tical tools (visual-statistical), and automated quantitative analysis, but the relationship between the latter 2 of these approaches has been little explored, to our knowledge. Visual interpretation features comprehensive and flexible assessment of the qualitative radioactivity distribution by the reader, who may look into all features across the brain. This approach appears effective because patients with AD typically present with characteristic temporoparietal hypometabolism known as the "AD pattern." However, inter-rater variability inevitably occurs because each rater has his or her own experience and criteria, especially for borderline cases, and this variability can potentially be increased or decreased when the reader also takes into account statistical information provided by various software display tools.

On the other hand, quantitative analysis traditionally extracts radioactivity uptake values of the region of interest, placement of which is a subjective matter requiring experience. Although a recently developed anatomic standardization technique can define ROIs automatically and further allows voxelwise statistical analysis to generate *Z*-maps, standardization may not always be accurate and may require adjustment by a human observer. Although these region-of-interest values can be processed into a numeric indicator such as an FDG-PET score[4,5] and a cutoff level can be determined, a single indicator may not be as accurate as complex and comprehensive evaluation by expert readers. As a result, a "combined" approach of visual and quantitative evaluation is often used during image interpretation, in which the readers examine both the tomographic PET images and the result of region-of-interest analysis and/or a *Z*-map.

Inter-rater variability and comparison between visual reading and software-based evaluation have been studied by some investigators on brain ^{18}F-FDG-PET. Ng et al[6] studied the inter-rater variability of 15 patients with AD and 25 cognitively normal subjects (NCs) and reported that visual agreement between 2 readers was good ($\kappa = 0.56$). Tolboom et al[7] studied the variability of 20 patients with AD and 20 NCs and reported that agreement between 2 readers was moderate ($\kappa = 0.56$). Rabinovici et al[8] also reported the inter-rater agreement of ^{18}F-FDG ($\kappa = 0.72$). However, the data of these preceding studies were acquired with a single scanner in a single site and were evaluated by the readers belonging to the institution who were used to the scanner and its image quality. In addition, the studied subjects did not include patients with MCI, in whom PET findings featuring AD, if any, are mild and may make the discrimination challenging. Furthermore, inter-rater variability for combined interpretation of visual and statistical analysis has never been reported, to our knowledge.

In the present study, we analyzed the baseline scans of ^{18}F-FDG in a multicenter clinical project named Japanese Alzheimer's Disease Neuroimaging Initiative (J-ADNI)[9] and evaluated the inter-rater variability among 3 independent expert raters who were blinded to the clinical information and interpreted the PET images to evaluate the characteristic AD pattern in ^{18}F-FDG-PET on the basis of a combined visual-statistical evaluation. The raters looked at the 3D stereotactic surface projection *Z*-map of ^{18}F-FDG-PET visually as well as the ^{18}F-FDG tomographic images because it is considered the standard means of human interpretation of ^{18}F-FDG-PET images in Japan and therefore was adopted as the official interpretation method in J-ADNI. Images were also assessed by automated quantitative analysis by using an FDG-PET score, which was derived from ADtsum,[4,5] and were compared with the visual-statistical rating by the 3 raters and with their consensus.

MATERIALS AND METHODS

Subjects

Data used in the present study were obtained from J-ADNI.[9] This project was approved by the ethics committee of each site in which J-ADNI data were acquired, and written informed consent was obtained from each subject before participating in J-ADNI. All subjects were native Japanese speakers, 60–84 years of age, and were registered as 1 of 3 clinical groups (mild AD, MCI, or NC). Subjects of the mild AD group scored 20–26 in Mini-Mental State Examination-Japanese and 0.5–1.0 in the Clinical Dementia Rating-Japanese and were compatible with the probable AD criteria in the National Institute of Neurologic and Communicative Disorders and Stroke and the Alzheimer Disease and Related Disorders Association.[10] Subjects of the MCI group scored 24–30 in the Mini-Mental State Examination-Japanese and 0.5 in the Clinical Dementia Rating-Japanese. Subjects of NC group scored 24–30 in the Mini-Mental State Examination-Japanese and 0 in the Clinical Dementia Rating-Japanese. The exclusion criteria were depression (Geriatric Depression Scale-Japan $\geq$ 6), cerebrovascular disorders (Hachinski Ischemic Score $\geq$ 5), and other neurologic or psychiatric disorders.

Enrollment in each clinical group for J-ADNI was primarily determined by the referring physician, and 303 consecutive subjects entered the study to undergo ^{18}F-FDG-PET scanning. A thorough central review of the clinical and behavioral data by expert psychiatrists and psychologists excluded 29 cases that had erroneous assessment of the cognitive test results, depression or cerebrovascular disorders that had been overlooked, prohibited concomitant medications, or other deviations from the criteria. As a result, 274 baseline ^{18}F-FDG-PET scans (67 mild AD, 100 MCI, and 107 NC) were analyzed in the present study.

PET Imaging

As a quality assurance measure necessary for the multicenter study, all PET sites in J-ADNI were qualified for the PET scanner and other devices, resting-state environment, quality of the on-site-produced PET drugs, and so forth before scanning of the first subject. Intersite differences were minimized by standardizing the imaging protocol, and interscanner differences were addressed with the Hoffmann 3D phantom data.[11] The data used for the analysis in the present study were acquired with 14 types of PET or PET/CT scanners in 23 PET centers.

In the ^{18}F-FDG-PET scans, all subjects fasted for at least 4 hours and their preinjection blood glucose levels were confirmed to be <180 mg/dL. Intravenous administration of ^{18}F-FDG (185 $\pm$ 37 MBq) was followed by a resting period of 30 minutes in a dimly lit and quiet room. Dynamic scans (300 seconds $\times$ 6 frames) were obtained starting 30 minutes postinjection in the 3D mode. Attenuation was corrected for by a transmission scan with segmentation for dedicated PET and by a CT scan for PET/CT.

All the PET images acquired in each PET site went through the J-ADNI PET quality control process,[11] in which head motion between frames was corrected for and bad frames were removed to create sum frame images. Then the images were reoriented to the anterior/posterior commissure line with the same matrix size and

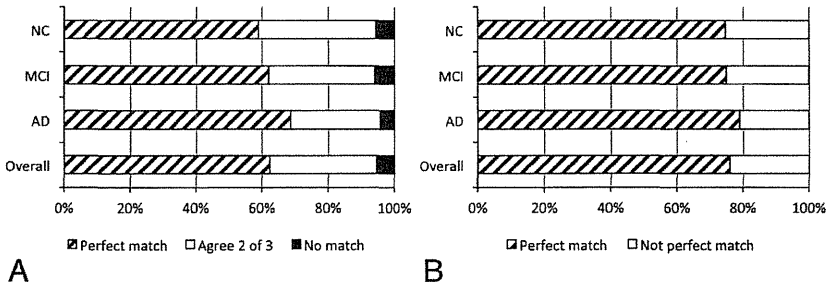

FIG 1. Breakdown of the ^{18}F-FDG-PET cases into degree of match by 3 raters in a combined visual-statistical human classification into 7 (FDG-7) (*A*) or 2 (FDG-2) (*B*) categories. A perfect match by the 3 raters is observed for 62% of the cases for FDG-7 and 76% for FDG-2 in total. The AD group shows the highest concordance followed by the MCI and NC groups, in this order, both for FDG-7 and FDG-2.

voxel size so that all camera models presented images of similar orientation and appearance to the viewer and were then passed on to image interpretation.

The ^{18}F-FDG-PET images that had passed through the quality control process above were also treated with a 3D stereotactic surface projection technique to generate *z* score maps (displayed with upper = 7 and lower = 0) by using iSSP software, Version 3.5 (Nihon Medi-physics, Tokyo, Japan). The normal data base used for generating the *Z*-maps was made by a method of leave-one-out cross-validation based on 25 healthy subjects of J-ADNI (11 men and 14 women; mean age, 66.0 ± 4.8 years) who were interpreted as having a normal pattern by one of the coauthors of the study. The *Z*-maps were used not for the automated quantification but for a part of the information for human raters in the visual-statistical interpretation.

Human Interpretation

Those ^{18}F-FDG images generated through the quality control process above were independently interpreted with the combined visual-statistical method by 3 expert raters blinded to the clinical group and other clinical and laboratory data. The raters were provided with the ^{18}F-FDG tomographic images on the viewer as well as the *Z*-map images in PDF format. Information about the age and sex was also provided to the raters. Moreover, T1-weighted MR images acquired in 3D mode by using MPRAGE or its equivalent and reformatted in axial sections were also provided together with axial T2WI and proton-attenuation images, in which the MR imaging sections did not correspond to the PET section positions. The experience of the 3 raters as physicians specializing in nuclear neuroimaging was 17, 19, and 19 years, respectively, when this project started.

After independent interpretation, consensus reads were performed by the 3 raters and 2 other discussants who are experienced nuclear medicine physicians specialized in neuroimaging. The experience of both discussants as physicians specializing in nuclear neuroimaging was 20 years. The same images and information as that in the independent interpretation were also provided for the discussants in the consensus reads. The 7 sessions of consensus reads lasted for 1.5 years in the order of subject enrollment in J-ADNI. In the consensus reads, the cases in which the evaluations by the 3 raters did not completely match were discussed, and the unified visual-statistical interpretation was determined as an official judgment by the J-ADNI PET Core.

For classification of ^{18}F-FDG-PET, the criteria of Silverman et al[1] were adopted for classifying the uptake pattern in J-ADNI. All 3 expert raters and the 2 discussants had attended a training course for the criteria organized by Silverman et al before starting the J-ADNI project. In the criteria of Silverman et al, ^{18}F-FDG uptake patterns were classified into 7 categories: progressive patterns: P1, P1+, P2, and P3, in which P1 represents the characteristic AD pattern and P1+ represents AD-variant pattern, including the characteristic Lewy body dementia pattern; and nonprogressive patterns: N1, N2 and N3, in which N1 represents the characteristic normal pattern. In addition to these original 7 categories (FDG-7), the present study defined a binary criteria (FDG-2) in which the 7 categories were dichotomized into posterior-predominant hypometabolism (AD and AD-variant) patterns (P1, P1+) and the other patterns (N1, N2, N3, P2, and P3).

Automated Quantitative Evaluation

In the automated quantitative analysis, the FDG-PET score, as a measure of the AD pattern, was calculated from ADtsum[4] by using the Alzheimer's Discrimination Tool in PMOD, Version 3.12 (PMOD Technologies, Zurich, Switzerland)[4,5] by using the following equation: FDG-PET score = log2 {(ADtsum / 11,089) + 1}. The FDG-PET score was not calculated in 1 case because no significant clusters were determined for the image.[4] This case was excluded from the quantitative analysis.

Statistical Analysis

Concordance among the 3 raters was evaluated by Cohen κ statistics. As comparisons between human and automated evaluation, the association between the FDG-PET score and the number of the raters who interpreted the case as P1 (AD pattern) in FDG-7 was evaluated by the Spearman rank correlation coefficient. Likewise, association between the FDG-PET score and the number of the raters who interpreted the case as N1 (normal pattern) was evaluated. The association was also examined between the FDG-PET score and the number of raters in FDG-2 classification (ie, how many raters judged the case as the AD and AD-variant patterns [P1, P1+] versus the other patterns [N1, N2, N3, P2, and P3]). A *P* value < .05 was considered significant. In addition, the FDG-PET score was compared with the final combined visual-statistical interpretation determined by the consensus read and with the clinical group. Receiver operating characteristic analysis was used to obtain the optimum cutoff level for the quantitative index for discrimination.

Neither iSSP nor the PMOD Alzheimer's Discrimination Tool was approved for clinical use by the US Food and Drug Administration.

RESULTS

Figure 1 summarizes concordance rates among the 3 raters. Agreement among the 7 visual-statistical categories by at least 2 of the 3 readers occurred in >94% of cases for all groups: NC, MCI,

and AD. The κ statistic $\pm$ SE for each pair of the 3 raters was 0.59 $\pm$ 0.04, 0.54 $\pm$ 0.04, and 0.58 $\pm$ 0.04 in FDG-7 (average, 0.57), and 0.73 $\pm$ 0.04, 0.65 $\pm$ 0.0, and 0.64 $\pm$ 0.05 in FDG-2 (average, 0.67), respectively.

Figure 2 illustrates the relationship between the FDG-PET score and the number of raters who visually-statistically interpreted the ^{18}F-FDG-PET image as P1 (Fig 2*A*) and N1 (Fig 2*B*). A significant positive association was observed between the FDG-PET score and the number of P1 interpretations ($\rho = 0.59$, $P < .0001$). The mean FDG-PET score was 0.46 $\pm$ 0.37 ($n = 103$) for the scans no raters interpreted as P1, but it increased to 0.723 $\pm$ 0.39 ($n = 34$) for those that 1 rater interpreted as P1, to 0.99 $\pm$ 0.45 ($n = 31$) for 2 raters, and to 1.21 $\pm$ 0.73 ($n = 105$) for all 3 raters. Likewise, a significant negative association was observed between the FDG-PET score and the number of N1 interpretations ($\rho = -.64$, $P < .0001$). The FDG-PET score was 1.15 $\pm$ 0.69 ($n = 146$) for the scans no raters interpreted as N1, but it decreased to 0.80 $\pm$ 0.39 ($n = 28$) for those 1 rater interpreted as N1, 0.50 $\pm$ 0.25 ($n = 40$) for 2 raters, and 0.34 $\pm$ 0.22 ($n = 59$) for all 3 raters. A similar association was observed between the FDG-PET score and the number of raters who interpreted the case as AD and AD-variant patterns, including the Lewy body dementia pattern (P1, P1+) or the other patterns (N1, N2, N3, P2, and P3); and both showed significant positive and negative associations ($\rho = 0.60$, $P < .0001$; and $\rho = -0.60$, $P < .0001$).

Figure 3 illustrates scatterplots of the FDG-PET scores as contrasted to the combined visual-statistical interpretation determined by the consensus read of ^{18}F-FDG-PET for each clinical group. For each group as well as for all subjects, cases with P1 interpretation showed higher FDG-PET scores than those with N1. Receiver operating characteristic analysis on P1 and N1 cases led to a cutoff FDG-PET score of 0.67 for discrimination between P1 and N1. As was expected, NC cases with P1 interpretation had lower FDG-PET scores than MCI and AD cases with P1 interpretation, and the ratio of the cases above-to-below the cutoff level was also lower. As for the cases with other patterns, a large fraction of the cases with N2 interpretation had FDG-PET scores above the cutoff level, though most were below 1.0. The FDG-PET scores of the cases with P1+ and P2 were variable.

DISCUSSION

Matches among 7 visual-statistical categories by at least 2 of 3 readers occurred in >94% of cases for each clinical group, and perfect matches among the 3 raters were observed for 62% of the cases for FDG-7 and 76% for FDG-2 categorization schemes in total. The mild AD group showed the highest concordance, followed by MCI and NC, in order, for both FDG-7 and FDG-2. The AD pattern in ^{18}F-FDG-PET is usually seen in the early stage of AD and is expected to predict the onset of AD.[1,12] Because most of the subjects who are clinically diagnosed as having AD may have had an established AD pattern in ^{18}F-FDG-PET, it is reasonable for these results that AD showed the highest concordance.

Based on the classification of κ values described by Landis and Koch,[13] agreements were considered to be moderate for FDG-7 and substantial for FDG-2. Inter-rater variability is one of the indices that are often used to evaluate the validity of methods of image interpretation, and it facilitates comparison with the other studies. The κ index of FDG-2 ($\kappa = 0.67$) of the present study showed values similar to those of the other studies ($\kappa = 0.56$-$.72$) evaluated by the bi-

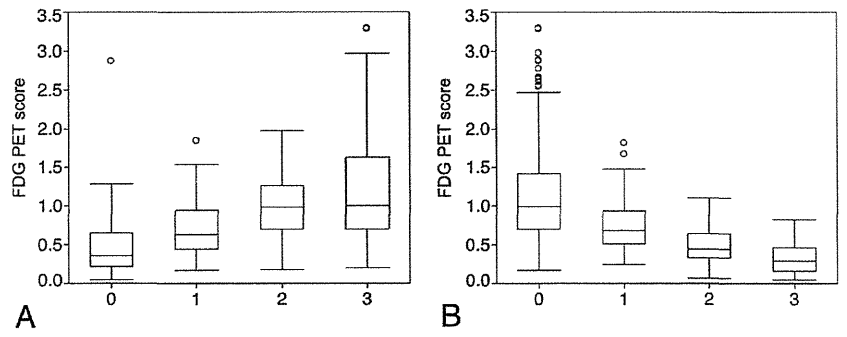

FIG 2. Boxplots of the FDG-PET score against the number of raters who interpreted the ^{18}F-FDG-PET images as P1 (*A*) and N1 (*B*) based on the FDG-7 criteria. The FDG-PET score gradually increases as the number of P1 (AD pattern) interpretations increases (Spearman rank correlation coefficient: $\rho = 0.59$, $P < .0001$). On the other hand, FDG-PET score gradually decreases as the number of N1 (normal pattern) interpretations increases ($\rho = -.64$, $P < .0001$).

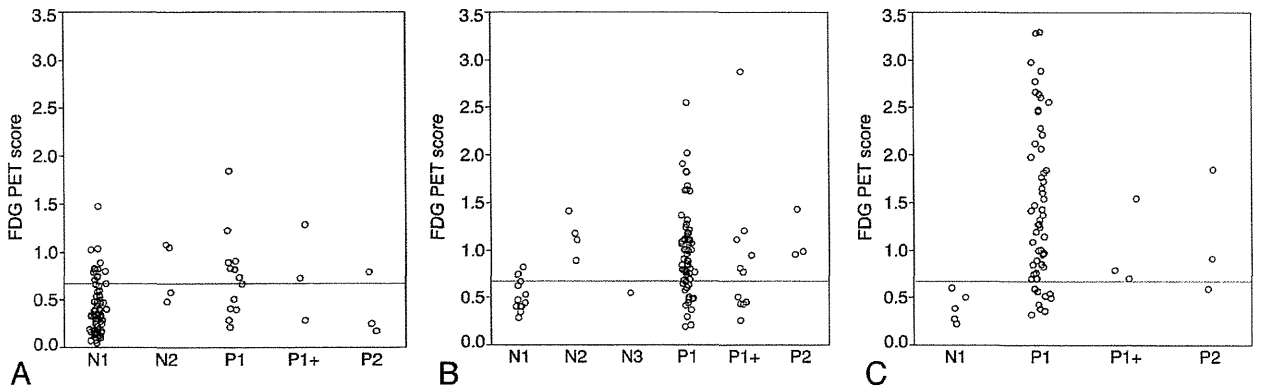

FIG 3. Scatterplot of the FDG-PET score as contrasted with the combined visual-statistical interpretation determined by the consensus read of ^{18}F-FDG-PET for each clinical group (*A*, NC; *B*, MCI; and *C*, AD). The horizontal line indicates the cutoff level of 0.67 derived by receiver operating characteristic analysis on P1 and N1 cases.

nary criteria.[6-8] However, the values observed in the other studies are not the same as those in the present study because we analyzed the interpretation both visually and statistically. Recent studies have shown that the diagnostic capability of visual analysis of ^{18}F-FDG-PET increases when the raters interpret the images in combination with 3D stereotactic surface projections.[14,15] These kinds of visual-statistical methods seem to be a standard approach in clinical settings.

To increase the concordance rate and diagnostic capability, we need to overcome some problems. We had to degrade the image quality according to the PET with the lowest quality among the 23 facilities of J-ADNI.[11] Therefore, the quality of the images may be improved in the future. In addition to the image quality, development of new methods or new approaches to image interpretation may contribute to increasing the concordance.

This study showed a relationship between combined visual-statistical interpretation and automated quantitative assessment regarding the characteristic AD pattern in brain ^{18}F-FDG-PET. Significant association was observed between the quantitative index (FDG-PET score) and the number of raters who interpreted the scans accordingly. This correlation may have been something expected from reports on similar/automated analysis.[5,6] However, this association was observed in a large-scale multicenter study by using various camera models on a wide spectrum of subjects in the present study.

From the standpoint of detecting the AD pattern, cases evaluated as having positive AD findings by complete agreement of all 3 raters tended to show a higher quantitative index than the cases that fewer than 3 raters interpreted as having positive AD findings. From the standpoint of ruling out the AD pattern, cases evaluated as having negative AD findings by complete agreement of all 3 raters also tended to show a lower quantitative index than the cases that fewer than 3 raters interpreted as having negative AD findings. Therefore, the results suggest that interpretation by 3 raters may be better than that by 2 or fewer raters. The results also indicate that cases that only 1 rater interpreted as having positive (or negative) AD findings presented a different quantitative index from those that no raters interpreted as having positive (or negative) findings. This outcome suggests that there are cases in which the "minority opinion" may not be ignored.

Generally, the minority opinion is somewhat important when a subtle but definite finding is evaluated. However, most of the ^{18}F-FDG-PET images for which the judgment did not agree among the raters showed ambiguous findings. Ng et al[6] reported that experienced raters scored higher accuracy than nonexperienced raters in the interpretation of brain ^{18}F-FDG-PET images for the diagnosis of AD.[6] Such subtle findings in brain ^{18}F-FDG-PET may be difficult to interpret. We need to analyze the difference in detail and develop new methods for interpretation or new diagnostic tools.

When the FDG-PET score of the cases judged as P1 in the consensus read were examined, NC subjects with P1 interpretation showed lower FDG-PET scores than MCI and AD subjects. This result is probably because many of the NC subjects with P1 interpretation presented with a very mild AD pattern that influenced the FDG-PET score to only a small extent. Those cases, however, presented characteristic findings such as posterior cingulate hypometabolism, which led to the P1 interpretation.

The criterion standard used in this study was the clinical diagnosis at enrollment. Although dementia with Lewy body cases with the specific symptoms were excluded from enrollment in the J-ADNI beforehand, differentiating Lewy body dementia from AD is occasionally difficult in clinical settings.[16] The typical Lewy body dementia pattern of ^{18}F-FDG-PET, evaluated as occipital hypometabolism, is classified into P1+ by the criteria of Silverman et al.[1] Some cases classified into P1+, though limited in the present study, seem to have the possibility of Lewy body dementia. Moreover, the consensus read judged 16 of 107 cases of the NC group to be the AD pattern (P1 and P1+), and 8 of 67 cases in the AD group to be a non-AD pattern (N1 and P2). These disagreements might be either caused by inappropriate clinical diagnosis at enrollment or reflecting the limitation of FDG-PET as a diagnostic tool. While these diagnostic discrepancies are not critical in the present study, which analyzed inter-rater concordance, comparison with other criterion standards such as long-term follow-up or postmortem examination is important for this kind of multicenter study in the future.

The FDG-PET score of 1.0, by definition, is proposed as an optimum threshold for the differential diagnosis of AD from healthy subjects.[5] Because the present study deals with comparison of combined visual-statistical human interpretation with automated quantitative analysis, we derived a cutoff level of 0.67 based on discrimination of the P1 from the N1 pattern. This discrepancy may be explained by the difference in the target of discrimination as well as in the profile of subjects, and the lower cutoff would be consistent with a higher sensitivity for visually detecting the AD pattern than for clinically identifying the diagnosis of AD, for which the 1.0 cutoff is designed. In addition, one of the essential factors for this discrepancy seems to be that decisions by visual-statistical interpretation are not completely consistent with the actual clinical diagnosis. Because the diagnostic capability of ^{18}F-FDG-PET is not the subject of the present study, further studies are needed to elucidate the discrepancy.

CONCLUSIONS

Inter-rater agreement was moderate to substantial regarding the combined visual-statistical human interpretation of the characteristic AD pattern in ^{18}F-FDG-PET. In addition, a significant relationship between human interpretation and automated quantitative assessment was found. The human rating as an AD or normal pattern was best predicted by the FDG-PET score when using a cutoff of 0.67.

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C-reactive protein levels and risk of disabling dementia with and without stroke in Japanese: The Circulatory Risk in Communities Study (CIRCS)

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ABSTRACT

Objective: Studies have shown that elevated high-sensitivity C-reactive protein (hs-CRP) predicts stroke, which is a risk factor for dementia. It remains, however, unclear whether hs-CRP increases risk of dementia.

Methods: A prospective nested case–control study of Japanese 40–69 years of age was conducted using frozen serum samples collected from approximately 7531men and women who participated in cardiovascular risk surveys from 1984 to 1994 in one community and 1989–1995 in another community under the Circulatory Risk in Communities Study (CIRCS). Two control subjects per case were matched by sex, age, community, and year of serum storage. The hs-CRP was measured using a latex particle-enhanced immunonephelometric assay.

Results: Between 1999 and 2013, we identified 275 disabling dementia cases (96 cases with history of stroke and 179 without it). There was a positive association between hs-CRP levels and risk of dementia with history of stroke. No significant association was observed between hs-CRP levels and risk of dementia without history of stroke. After adjustment for hypertension, diabetes and other confounding variables, the positive association remained statistically significant. The multivariable odds ratios associated with 1-SD increment of log hs-CRP were 1.02 (0.87–1.20) for total dementia, 1.35 (1.02–1.79) for dementia with history of stroke, and 0.89 (0.72–1.10) for dementia without history of stroke.

Conclusion: Elevated hs-CRP levels were associated with increased risk of disabling dementia in individuals with history of stroke but not in those without it.

1. Introduction

As a consequence of a rapidly growing elderly population, the number and proportion of individuals with dementia is dramatically expanding worldwide. In Japan, where the elderly population has been increasing faster than in other countries, it is projected that the number of elderly with dementia will increase from 30 million in 2010 to 36 million in 2020 [1]. This has led to intense efforts to identify factors that distinguish persons who are at higher or lower risk for developing dementia.

Cardiovascular diseases are the leading cause of disability and death in Japan [2,3] and Japanese populations have a higher incidence of stroke compared to western populations [4]. Patients with history of stroke have a 5 fold increased risk of developing dementia compared with patients without it [5]. Cardiovascular risk factors profiles for dementia with history of stroke and dementia

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without it may be different. The pooled results from a meta-analysis study revealed that midlife total cholesterol was positively associated with risk of dementia without history of stroke, but not dementia with it [6]. However, follow-up studies of elderly (65 years or older) found that low-density lipoprotein (LDL) cholesterol was associated with risk of dementia with history of stroke (with a relative risk range from 2.5 to 2.6) for the highest compared with the lowest quartile of LDL cholesterol but not in dementia without it [7,8]. Elevated high sensitive C-reactive protein (hs-CRP), an acute-phase reactant and a marker of systemic inflammation, has been associated with risk of stroke in western [9,10] and Japanese populations [11,12]; however, the connection between elevated serum hs-CRP levels and risk of developing dementia still remains controversial.

In the Honolulu-Asia Aging study [13], 1050 Japanese American men (mean age = 54–58 years) were followed-up for 25 years, those with elevated serum hs-CRP in midlife were associated with higher risk of developing dementia in later life (OR:2.8, 95% CI 1.6–5.1). The Rotterdam study [14] and the Conselice Study of Brain Aging [15] also found an association between elevated hs-CRP levels and increased risk of dementia. The pooled hazard ratio from meta-analysis for hs-CRP and the incidence of dementia was 1.5 (95% CI 1.1–1.9) [16]. However, in the Women's Health Study [17], hs-CRP levels were not associated with cognitive function in older women (age 60–90 years) and in the 90 + Study [18], hs-CRP was not associated with dementia in the oldest elderly individuals. Other prospective studies [19,20] also failed to find such an association. In contrast, in the Framingham Heart Study [21] elevated serum hs-CRP levels were associated with reduced risk of dementia, however, after adjusting for additional risk factors such as *APOE4* allele, the results were no longer statistically significant. To date, age-stratified analysis on the association between hs-CRP levels and risk of dementia with history of stroke and without it has not been undertaken with a large Japanese cohort.

Inflammatory process may directly or indirectly relate to dementia risk via their role in initiation of athero- and arteriolesclerotic lesions in the cerebral vascular system, and may contribute to the development of dementia. Our hypothesis is that elevated hs-CRP levels, a biomarker of inflammation, increased risk of dementia with history of stroke. We measured hs-CRP from stored serum samples of two Japanese communities of the Circulatory Risk in Communities Study (CIRCS), and examined the association between hs-CRP levels and risk of disabling dementia with history of stroke and without it.

2. Materials and methods

2.1. Subjects

The present study was an ancillary study of the CIRCS. The details of this study have been described previously [22]. Participants in the present study were recruited from all residents who participated in cardiovascular risk surveys in two communities of CIRCS. The surveyed populations comprised approximately 7531 men and women 40–69 years old who participated in the surveys between 1984 and 1994 in a mid-eastern rural community (Kyowa; $n = 5349$) and between 1989 and 1995 in northeastern rural community (Ikawa; $n = 2182$). These two cohorts have been followed up with annual cardiovascular surveys and surveillance for incidence and mortality of stroke and coronary heart disease systematically, as described elsewhere. We excluded persons aged 70 old or older at baseline, since serum hs-CRP is likely to be an indicator of advanced age rather than inflammatory process for the elderly [23–25]. Within these two cohorts, elderly persons aged ≥ 65 years with disabling dementia requiring care were identified under the national long-term care insurance program, between 1999 and 2005 in Kyowa, and between 1999 and 2013 in Ikawa. We did not have information on cognitive function prior to dementia. The mean duration of follow-up was 14 years, with 6 being the minimum and 24 the maximum. Informed consent was obtained by community leaders and by individual participants verbally, which was common practice in Japanese communities at that time. The Ethics Committees of the Osaka Center for Cancer and Cardiovascular Disease Prevention and University of Tsukuba approved the study procedures.

2.2. Long-term care insurance program for elderly aged ≥65

Details of the national long-term care insurance program for elderly aged ≥65 have been reported elsewhere [26,27]. In brief, the insurance program began in Japan from April 2000. This program was essentially an extension of the national health insurance system, and partially relied on subsidies from general revenue from the national government, prefectures and municipalities. All individuals aged ≥40 are required to pay a supplement to their health insurance, which is transferred to a long-term care fund. The payment is directly withdrawn from their monthly income, shared with the employer or deducted from their public pension. All individuals are able to receive long-term care through their resided municipalities when they turn 65 and also if they have disabling dementia and/or reduced capacity for daily living.

2.3. Case selection

The cases were selected among subjects aged ≥65 years between 1999 and 2005 in Kyowa, between 1999 and 2013 in Ikawa, who were regarded as suffering from dementia under the long-term insurance program. The dementia status was classified into six ranks (0–V) and was reported by their primary care physicians according to a standardized physicians' manual issued by the Health and Welfare Bureau for the Elderly of Japan [28]. The dementia status was usually updated annually and was reviewed until the patients were withdrawn due to death or move out of the study area. Individuals without dementia were classified as rank 0. Individuals who were diagnosed with mild cognitive dysfunction, but who had no dementia-related symptoms or behavioral disturbance and were capable of living independently, were classified as rank I. Individuals who had moderate dementia-related behavioral disturbance and cognitive impairment with slight dependence were classified as rank II. Individuals who had moderate to severe dementia-related behavioral disturbance and cognitive impairment with moderate dependence were classified as rank III. Individuals who had severe dementia-related behavioral disturbance and cognitive impairment with heavy dependence were classified as rank IV. Finally, individuals with severe dementia-related behavioral disturbance and cognitive impairment who required medical treatment were classified as rank V. Individuals who were ranked II or greater for the first time were regarded as incident disabling dementia cases in the present analysis. The validation for the cut-off point was determined by neuropsychiatrists of the subjects' cognitive functions (attention, memory, visuospatial function, language and reasoning) based on their aging-associated cognitive decline, as defined by the International Psychogeriatric Association [29]. The calculated sensitivity and specificity values were 36% and 90%, respectively, from the preliminary validation study of 34 disabling subjects. [30].

2.4. History of stroke identification

The history of stroke was obtained from the annual cardiovascular surveys and/or surveillance of the cardiovascular disease

registration system [11] from 1981 to the present. In the present study, 90% of stroke occurrence was confirmed based on CT or MRI using standardized criteria [11,31]. The determination of stroke without imaging studies was conducted based on the clinical criteria [32]. Stroke was defined as rapid-onset focal neurological disorder persisting for ≥ 24 h, or until death. Transient ischemic attack was not included.

2.5. Control selection

We employed risk set sampling [33] of controls, i.e. matching each case of dementia randomly with two of all other individuals with no diagnosis of dementia in the study cohort who were alive and resident within the same community on the date of diagnosis of dementia for the case, age (±2 years) and who had the same gender as the control. The vital status of controls was assessed before control selection.

2.6. Determination of serum high-sensitivity C-reactive protein

Non-fasting venous blood was collected in a 7- to 10- mL plain tube and allowed to stand for <30 min for serum separation. The serum samples were aliquoted immediately and placed on dry ice at the survey sites and then stored at −80 °C until analysis. Serum hs-CRP was measured using latex particle-enhanced immunonephelometric assays on the BN Prospec nephrometer Behring II (Dade Behring). In this method, monoclonal anti-CRP antibodies coated with polystyrene particles formed a complex with CRP present in the measured study sample. The amount of scattered light was directly proportional to the size of the antigen–antibody complex and reflected the hs-CRP concentration present in the study sample [34]. For results under the measurement limit of hs-CRP; hs-CRP>0.500 mg/dL or hs-CRP< 0.004 mg/dL, the values of 0.500 mg/dL or 0.004 mg/dL were used respectively.

2.7. Determination of confounding variables

Confounding variables were collected in the same year of blood collection. An interview was conducted to ascertain histories of cigarette smoking, ethanol intake and medication use for hypertension and diabetes. Height in stocking feet and weight in light clothing were measured. Body mass index (BMI) was calculated as weight (kg)/height (m^2). Systolic and diastolic blood pressures were measured by trained observers using a standard mercury sphygmomanometer on the right arm of seated participants after a 5-min rest. Use of antihypertensive medication was defined as systolic blood pressure ≥140 mmHg and diastolic blood pressure ≥90 mmHg and as taking antihypertensive medication. Serum total cholesterol was measured by enzymatic method. Serum glucose was measured by the hexokinase method. Borderline diabetes was defined as a fasting glucose of 6.1–6.9 mmol/L and/or a non-fasting glucose level of 7.8–11.0 mmol/L, without medication use for diabetes. Diabetes was defined as a fasting glucose level of ≥7.0 mmol/L and/or a non-fasting glucose level of ≥11.0 mmol/L and/or use of medication for diabetes. Atrial fibrillation was defined using Minnesota Codes 8-3-1 or 8-3-2 in electrocardiogram.

2.8. Statistical analysis

The unpaired student's *t* test and Wilcoxon rank sum test were used to compare the mean values of baseline dementia risk factors and median variables of hs-CRP between incident cases and control subjects. The χ^2 test was used to compare proportions between cases and control subjects. Potential confounding factors according to hs-CRP quartiles were investigated using the analysis of variance for continuous variables and χ^2 test for categorical variables. The conditional odds ratios (OR) and 95% confidence intervals (CI) for disabling dementia, dementia with stroke and dementia without stroke were estimated according to quartiles of hs-CRP levels and 1-SD increment of log transformed hs-CRP (antilog of SD = 3.3 mg/dL) of control subjects with conditional logistic regression models. Adjustment was made for systolic blood pressure (mmHg), use of antihypertensive medication (yes and no), BMI (kg/m^2), ethanol intake (never, former, current: less than 46 g/day, and 46 g/day or more ethanol), cigarette smoking status (never, ex-, and current smokers; 20 cigarettes/day or less, and more than 20 cigarettes/day), serum total cholesterol levels (mmol/L), borderline diabetes (yes and no) and diabetes (yes and no). Linear regression was employed to test for linear trends across the hs-CRP categories by using a median variable of hs-CRP for each hs-CRP category. The significance of the interactions for sex, use of antihypertensive medication (yes and no) and diabetes (yes and no) were tested using cross-product terms of sex, use of antihypertensive medication and diabetes with hs-CRP levels. All probability values of statistics were two- tailed, and values of $P < 0.05$ were regarded as statistically significant. The SAS statistical package version 9.1.3 (Statistical Analysis System Inc., Cary, NC) was used for analyses.

3. Results

Table 1 shows the risk characteristics of total dementia cases, dementia cases with history of stroke and dementia cases without history of stroke compared with control subjects. The average age for both cases and control of total dementia and dementia subtypes was 63 years. The proportion of men was higher in dementia with history of stroke (46%) than dementia without it (27%). Mean systolic blood pressure levels, diastolic blood pressure levels and median hs-CRP levels were higher in dementia with history of stroke than in controls; however this trend was not observed in dementia without it. The prevalence of diabetes was higher in total dementia than in controls, and these cases-controls differences were more evident in dementia with history of stroke than dementia without it.

During the follow-up period, we identified 275 dementia cases, comprising 96 dementia cases with a history of stroke and 179 dementia cases without such history.

Table 2 shows age-, sex-, community-, and survey year-matched and multivariable-adjusted odds ratios (95% CI) for total and subtypes of dementia according to quartiles of hs-CRP levels and 1-SD increment in log transformed hs-CRP levels. There was a significant association between elevated hs-CRP levels and dementia with history of stroke. After adjustment for hypertension, diabetes and other confounding variables, these positive associations remained statistically significant. The multivariable odds ratios associated with 1-SD increment of hs-CRP were 1.02 (0.87–1.20) for total dementia, 1.35 (1.02–1.79) for dementia with history of stroke, and 0.89 (0.72–1.10) for dementia without history of stroke. The multivariable odds ratios of dementia with history of stroke for the highest vs. lowest quartiles of hs-CRP levels were 0.99 (0.61–1.60) for total dementia, 2.72 (1.12–6.64) for dementia with history of stroke, and 0.63 (0.34–1.14) for dementia without history of stroke.

We examined potential effect modification by stratifying the analyses for use of antihypertensive medication (yes and no) and diabetes (yes and no) (data not shown) for dementia with history of stroke. There were no statistically significant interactions between hs-CRP levels and use of antihypertensive medication (p for interaction = 0.48) or diabetes (p for interaction = 0.12).

Table 1
Risk characteristics among cases and control subjects of total dementia stratified by the presence of stroke history.

	No	Age y	Men, %	Systolic BP, mm Hg	Diastolic BP, mm Hg	Antihypertensive medication use, %	BMI, kg/m2	Ethanol intake, g/d	Current smokers, %	Serum cholesterol mmol/L	Impaired glucose tolerance %	Diabetes mellitus %	Atrial fibrillation %	Median hs-CRP (mg/dL)
Total dementia														
Cases	275	62.8 ± 5.2	33.8	137.2 ± 18.4‡	79.9 ± 10.8*	32.4	23.9 ± 3.5	0.48 ± 0.90	23.3	200.1 ± 32.9	16.7	10.2*	0.4	0.041
Control subjects	550	62.6 ± 5.2	33.8	132.7 ± 16.7	78.1 ± 10.5	30.9	23.9 ± 3.2	0.43 ± 0.84	19.1	198.2 ± 33.3	15.6	5.3	0.4	0.042
Dementia with history of stroke														
Cases	96	62.4 ± 4.3	45.8	140.8 ± 19.9‡	82.5 ± 10.5‡	39.6	23.7 ± 3.3	0.65 ± 0.97	29.2	198.2 ± 32.8	16.7	9.4*	1.1	0.050*
Control subjects	192	62.2 ± 4.4	45.8	132.1 ± 16.1	78.0 ± 11.4	32.3	23.8 ± 3.1	0.57 ± 0.97	26.0	193.0 ± 32.8	17.7	2.6	0.5	0.036
Dementia without history of stroke														
Cases	179	63.1 ± 5.6	27.4	135.4 ± 17.3	78.6 ± 10.8	28.5	24.0 ± 3.6	0.39 ± 0.85	20.1	201.1 ± 32.4	16.8	10.6	0	0.037
Control subjects	358	62.8 ± 5.6	27.4	132.9 ± 17.1	78.1 ± 10.0	30.2	24.0 ± 3.2	0.35 ± 0.75	15.4	201.0 ± 33.2	14.5	6.7	0.3	0.044

Data are shown as mean ± SD, frequency as a number (%).
hs-CRP levels are expressed as median (interquartile range).
P values for differences from control subjects : $^*P < 0.05$, ‡$P < 0.001$.

4. Discussion

The present study is the first study to provide evidence that elevated hs-CRP levels were associated with increased risk of disabling dementia in individuals with history of stroke. These associations remained unchanged even after adjustment for risk factors of dementia and the matching variable of age, sex, years of serum storage, and community. In addition, this association does not vary according to use of antihypertensive medication or whether a person has diabetes. However, no significant association was observed between hs-CRP levels and risk of disabling dementia in individuals without history of stroke.

The most important findings in the present study were that elevated hs-CRP in midlife was associated with the increased risk of developing dementia in individuals with history of stroke but not in individuals without such history. The evidence from Honolulu–Asia Aging study [13] indicated that elevated hs-CRP levels in midlife increased the risk of developing dementia in later life. That study, however, did not stratify individuals with dementia by stroke history but included stroke as a mediating variable in their model. Previous prospective studies [13–15] of hs-CRP and dementia showed a 1.5–2.8-fold increased risk of dementia with elevated hs-CRP levels whereas other studies [20,21] failed to find such associations. Again, those studies did not stratify individuals with dementia by stroke history. The inconsistent results across previous studies may suggest that the interaction effect of hs-CRP and cardiovascular disease on developing dementia was often insufficiently considered. One reason to explain our findings is the fact that Japan populations have the higher incidence of stroke compared to Western countries [4]. The comparatively larger number of stroke cases in our sample could account for the positive association we found between hs-CRP levels and dementia in individuals with history of stroke, and a result not found in studies conducted in Western countries.

Inflammatory process may directly or indirectly relate to dementia risk via their role in the initiation of athero- and arteriolesclerotic lesions in cerebrovascular system [25], which subsequently may increase risk of developing dementia in individuals with stroke history. High CRP facilitates the formation of foam cells in the process of atherogenesis [25] and also impairs endothelial function by attenuating the production of nitric oxide [35]. Both processes contribute the cognitive decline in older adults [36]. In addition, increased myo-inositol signal is a neurochemical abnormality associated with cognitive decline and Alzheimer's disease [37]. A recent cross-sectional study found that higher serum CRP was associated with higher ratio of cerebral myo-inositol/creatine concentrations in cognitively normal middle-aged adults, suggesting the linkage of high CRP to neurochemical changes [38].

Previous cohort studies reported that CRP levels were positively associated with risk of vascular dementia [13–15] but not associated with risk of Alzheimer's disease [13–15,21,39]. These findings corresponded to our present result that CRP levels were positively associated with risk of dementia with history of stroke, but not with dementia without history of stroke, presumably Alzheimer's disease. Although the carrying of *APOE4 allele* is a major risk factor for Alzheimer's disease [40] and *APOE4* carriers had lower levels of CRP compared to non *APOE4 allele* carriers [41–43], the effect of *APOE4 allele* to the association between CRP levels and Alzheimer's disease still remains murky. In the ULSAM-study [39] CRP levels were not associated with Alzheimer's disease after accounted for *APOE* genotype (*APOE4* allele carriers *versus* non carries). However, in a cohort study of Mexican Americans aged 60-101, CRP levels were found inversely associated with the risk of Alzheimer's disease (HR:0.74 (95% CI 0.35–0.90)) in *APOE4* carriers (13% of total subjects) and were positively associated with

Table 2
Odd ratios (95% confidence intervals) of total dementia, stratified by the presence of stroke history according to quartiles of serum hs-CRP levels of control subjects.

	Quartiles of hs-CRP, mg/dL					OR for 1SD increment of log hs-CRP
	1 (low)	2	3	4 (high)	*P* for trend	
Serum hs-CRP						
Median (mg/L)	0.01	0.03	0.06	0.152		
Range (mg/L)	0.002–0.016	0.017–0.041	0.042–0.088	0.090–3.11		
Total dementia						
No. of case	55	83	74	63		
No. of control	134	141	138	137		
Age-, sex, and community-matched OR	1.00	1.44 (0.95–2.18)	1.32 (0.86–2.04)	1.13 (0.73–1.74)	0.69	1.06 (0.92–1.22)
Multivariable OR[a]	1.00	1.34 (0.86–2.07)	1.16 (0.73–1.85)	0.99 (0.61–1.60)	0.82	1.02 (0.87–1.20)
Dementia with history of stroke						
No. of case	11	34	24	27		
No. of control	49	56	44	43		
Age-, sex, and community-matched OR	1.00	2.71 (1.22–6.03)*	2.43 (1.06–5.60)*	2.72 (1.21–6.10)*	0.02	1.33 (1.04–1.71)*
Multivariable OR[a]	1.00	2.15 (0.90–5.15)	2.06 (0.82–5.21)	2.72 (1.12–6.64)*	0.04	1.35 (1.02–1.79)*
Dementia without history of stroke						
No. of case	44	49	50	36		
No. of control	85	85	94	94		
Age-, sex, and community-matched OR	1.00	1.10 (0.66–1.83)	1.04 (0.62–1.74)	0.74 (0.44–1.27)	0.27	0.94 (0.78–1.12)
Multivariable OR[a]	1.00	1.08 (0.64–1.84)	0.93 (0.54–1.62)	0.63 (0.34–1.14)	0.11	0.89 (0.72–1.10)

*$P < 0.05$.
[a] Adjusted for systolic blood pressure, antihypertensive medication use, borderline diabetes, diabetes, BMI, alcohol intake categories, cigarette smoking status, serum total cholesterol levels as well as matching for sex, age, community, year of serum stored, and fasting status.

the risk of Alzheimer's disease (HR 1.24 (95% CI 1.29–1.40)) in non *APOE4* carriers [43].

The strengths of the present study were its prospective design, the comprehensive nature of cardiovascular surveys, storage of serum blood samples and the large number of strokes confirmed by imaging studies. These allowed us to investigate the association between hs-CRP levels and risk of dementia with history of stroke and without such history and to adjust for important potential confounding variables. Moreover, because we used annual cardiovascular surveys that were carried out at least 5 years before the endpoint determination, severe dementia was unlikely to be present at the time of the risk factor assessment. This would enhance our confidence that elevated hs-CRP was not influenced by subclinical dementia.

There are some limitations of the current study. First, we did not have the data of *APOE* genotype, and could not examine an effect modification by *APOE4* allele. Further studies are required to confirm the potential effect of CRP levels on Alzheimer's disease in the presence and absence of *APOE4* allele. Second, we did not assess the cognitive functions of studied subjects at baseline. Therefore, we cannot completely exclude the effects of co-existing subclinical dementia at the time of blood serum collection. However, participants were presumed fit and able to attend the annual cardiovascular checkup for data collection. We conducted another analysis that excluded 6 cases that attended the annual cardiovascular survey within at least 5 years of the diagnosis of dementia, and the results remained unchanged (data not shown). Third, we had a systematic survey and surveillance for stroke incidence, but did not obtain the imaging study results for 10% of the stroke cases because CTs and MRIs were not common at local hospitals in the early 1980s. However, the diagnosis for stroke based only on clinical evidence (excluding medical imaging) was found to be in agreement with 97% of the autopsy results in a previous Japanese study [32]. Therefore, false-negative stroke cases were less likely to exist in our dementia cases. Fourth, we used frozen serum to estimate hs-CRP levels and we did not examine long-term changes in hs-CRP levels in stored serum samples. However, hs-CRP levels were reported to be stable at −70 °C which were stored for 8–11years [44]. Finally, these findings are based on a single measure of hs-CRP. CRP levels of subjects in the general population tend to be stable, although CRP levels may spike occasionally in the presence of minor or subclinical infections, inflammation or trauma [45].

In conclusion, elevated hs-CRP levels were associated with increased risk of dementia among those with history of stroke. No significant association was observed among those without history of stroke. The current study highlights that the risk of developing dementia in the elderly could be predicted in part through CRP measures.

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Disclosure

None.

Conflict of interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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Appendix I

The Circulatory Risk in Communities Study (CIRCS) is a collaborative study managed by the Osaka Center for Cancer and Cardiovascular Disease Prevention, University of Tsukuba, Osaka

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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: $p_{\text{allele}} = 6.71\text{E-}171$ 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.

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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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Serum coenzyme Q10 and risk of disabling dementia: The Circulatory Risk in Communities Study (CIRCS)

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ABSTRACT

Objective: To examine whether coenzyme Q10, a potent antioxidant, is associated with risk of dementia, which has not yet been elucidated. **Approach and results**: We performed a case–control study nested in a community-based cohort of approximately 6000 Japanese aged 40–69 years at baseline (1984–1994). Serum coenzyme Q10 was measured in 65 incident cases of disabling dementia with dementia-related behavioral disturbance or cognitive impairment incident between 1999 and 2004, and in 130 age-, sex- and baseline year-matched controls. Serum coenzyme Q10 was inversely associated with dementia: the multivariate odds ratios (95% confidence intervals) were 0.68 (0.26–1.78), 0.92 (0.33–2.56), and 0.23 (0.06–0.86) for individuals with the second, third, and highest quartiles of coenzyme Q10, respectively, as compared with the lowest quartile (P for trend = 0.05). A similar association was found for the coenzyme Q10/total cholesterol ratio: the respective ORs were 0.67 (0.25–1.78), 0.73 (0.28–1.92), and 0.21 (0.05–0.90) (P for trend = 0.04). **Conclusions**: Serum coenzyme Q10 levels were inversely associated with risk of disabling dementia.

1. Introduction

Coenzyme Q10 (CoQ10), or ubiquinone, is a vitamin-like substance synthesized by animal cells. CoQ10 largely exists in the myocardium and plays a role in mitochondrial energy production. It also has an antioxidant function and is widely consumed as a supplement in the United States [1]. In addition to the prescription of CoQ10 as an orphan drug for mitochondrial encephalomyopathy, some evidence exists for a beneficial effect of CoQ10 on several neurologic diseases such as Parkinson disease [2], Huntington disease [3], and Friedreich ataxia [4,5] as well as on improved physical exercise capacity [6] and lowered blood pressure [6,7]. Animal studies have shown a potential benefit of CoQ10 on cognitive function [8–11]. A randomized controlled trial, however, has shown that the supplementation of CoQ10 did not influence cerebrospinal fluid biomarkers in patients with mild-to-moderate Alzheimer disease [12]. Yet, evidence on this issue is still limited, and no prospective study has been performed on the preventive effect of CoQ10 on risk of incident dementia in the general population.

In the present study, we hypothesized that because of its antioxidant effect, serum level of CoQ10 is inversely associated with disabling dementia. To test this hypothesis, we conducted a nested case–control study in the Circulatory Risk in Communities Study (CIRCS), a large community-based cohort study of Japanese population.

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