefficients were obtained between the social-psychological-cognitive factors and the SDL score for each of the two groups. The factors with significant coefficients were entered in a stepwise manner into the multiple linear regression model as independent variables, with the SDL score as the dependent variable. Then, we obtained the partial correlation coefficient between the SDL score and each of the variables within the final model of multiple regression. The data were analyzed using the Japanese version of SPSS for Windows version 19.0 (IBM, Armonk, New York, USA) and level of statistical significance was set as $P < 0.05$.

RESULTS

Characteristics of subjects

Table 1 contains descriptive statistics for participants and the outcome variables. SDL scores were 44.3 ± 5.6 in the NC group and 44.2 ± 7.1 in the MCI group,

Table 1 Demographic data and correlation with quality of life (QOL)

Stage	NC (n = 120) (Men/women) (30/90)		MCI (n = 37) (Men/women) (17/20)		NC vs MCI
Gender	Mean ± SD	$r^{\dagger}$	Mean ± SD	$r^{\dagger}$	P-value‡
QOL (SDL)§	44.3 ± 5.6		44.2 ± 7.1		0.926
Age	71.9 ± 4.1	−0.183*	73.1 ± 4.4	0.261	0.127
Years of education	11.9 ± 2.2	−0.019	11.5 ± 3.0	0.067	0.372
Memory complaints (Q-SMC)§	6.3 ± 1.7	−0.211*	6.9 ± 2.2	−0.653***	0.082
Psychological factors					
Depressive mood (GDS)§	3.3 ± 3.0	−0.715***	4.1 ± 3.4	−0.550***	0.141
Self-efficacy (SE)§	76.7 ± 12.1	0.489***	74.0 ± 12.1	0.623***	0.245
Personality traits (Big Five)§					
Extraversion	51.0 ± 9.5	0.425***	51.1 ± 7.3	0.372*	0.946
Neuroticism	48.8 ± 9.4	−0.332***	49.8 ± 9.3	−0.439**	0.581
Intellect	48.5 ± 8.7	0.186*	49.7 ± 9.1	0.027	0.474
Conscientiousness	53.4 ± 8.3	0.099	49.9 ± 7.8	0.495**	<0.05
Agreeableness	56.2 ± 8.2	0.185*	53.0 ± 5.6	0.622***	<0.05
Cognitive function					
MMSE	28.4 ± 1.5	−0.082	25.7 ± 1.9	0.135	<0.001
Memory test	15.2 ± 4.5	0.038	8.8 ± 3.5	0.222	<0.001
Executive function test	21.9 ± 6.4	−0.055	14.7 ± 7.2	0.026	<0.001
Functional capacity					
TMIG-IC	12.0 ± 1.4	0.278**	11.7 ± 1.6	0.380*	0.302
Social factors					
Lubben social network size	16.8 ± 6.0	0.431***	16.5 ± 5.5	0.597***	0.810
Life-space assessment (LSA)§	90.8 ± 19.7	0.366***	96.3 ± 17.4	0.370*	0.128

*P < 0.05, **P < 0.001, ***P < 0.001. †Correlation coefficients with SDL, level of significance. ‡Comparison between scores of NC and MCI by two sample t-test. §Related test appears in parentheses. GDS, the Japanese version of Geriatric Depression Scale; LSA, Life-Space Assessment; Lubben, the Japanese version of the abbreviated Lubben Social Network Scale; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; NC, normal controls; Q-SMC, the Questionnaire for Subjective Memory Complaint; SDL, Satisfaction in Daily Life; SE, the Japanese version of the General Self-Efficacy scale; TMIG-IC, Tokyo Metropolitan Institute of Gerontology Index of Competence.

and were not significantly different between the NC and MCI groups (P = 0.926).

Possible QOL predictors

The factors showing significant correlation with SDL scores were similar in the NC and MCI groups, with the exception of personality traits (Table 1). There were positive correlations between SDL scores and self-evaluation scores of remaining function (i.e. sense of self-efficacy and daily functioning (TMIG-IC)), social factors of social network size (Lubben Social Network Scale), and range of activity (LSA). A negative correlation was observed between SDL scores and both scores related to memory complaints (Q-SMC) and depressive mood (GDS).

No significant correlation was observed between SDL scores and either cognitive scores (MMSE, memory test and executive function test) or years of education (Table 1).

There was also no correlation between memory complaints (Q-SMC) and MMSE (NC: r = −0.082; MCI: r = 0.135), memory function (NC: r = 0.038; MCI: r = 0.222), or executive function (NC: r = −0.055; MCI: r = 0.026). The gender difference on the SDL was not significant in either the NC or MCI group.

QOL predictors after controlling for other factors

There were several independent variables in the final models of the stepwise multiple regression analyses for the NC and MCI groups. Memory complaint (Q-SMC) was a negative predictor in the MCI group. The positive predictors were range of activity (LSA) in the NC group and sense of self-efficacy (SE) in the MCI group. Depressive mood (GDS) was a common negative predictor in both groups (Table 2). The partial correlation coefficient between the SDL and Q-SMC scores in the MCI group was −0.62 (P < 0.001), when the SE and GDS scores were controlled. In the same way, the coefficient between the SDL and GDS scores was −0.51 (P < 0.01) after the SE and Q-SMC scores were controlled. The coefficient between the SDL and SE scores was −0.37 (P < 0.05) after the GDS and

Table 2 Regression models of factors predicting QOL score

A. NC group

Predictors	Unstandardized β	Unstandardized SD	Standardized β	*t*-value	*P*-value
(Constant)	43.912	2.190		20.051	<0.001
GDS	−1.251	0.133	−0.662	−9.389	<0.001
LSA	0.051	0.021	0.168	2.380	<0.05

B. MCI group

Predictors	Unstandardized β	Unstandardized SD	Standardized β	*t*-value	*P*-value
(Constant)	45.557	6.728		6.771	<0.001
Q-SMC	−1.439	0.319	−0.490	−4.506	<0.001
GDS	−0.757	0.225	−0.374	−3.369	<0.01
SE	0.156	0.070	0.268	2.233	<0.05

GDS, the Japanese version of Geriatric Depression Scale; LSA, Life-Space Assessment; MCI, mild cognitive impairment; NC, normal controls; Q-SMC, the Questionnaire for Subjective Memory Complaint; SE, the Japanese version of the General Self-Efficacy scale.

Q-SMC scores were controlled. In the NC group, the partial correlation coefficient between the QOL and GDS scores was −0.67 ($P < 0.001$) after the LSA scores were controlled, whereas the coefficient between the QOL and LSA scores was not significant ($r = 0.17$) after the GDS scores were controlled.

DISCUSSION

Memory complaints had a negative impact on self-rated QOL in the MCI group, whereas a negative correlation was weak in the NC group. The QOL scores did not significantly correlate with the memory test in either the MCI or NC group. In multiple linear regression analysis, subjective memory complaint was found to be a negative predictor of QOL. This was further confirmed by partial correlation analysis. The QOL scores were significantly correlated with the scores of subjective memory complaints after the scores of self-efficacy and depressive mood were controlled.

These results suggest that those with MCI consider their awareness of memory decline seriously enough to affect their QOL. When self-awareness of memory decline is considered, it should be taken into account whether one can evaluate one's own memory function properly. Deterioration of self-awareness of memory decline is characteristic of patients with Alzheimer's disease and other types of dementia. Individuals with dementia tend to overestimate their capacity and ignore their deficits,[24] whereas those with MCI retain the ability to estimate their own memory function in most cases.[25] Consequently, those with MCI may recognize that their own memory decline is more severe than age-related decline, and they are all the more afflicted with fear of developing dementia.

Depressive state was a negative predictor in the MCI group. It is well established that QOL is intrinsically related to depressive mood,[26–28] which is also highly associated with the personality trait of neuroticism among elderly individuals.[29,30]

With regard to positive predictors, sense of self-efficacy was shown to be a positive predictor of self-rated QOL in the MCI group. A higher sense of self-efficacy was reported as a positive predictor of QOL in demented individuals.[31] As autonomy becomes limited among those with MCI, they are confronted by limits in their social lives. Thus, a higher sense of self-efficacy would contribute to higher life satisfaction.

For those with MCI, an approach that aims to improve memory function could soothe the fear of memory decline, and cognitive stimulation, including memory training, has been widely practised in individuals with MCI to prevent the development of dementia. However, it should be noted that such approaches inevitably identify what those with MCI are incapable of doing and could aggravate their awareness of memory decline. The fear of developing dementia and the realization of their memory deficits can devastate the self-confidence of those with MCI and worsen their depressive tendency. Indeed, adverse effects of cognitive training, such as frustration, anxiety, depression, and reduced self-esteem, have been reported,[32,33] and the consensus statement of the American Association for Geriatric Psychiatry warned of the potentially harmful effects of cognitive training.[34] Thus, cognitive training should be conducted with full attention to the mental state of the individual, so as to avoid exacerbating his or her depressive state or damaging his or her sense of self-efficacy for the improvement of QOL.

Limitations

With regard to limitations, the questionnaires used in the study, including QOL, depressive mood, and personality assessments, were self-rated, and it is necessary to confirm the results using a more objective evaluation of QOL. Additionally, those in the NC group in this study subjectively perceived cognitive decline, although they showed no objective cognitive decline.

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ORIGINAL ARTICLE

CSF levels of Aβ1-38/Aβ1-40/Aβ1-42 and ^{11}C PiB-PET studies in three clinical variants of primary progressive aphasia and Alzheimer's disease

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Abstract

Primary progressive aphasia (PPA) is a cognitive syndrome characterized by progressive and isolated language impairments due to neurodegenerative diseases. Recently, an international group of experts published a Consensus Classification of the three PPA clinical variants (naPPA, svPPA and lvPPA). We analyzed 24 patients with PPA by cognitive functions, neuroimaging (MRI, ^{99m}Tc ECD-SPECT, ^{11}C PiB-PET and FDG-PET) and cerebrospinal fluid (CSF) analysis (ptau-181, Aβ1-42, Aβ1-40 and Aβ1-38), to elucidate relationships between neuroimaging studies and biochemical findings in the three PPA clinical variants. Cognitive and speech functions were measured by mini-mental state examination and standard language test of aphasia. The patients with lvPPA showed significant decreases in CSF Aβ1-42 and ratios of Aβ1-42/Aβ1-40 and Aβ1-42/Aβ1-38, and significant increases in CSF ptau-181 and ratios of ptau-181/Aβ1-42 and ptau-181/Aβ1-38; these findings were similar to those of patients with Alzheimer's disease (AD). We observed a higher frequency of the ApoE ε4 allele in the lvPPA patients relative to the two other PPA variants. In ^{11}C PiB-PET of lvPPA patients, PiB positive findings were detected in cortices of frontal, temporal and parietal lobes and the posterior cingulate, where massive Aβ may accumulate due to AD. Our results of AD-CSF markers including Aβ1-38 and ^{11}C PiB-PET in the lvPPA patients demonstrate a common pathological mechanism with the occurrence of AD.

Abbreviations: Aβ: β amyloid β protein; AD: Alzheimer's disease; AOO: age of onset; AOS: apraxia of speech; Apo E: apolipoprotein E; ^{11}C PiB-PET: ^{11}C Pittsburgh compound B-positron emission tomography; ^{11}C PBB3-PET: ^{11}C Pyridinyl-Butadienyl-Benzothiazole-positron emission tomography; CSF: cerebrospinal fluid; ELISA: enzyme-linked immunosorbent assay; EOSAD: early-onset sporadic AD; FDG-PET: ^{18}F-fluorodeoxy glucose-positron emission tomography; FTD: frontotemporal dementia; FTLD: frontotemporal lobar degeneration; ^{123}I IMP-SPECT: N-isopropyl-p-(iodine-123)-iodoamphetamine; LOSAD: late-onset sporadic AD; lvPPA: logopenic variant PPA; MMSE: mini-mental state examination; naPPA: non-fluent/agrammatic variant PPA; ND: non-demented subject; PCA: posterior cortical atrophy; PPA: primary progressive aphasia; ptau: phosphorylated tau; S.D.: standard deviation; SLTA: Standard Language Test of Aphasia; svPPA: semantic variant PPA; ^{99m}Tc-ECD SPECT: ^{99m}Tc-ethyl cysteinate dimer single photon emission computerized tomography.

Keywords

^{11}C PiB-PET, amyloid β protein, Alzheimer's disease, cerebrospinal fluid, primary progressive aphasia

History

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Introduction

Primary progressive aphasia (PPA) is a cognitive syndrome characterized by a progressive and initially isolated language impairment caused by a neurodegenerative disease [1]. The non-fluent/agrammatic variant of PPA (naPPA) is characterized by agrammatism and/or motor speech articulatory errors due to an apraxia of speech (AOS), in which impairment of sentence comprehension for difficult syntactic constructions may also be present [2,3]. The core features of the semantic variant of PPA (svPPA) are impaired confrontation naming and single-word comprehension [4], while object knowledge

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is usually affected but repetition and motor speech are spared. The consensus neuroimaging markers for naPPA are atrophy and/or functional abnormalities in the left posterior fronto-insular region [2,3]. A consensus meeting developed criteria for these conditions in relation to frontotemporal lobar degeneration (FTLD) [5]. Later, other affected cognitive domains and different accompanying language disorders were recognized. The logopenic variant of PPA (lvPPA) was defined by hesitant speech with word-finding pauses due to impaired single-word retrieval and difficulty in sentence repetition, without object knowledge and motor deficits of speech [6]. For lvPPA, the MRI findings are predominant in left posterior perisylvian or parietal atrophies [6]. Consequently, functional neuroimaging studies have established consistent neuroanatomical correlations in three clinical variants of PPA [7–9]. According to these defining characteristics, an international group of experts published a Consensus Classification of the most accepted three clinical variants of PPA (naPPA, svPPA, lvPPA) [10]. In the last decade, cerebrospinal fluid (CSF) biomarkers [11] and amyloid positron emission tomography (^{11}C PiB-PET) [12,13] have been developed in research settings to elucidate clinical–pathological correlations of Alzheimer's disease (AD). So far, some subgroup of patients with PPA have high association with the CSF diagnostic AD markers [14,15], and the neuroimaging biomarkers of amyloid PET/FDG-PET [7,8,16,17] and MRI [8,9,18].

Materials and methods

Subjects

This study complied with the Declaration of Helsinki and was approved by the Institutional Review Boards (IRB) of Gunma University Graduate School of Medicine, Geriatrics Research Institute and Hospital, and Maebashi Red Cross Hospital. The spouse or family members of each AD patient provided written informed consent for the patient to participate in the study. The subjects who underwent lumbar punctures were recruited at Gunma University Graduate School of Medicine, Geriatrics Research Institute and Hospital, and Maebashi Red Cross Hospital (Maebashi, Gunma, Japan). Upon entering the study, subjects underwent a standardized clinical assessment, including medical history, physical and neurological examinations, Mini-Mental State Examination (MMSE) [19], brain MRI and/or computed tomography (CT) scan. AD was diagnosed for patients scoring 23 points or fewer on the MMSE [20], combined with caregivers' information of patients' daily activities. Diagnostic criteria of the National Institute of Neurological and Communicative Diseases and Stroke-Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) [21] were used for AD diagnosis. Subjects were classified as non-demented (ND) if they scored more than 24 points on the MMSE, and if, based upon information on activities of daily living (ADL) provided by the family, they were considered to have a normal daily life not requiring any intellectual assistance. Speech function of patients was estimated by the Standard Language Test of Aphasia (SLTA) [22,23]. SLTA is a test battery originally developed for language function to estimate multi-domains, including ''Confrontation naming'', ''Word repetition'', ''Sentence repetition'', ''Auditory single-word comprehension'', and ''Auditory complex sentence comprehension commands''. Three variants of PPA patients were diagnosed clinically, based on the Consensus Classification of the three most accepted PPA clinical variants [10].

Demographics of PPA patients and AD

The number of patients in each study group was as follows: 10 for naPPA, 4 for svPPA, 10 for lvPPA, and 50 for AD patients. Age of onset (AOO, years old, mean ± SD) was 63.50 ± 5.06 in naPPA patients, 62.00 ± 0.82 in svPPA patients, 64.70 ± 4.97 in lvPPA patients and 64.8 ± 8.01 in AD patients. Duration of the disease (years) was 5.60 ± 1.78 in naPPA patients, 4.00 ± 1.83 in svPPA patients, 4.00 ± 1.16 in lvPPA patients and 3.06 ± 1.99 in AD patients. The male ratio to total patient number was 0.50 in naPPA patients, 1.00 in svPPA, 0.50 in lvPPA patients and 0.40 in AD patients. The years of attained education were 11.70 ± 0.95 for naPPA patients, 13.00 ± 2.00 for svPPA patients, 12.10 ± 1.66 for lvPPA patients and 12.25 ± 2.13 in AD patients.

Neuroimaging studies

MRI or CT scan, ^{99m}Tc ECD-SPECT and ^{11}C PiB-PET and FDG-PET neuroimaging studies were performed for the patient study groups. Each MRI and SPECT/PET scan was evaluated by an experienced radiologist or nuclear medicine clinician and two neurologists; all evaluators were blinded to the patients' data on neurological findings, cognition and linguistic assessment (MMSE and SLTA). For each patient in the PPA variant and AD study groups, we assessed the presence or absence of imaging-supported diagnostic biomarkers by MRI, SPECT and FDG-PET [7–10,16–18]: (A) predominant atrophy and/or hypoperfusion/hypometabolism in the left posterior fronto-insular region (naPPA), (B) predominant atrophy and hypoperfusion/hypometabolism in the left anterior temporal lobe (svPPA), (C) predominant atrophy and hypoperfusion/hypometabolism in the left posterior perisylvian or parietal region (lvPPA), (D) hypoperfusion/hypometabolism in bilateral posterior cingulate gyrus and precuneus (AD) (Figure 1A–D).

PiB (2-(4-aminophenyl)-6-hydroxybenzothiazole) was synthesized for ^{11}C PiB-Positron Emission Tomography (^{11}C PiB-PET) [12]. After an intravenous injection of ^{11}C-PiB (550 MBq), a dynamic 70-min scan was acquired in the three-dimensional mode without arterial sampling using an Eminence-B PET scanner (General Electric, CT, USA). CT scans were co-registered with the respective PET images using the PMOD image-fusion tool (PMOD Technologies Ltd., Zurich, Switzerland). The PET images were reconstructed using a filtered back-projection algorithm for attenuation and scatter corrections. According to a previous study [24], in which the frame summation of the dynamic images was recorded for 70 min, Logan graphical analysis was used for determining the regional counts (SUVR)(distribution volume ratio, DVR = binding potential + 1) using the cerebellum as the reference region. For this purpose, the cortical lesions occurring in the frontal and temporal lobes and posterior cingulate gyrus were selected. The mean cortical DVR (MCDVR) was the mean of the DVR values

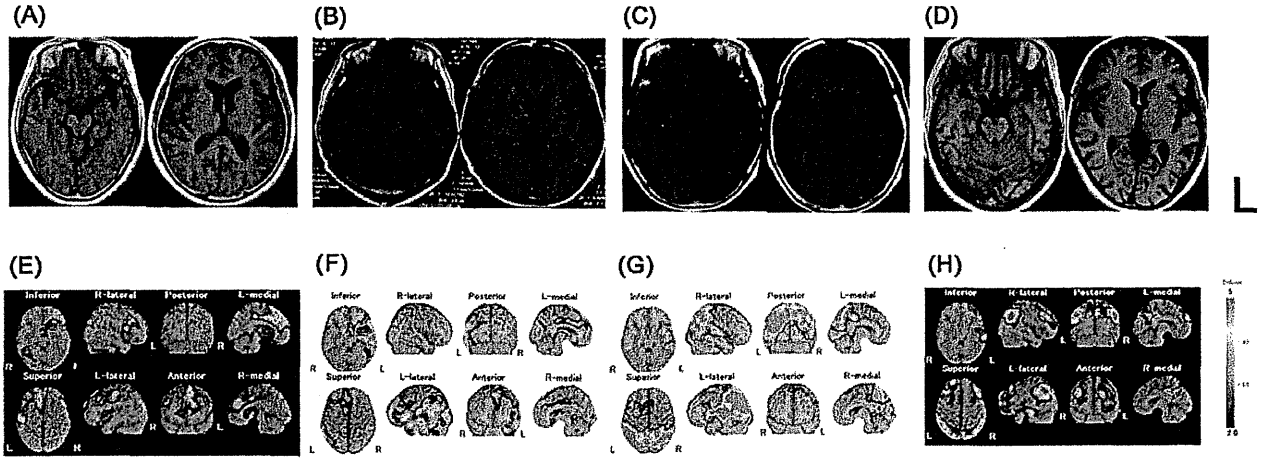

Figure 1. MRI and ^{99m}Tc ECD-SPECT.Brain MRI of PPA patients for naPPA (A), lvPPA (B), svPPA (C) and early-onset AD (D). ^{99m}Tc ECD-SPECT of patients for naPPA (E), lvPPA (F), svPPA (G) and AD (H). naPPA is characterized by predominant atrophy (A) and hypoperfusion (E) in the left posterior fronto-insular region, svPPA by predominant atrophy (B) and hypoperfusion in the left anterior temporal lobe (F), and lvPPA by predominant atrophy (C) and hypoperfusion in the left posterior perisylvian or parietal region (G). An early-onset AD patient showed frontal and temporal lobes atrophy (D), and hypoperfusion in the frontal lobes and parietal lobes cortices and the posterior cingulate (H).

of these lesions. Positive ^{11}C PiB binding indicated that the visible cortical ^{11}C PiB accumulation was higher than that of the white matter or that the MCDVR of the cortex was larger than the cutoff index obtained at our hospital.

CSF biomarkers

Measurement of CSF Aβ1-42, Aβ1-40 and Aβ1-38

CSF was obtained by a lumbar puncture in the L3/L4 or L4/L5 intervertebral space. CSF samples were centrifuged for 10 min at 1800 *g* at 4 °C within 3 h of collection. Samples were divided into aliquots of 0.5 mL in polypropylene tubes and stored at −80 °C until analysis using an ELISA kit for human CSF Aβ1-40 (Wako Pure Chemical Industries, Tokyo, Japan), human CSF Aβ1-42 (Wako Pure Chemical Industries) and human CSF Aβ1-38 (IBL, Gunma, Japan) [25,26].

Measurement of CSF phosphorylated tau 181

Measurement of ptau-181 in CSF was performed by sandwich ELISA (Innogenetics, Ghent, Belgium) as described elsewhere [27].

Genetic analysis of apolipoprotein E

After obtaining informed consent for genetic testing, we purified genomic DNA from lymphocytes in the peripheral blood of affected subjects. For the analysis of apolipoprotein E genotype, purified genomic DNA was examined as previously described [28].

Results

Mini-mental state examination

Scores (full score 30: mean ± S.D.) of mini-mental state examination (MMSE) were 17.20 ± 7.47 in naPPA patients, 6.75 ± 5.56 in svPPA patients, 15.70 ± 4.92 in lvPPA patients and 18.44 ± 4.74 in AD patients. The MMSE score for svPPA patients was lower than those of naPPA and lvPPA patients ($p < 0.0001$, respectively (Table 1).

Standard Language Test of Aphasia

Scores for "Naming" (% correct: mean ± S.D.) from the Standard Language Test of Aphasia (SLTA) were 39.00 ± 19.26 in naPPA patients, 16.25 ± 4.79 in svPPA patients and 59.00 ± 21.58 in lvPPA patients. Scores for "Single-word repetition" (% correct) from the SLTA were 76.00 ± 18.38 in naPPA patients, 75.00 ± 19.15 in svPPA patients and 75.00 ± 23.21 in lvPPA patients. Scores for "Sentence repetition" (% correct) from the SLTA were 30.0 ± 17.00 in naPPA patients, 40.00 ± 43.20 in svPPA patients and 32.00 ± 19.32 in lvPPA patients. Scores for "Auditory single-word comprehension" (% correct) from the SLTA were 76.00 ± 22.71 in naPPA patients, 42.50 ± 38.62 in svPPA patients and 77.00 ± 22.14 in lvPPA patients. Scores for "Auditory sentence comprehension command" (% correct) from the SLTA were 66.00 ± 28.75 in naPPA patients, 15.00 ± 10.00 in svPPA patients and 58.00 ± 30.48 in lvPPA patients. The scores for "Naming" and "Single-word comprehension" in svPPA patients were significantly lower than those of naPPA and lvPPA patients (*$p < 0.001$, **$p < 0.0001$, Mann–Whitney test, Table 1), while the scores for "Auditory single-word comprehension" and "Auditory sentence comprehension command" in svPPA patients were significantly lower than those of naPPA and lvPPA patients (**$p < 0.0001$, Mann–Whitney test, Table 1). The scores for "Calculation" in lvPPA patients were significantly lower than those for naPPA and svPPA patients (**$p < 0.0001$, Mann–Whitney test, Table 1).

Neuroimaging (MRI, ^{99m}Tc ECD-SPECT, FDG-PET and ^{11}C PiB-PET)

The 24 PPA patients were clinically subclassified into 10 naPPA patients, 4 svPPA patients and 10 lvPPA patients according to the Consensus classification of PPA [10]. All the

Table 1. Summary of clinical features, MMSE and SLTA for the 24 PPA patients.

	naPPA ($N=10$)	svPPA ($N=4$)	lvPPA ($N=10$)
Clinical information			
Age of onset (year)	63.50 ± 5.06	62.00 ± 0.82	64.70 ± 4.97
Disease duration (years)	5.60 ± 1.78	4.00 ± 1.83	4.00 ± 1.16
Male gender (%)	50	100	50
Education (years)	11.70 ± 0.95	13.00 ± 2.00	12.10 ± 1.66
MMSE	17.20 ± 7.47	6.75 ± 5.56**	15.70 ± 4.92
SLTA			
Naming (% correct)	39.00 ± 19.26*	16.25 ± 4.79**	59.00 ± 21.58
Single-word repetition (% correct)	76.00 ± 18.38	75.00 ± 19.15	75.00 ± 23.21
Sentence repetition (% correct)	30.00 ± 17.00	40.00 ± 43.20	32.00 ± 19.32
Auditory single-word comprehension (% correct)	76.00 ± 22.71	42.50 ± 38.62**	77.00 ± 22.14
Auditory sentence comprehension (% correct)	66.00 ± 28.75	15.00 ± 10.00**	58.00 ± 30.48
Calculation (% correct)	41.00 ± 27.67	40.00 ± 46.19	28.50 ± 27.79**

Figures indicate means ± SD or number with percentages in parentheses. MMSE = mini-mental state examination; SLTA = standard language test of aphasia. Asterisks denote significantly impaired at $*p<0.001$ and $**p<0.0001$ (Mann–Whitney test).

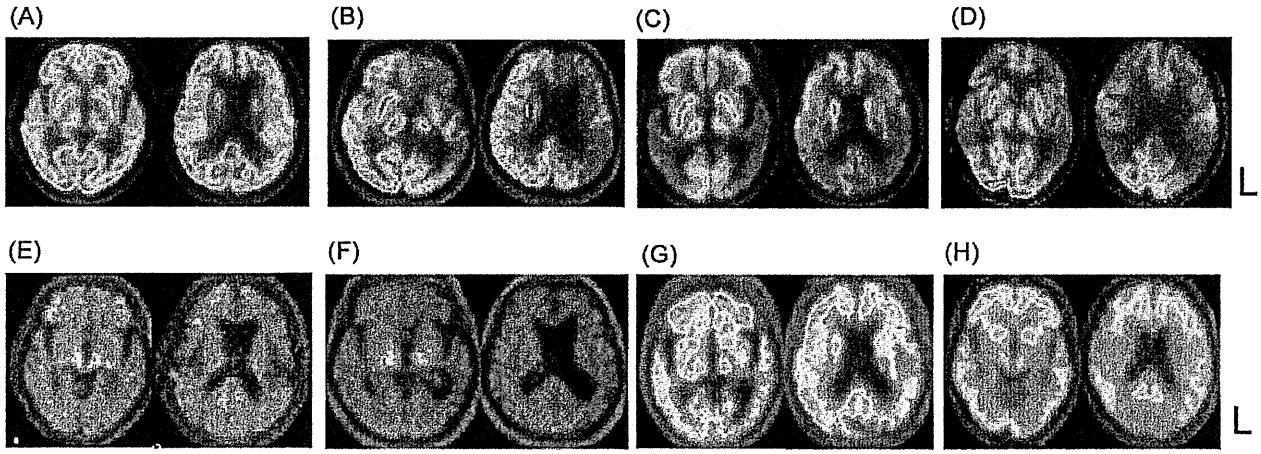

Figure 2. FDG-PET and ^{11}C PiB-PET. (A) In naPPA patients, FDG-PET analysis showed glucose hypometabolism in the left posterior fronto-insular region. (B) ^{11}C PiB-PET showed no abnormal signal lesion in the brain of naPPA patients. (C) In svPPA patients, FDG-PET showed glucose hypometabolism in the left anterior temporal lobe, while no PiB positive signal finding in cerebral cortices (D). (E) While lvPPA patients showed glucose hypometabolism in the left anterior temporal lobe by FDG-PET, ^{11}C PiB-PET showed abnormal high PiB signal findings in cerebral cortices of frontal lobes and temporal lobes and the posterior cingulate (F). (G) An early-onset AD patient showed glucose hypometabolism in bilateral frontal and temporal lobes, presenting abnormal PiB positive signal findings in cerebral cortices and the posterior cingulate in ^{11}C PiB-PET (H).

10 naPPA patients showed brain atrophy in the left posterior fronto-insular region by MRI (Figure 1A: a naPPA case). All the 10 naPPA patients showed hypoperfusion in the left posterior fronto-insular region by ^{99m}Tc ECD-SPECT (Figure 1E: a naPPA case). All the 4 svPPA patients showed atrophy in the left anterior temporal lobe by MRI or CT (Figure 1B: a svPPA case), and ^{99m}Tc ECD-SPECT showed hypoperfusion in the left anterior temporal lobe (Figure 1F: a svPPA case). All the 10 lvPPA patients showed brain atrophy in the left posterior perisylvian and parietal region by MRI or CT (Figure 1C: an lvPPA case) and hypoperfusion in the corresponding lesions by ^{99m}Tc ECD-SPECT (Figure 1G: an lvPPA case). An early-onset AD patient showed bilateral atrophy in the temporal and parietal lobes (Figure 1D), with bilateral hypoperfusion in the temporal and parietal lobes (Figure 1H).

All 7 naPPA patients showed glucose hypometabolism in the left posterior fronto-insular region by FDG-PET (Figure 2A). All 7 naPPA patients showed no abnormal signal lesion by ^{11}C PiB-PET (Figure 2E). All 4 svPPA patients showed glucose hypometabolism in the left anterior temporal lobe by FDG-PET (Figure 2B), while no PiB positive signal was found in the cerebral cortices (Figure 2 F). All 6 lvPPA patients showed glucose hypometabolism in the left anterior temporal lobe by FDG-PET (Figure 2C), and by ^{11}C PiB-PET showed PiB positive signal findings corresponding to Aβ accumulation bilaterally in the cerebral cortices (Figure 2G). By FDG-PET, an early-onset AD patient showed bilateral glucose hypometabolism in the frontal and temporal lobes (Figure 2D), and by ^{11}C PiB-PET presented bilateral PiB positive signal findings in the cerebral cortices of the frontal and temporal lobes, and also in the posterior cingulate (Figure 2H).

Comparative analysis of CSF data

The lvPPA patients showed lower levels of CSF Aβ1-42 and higher levels of CSF ptau-181 than ND. The CSF levels of ptau-181 (mean ± SD) were 33.59 ± 16.09 for naPPA ($N=10$), 42.24 ± 21.26 for svPPA ($N=4$), and

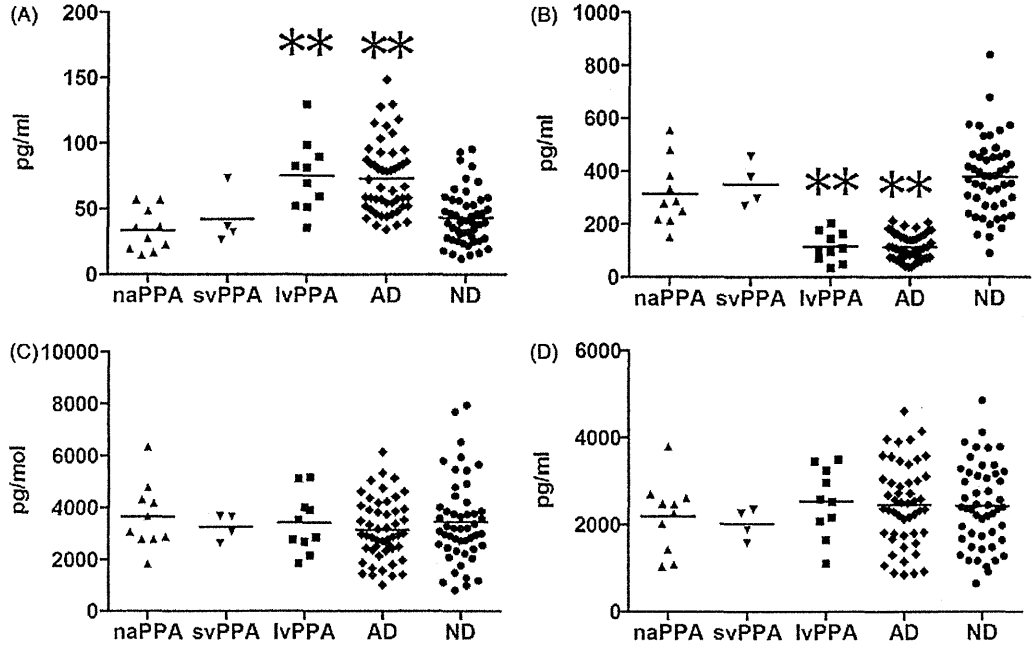

Figure 3. CSF levels of ptau-181, Aβ1-42, Aβ1-40 and Aβ1-38. (A) CSF levels of ptau-181 of lvPPA and AD showed significant increases compared to those of naPPA, svPPA and ND (**$p<0.0001$). (B) CSF levels of Aβ1-42 of lvPPA and AD showed significant decreases compared to those of naPPA, svPPA and ND (**$p<0.0001$). (C) CSF levels of Aβ1-40 were not significantly different amongst naPPA, svPPA, lvPPA and ND. (D) CSF levels of Aβ1-38 were not significantly different amongst naPPA, svPPA, lvPPA and ND. Bar in the Figure shows average data. Asterisks denote significantly impaired (**$p<0.0001$, Mann–Whitney test).

75.38 ± 27.32 for lvPPA ($N=10$), 73.14 ± 27.26 for AD ($N=50$) and 43.19 ± 20.49 for non-demented subjects ($N=50$) (Figure 3A). The CSF levels of ptau-181 for lvPPA and AD were significantly higher than those for naPPA and svPPA (Figure 3A). No abnormal findings for naPPA and svPPA patients were observed in the CSF levels of Aβ1-42, Aβ1-40, Aβ1-38 or ptau-181. In the CSF levels of Aβ1-42, average scores were 314.42 ± 125.83 for naPPA, 351.35 ± 84.21 for svPPA, 115.98 ± 56.46 for lvPPA, 113.82 ± 48.84 for AD and 379.25 ± 144.45 for ND (Figure 3B). The CSF levels of Aβ1-42 for lvPPA and AD were significantly lower than those for naPPA, svPPA and ND (Figure 3B). In the CSF levels of Aβ1-40, average scores were 3647.09 ± 1293.76 for naPPA, 3248.58 ± 504.53 for svPPA, and 3401.29 ± 1151.24 for lvPPA, 3126.24 ± 1185.32 for AD and 3439.24 ± 1611.39 for non-demented subjects (Figure 3C). In the CSF levels of Aβ1-38, average scores were 2190.12 ± 839.47 for naPPA, 2023.82 ± 356.92 for svPPA, and 2535.66 ± 790.99 for lvPPA, 2464.03 ± 946.80 for AD and 2435.37 ± 950.67 for non-demented subjects (Figure 3D). In either CSF levels of Aβ1-38 or Aβ1-40, no significant difference was observed amongst naPPA, svPPA and lvPPA patients (Figure 3C and D).

Ratios of CSF Aβ molecules (Aβ1-42, Aβ1-40 and Aβ1-38) and ptau-181

The ratio of Aβ1-42/Aβ1-40 (mean ± S.D.) was 0.09 ± 0.04 for naPPA, 0.11 ± 0.02 for svPPA, 0.04 ± 0.02 for lvPPA, 0.05 ± 0.04 for AD and 0.14 ± 0.11 for ND. The ratios of Aβ1-42/Aβ1-40 for lvPPA and AD were significantly lower than those for naPPA, svPPA and ND (**$p<0.0001$, respectively, Figure 4A). The ratio of Aβ1-42/Aβ1-38 was 0.16 ± 0.06 for naPPA, 0.17 ± 0.01 for svPPA, 0.05 ± 0.01 for lvPPA, 0.06 ± 0.04 for AD and 6.92 ± 3.37 for ND. The ratios of Aβ1-42/Aβ1-38 for lvPPA and AD were lower than those of those for naPPA, svPPA and ND (**$p<0.0001$, respectively, Figure 4B). The ratio of Aβ1-38/Aβ1-40 was 0.641 ± 0.273 naPPA, 0.64 ± 0.12 for svPPA, 0.81 ± 0.34 for lvPPA, 0.94 ± 0.62 for AD and 0.95 ± 0.92 for ND. No significant difference was observed among these ratios for naPPA, svPPA, lvPPA, AD and ND (data not shown). The ratio of ptau-181/Aβ1-42 was 0.12 ± 0.07 for naPPA, 0.12 ± 0.03 for svPPA, 0.83 ± 0.50 for lvPPA, 0.79 ± 0.54 for AD and 0.14 ± 0.13 for ND. The results of ptau-181/Aβ1-42 for lvPPA and AD were significantly higher than those for naPPA, svPPA and ND (**$p<0.0001$, respectively, Figure 4C). The ratio of ptau-181/Aβ1-38 was 0.02 ± 0.01 for naPPA, 0.02 ± 0.01 for svPPA, 0.03 ± 0.02 for lvPPA, 0.04 ± 0.03 for AD and 0.02 ± 0.01 for ND. The results of ptau-181/Aβ1-38 for lvPPA and AD were significantly higher than those for naPPA, svPPA and ND (**$p<0.0001$, respectively, Figure 4D). The results of Aβ1-42/Aβ1-40, Aβ1-42/Aβ1-38, ptau-181/Aβ1-42 and ptau-181/Aβ1-38 for AD and lvPPA were quite similar to those for EOSAD/LOSAD and ND in previous study [26].

Apolipoprotein E genotypes

The apoE ε4 allele frequency in the patient groups was 0.05 in naPPA, 0 in svPPA and 0.40 in lvPPA. In this study, the frequency of the ApoE ε4 allele in lvPPA is quite similar to

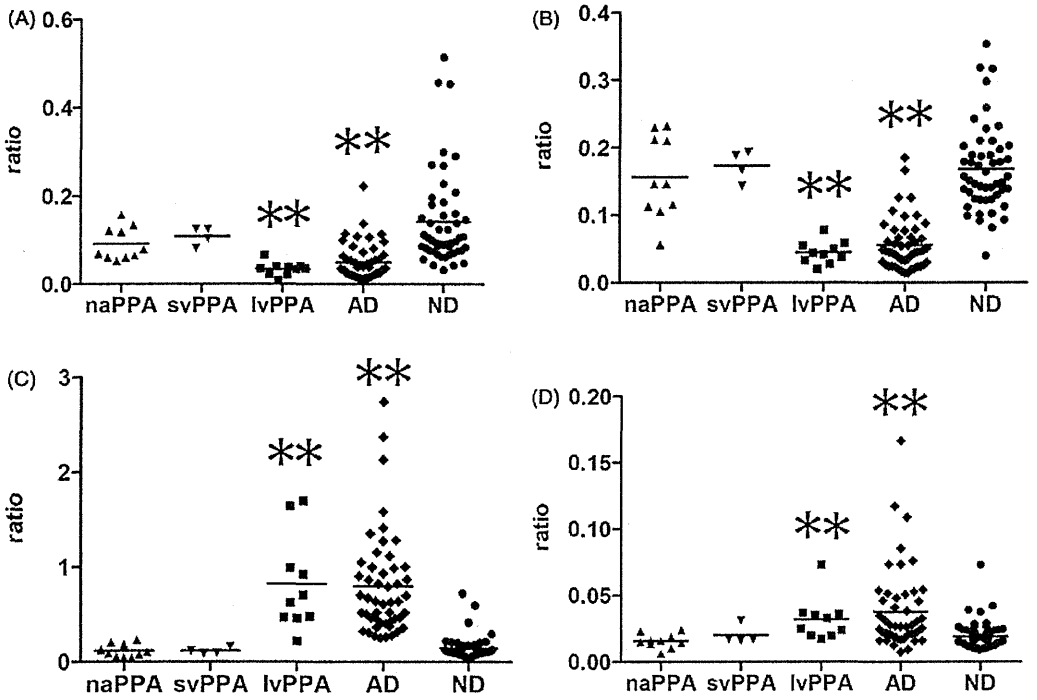

Figure 4. Ratios of CSF Aβ1-42/Aβ1-40, Aβ1-42/Aβ1-38, ptau-181/Aβ1-42 and ptau-181/Aβ1-38. (A) The ratio of Aβ1-42/Aβ1-40 in lvPPA and AD was significantly decreased compared to those of naPPA, svPPA and ND (**$p < 0.0001$). (B) The ratio of Aβ1-42/Aβ1-38 in lvPPA and AD was significantly decreased compared to those naPPA, svPPA and ND (**$p < 0.0001$). (C) The ratio of ptau-181/Aβ1-42 in lvPPA and AD was significantly increased compared to those naPPA, svPPA and ND (**$p < 0.0001$). (D) The ratio of ptau-181/Aβ1-38 in lvPPA and AD was significantly increased in lvPPA and AD compared to those naPPA, svPPA and ND (**$p < 0.0001$). The bar in Figure shows average data. Asterisks denote significantly impaired at **$p < 0.0001$ (Mann–Whitney test).

the frequency of the ApoE ε4 allele in AD patients in Japan [29,30]; however, it appears lower than a previous publication [6].

Discussion

Our study of PPA is based on the analysis of 24 patients diagnosed with a primary progressive language disorder. For the PPA patients in our study, there were clear clinical features, in which, SLTA scores of "Naming", "Word repetition", "Sentence repetition", "Auditory single-word comprehension" and "Auditory sentence comprehension commands" in svPPA were significantly less than those of naPPA and lvPPA. Relatively early age of onset, disease duration and education years were similar among the three clinical variants of PPA. These findings were compatible to those from a previous publication [6,9].

In our study, the MRI of PPA patients generally revealed left-sided dominant brain atrophy, with left posterior frontal lobe and insular atrophy for naPPA, left anterior temporal lobe atrophy for svPPA, and left temporal lobe and perisylvian region atrophy for lvPPA. ^{99m}Tc ECD-SPECT presented hypoperfusion in the left posterior frontal lobe/perisylvian region for naPPA, hypoperfusion in the left anterior temporal lobe for svPPA, and hypoperfusion in the left posterior perisylvian region/parietal lobe for lvPPA. In contrast, an early-onset AD patient showed bilateral hypoperfusion in the posterior cingulate to parietal lobe and frontal lobe. FDG-PET also showed hypometabolism in the left posterior frontal lobe for naPPA, left temporo-anterior lobe for svPPA, and left temporo-parietal lobe and the posterior cingulate for lvPPA. In contrast, an early-onset AD patient showed hypoperfusion/hypometabolism bilaterally in the frontal and temporal lobes and the posterior cingulate to parietal lobe. Our ^{11}C PiB-PET study showed PiB positive findings in the fronto-temporal cortices bilaterally and the posterior cingulate for lvPPA and AD, and PiB negative findings for naPPA and svPPA.

The results of AD-CSF biomarkers for the lvPPA patients were quite similar to those for AD patients, presenting significantly higher frequency of the ApoE ε4 allele in lvPPA patients than in naPPA and svPPA patients. Recently, the level of CSF Aβ1-38 for FTD patients was reported to be significantly lower compared to the other diagnostic groups of PPA patients (not classified clinical variants), AD and ND [14]. The AD-CSF markers are reported to be closely correlated to those of the lvPPA patients, while not correlated to those of naPPA and svPPA patients [15].

In our study, we observed no differences between the three PPA variants and AD in the levels of CSF Aβ1-38 and CSF Aβ1-40. Additionally, we have confirmed lower levels of CSF Aβ1-42 and higher levels of CSF ptau-181 and a higher ratio of ptau-181/Aβ1-42 for AD and lvPPA than those for the other two clinical variants (naPPA and svPPA) and ND, as previous reports [8,15,31].

Furthermore, we revealed that lvPPA patients showed significantly lower ratios of Aβ1-42/Aβ1-40 and Aβ1-42/Aβ1-38, whereas the ratios of ptau-181/Aβ1-42 and ptau-181/Aβ1-38 were significantly higher than those of naPPA, svPPA and ND. We observed neither higher levels of

CSF Aβ1-38 for lvPPA nor AD compared to naPPA, svPPA and ND. With a higher frequency of the ApoE ε4 allele in lvPPA, these patients might share a common pathological mechanism of Alzheimer's disease in biochemical pathways and pathology [31,32].

With the results of CSF and neuroimages including ^{11}C PiB-PET, we could diagnose lvPPA for AD and other variants of PPA more exactly. Our findings are the first report in Japan including Asian ethnics whose language structure differs from Western languages; which may support a common pathogenicity worldwide. The lvPPA might have a different pathogenesis from other two variants of PPA and may be a variant of AD in most cases from points of patho-biochemical findings [33]. Migliaccio et al. reported that lvPPA and posterior cortical atrophy (PCA) showed overlapping anatomic and biologic features with early age at onset of Alzheimer's disease [34]. Magnin et al. described that lvPPA is frequently found in PCA and may be associated with poor performance on verbal neuropsychological tasks, especially verbal memory [35]. They suggest that these clinical syndromes represent the spectrum of clinical manifestation of the non-typical form of AD that presents at early age.

Very recently, the use of PBB3 for tau PET study was developed, which enables detection of tau accumulation in mutant tau transgenic mice and AD patients [36]. By detecting the distribution and accumulation of Aβ and tau with ^{11}C PiB-PET and ^{11}C PBB3-PET for PPA patients, we could describe in more detail the correlation of both accumulation of Aβ and tau in brains of PPA patients, and better understand the pathogenesis of speech and dementia.

Conclusions

There were clear clinical features and neuroimaging findings in naPPA, svPPA and lvPPA, as well as changes in AD-CSF biochemical markers (decrease of Aβ1-42 and increase of ptau-181) in lvPPA as well as AD. In our studies, lvPPA showed a higher ratios of ptau-181/Aβ1-42, ptau-181/Aβ1-38 and a higher frequency of the ApoE ε4 allele as compared to naPPA and svPPA; these findings, accompanying the results of neuroimaging including ^{11}C PiB-PET, demonstrate a common mechanism of AD and lvPPA.

Declaration of interest

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Short Communication

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

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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: $p_{allele}=6.71E\text{-}171$ with χ^2 test (χ^2 value = 783.7, degree of freedom = 2), and $OR_{\varepsilon4/\varepsilon3}$ (95% confidence interval [CI]) = 4.81 (4.26–5.42) and $OR_{\varepsilon2/\varepsilon3}$ (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.

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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).

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SUPPLEMENTARY MATERIAL

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

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