suggest that for the depression scale, a cutoff of 3/4 identifies approximately 80% of depression cases.

Consensus diagnosis of depression. A group of psychiatrists and neuropsychologists reviewed the results of the interview using the PAS and the functional, medical, neurologic, psychiatric, and neuropsychological data gained during the first phase, and they reached a consensus diagnosis of MDE according to the DSM-III-R criteria. The subjects who did not qualify for the diagnosis of MDE, but scored 6 or higher on the GDS, were defined as the depressive symptoms cases (DSCs). Subjects who had dementia were not considered for a possible diagnosis of depression.

Statistical issues and analysis. Demographics (age, sex, and years of education), cognitive function (scores for all five cognitive domains of the 5-Cog), and GDS scores between participants and non-participants in the second phase, or ApoE4 carriers and non-carriers, were compared using non-paired t-test and χ^2 test.

We analyzed the relationship between ApoE4 and depression (DSC and MDE) by using multiple logistic regression analysis. According to mood state, we divided the subjects into three groups: normal, DSC, and MDE. The outcome variables were prevalence of DSC and MDE. The exposure variables were age, sex, years of education, prevalence of MCI, N-ADL scores, habitual alcohol drinking, habitual smoking, hypertension, diabetes, hyperlipidemia, and cerebral vascular disease.

The data were analyzed using SPSS 15.0J software (SPSS, Chicago, IL, USA) and STATVIEW 5.0J software (SAS Institute Inc., Cary, NC, USA). The results for continuous variables are given as mean ± SD. All tests were two-side, and alpha was set at 0.05.

Results

Survey population

Of the 3083 potential subjects, 132 (87 died and 45 moved before the initial examination) were excluded. An additional 253 residents were unreachable. The remaining 2698 residents were included as subjects at baseline. Among the 2698 residents, after excluding those who had been diagnosed as having dementia and who did not complete the questionnaire and could not complete a series of tests, 1619 subjects with complete data remained for the final analysis.

Demographics and cognitive function for the second phase participants and the non-participants

Table 2 shows the comparison of demographics (age, sex, and years of education) and cognitive function (scores of five cognitive domains), and GDS scores for 738 second-phase participants and 881 non-participants for the second phase. As shown in Table 2, there were significant differences in age ($t=3.32$, $p<0.01$) and scores for all of five cognitive domains of the 5-Cog: attention (-3.80, $p<0.01$), memory (-5.01, $p<0.01$), visuospatial function (-2.03, $p<0.05$), language (-3.80, $p<0.01$), and reasoning (-3.80, $p<0.01$). Overall, in comparison with the non-participants, the second-phase participants were younger, and their cognitive function was better.

Prevalence and characteristics of depression

Of the 738 second-phase participants, 147 passed or exceeded the cutoff value for GDS, and 591 scored less than 6. Twenty-four of the 147 (16.3%) subjects with

Table 2 Demographics and cognitive function for the second phase participants and the non-participants

Characteristics	Participants	Non-participants	p-Value
	$n=738$	$n=881$	
Age, years (mean ± SD)	73.6 ± 5.6	74.6 ± 6.6($n=881$)	<0.01
Sex (male/female)	313(42.4)/425(57.6)	344(39.0)/537(61.0)	0.17
Years of education (mean ± SD)	10.0 ± 2.6	9.7 ± 2.7($n=844$)	0.06
Attention (mean ± SD)	16.2 ± 8.3	14.9 ± 8.4($n=747$)	<0.01
Memory (mean ± SD)	10.9 ± 5.0	9.6 ± 5.3($n=756$)	<0.01
Language (mean ± SD)	13.4 ± 4.7	12.4 ± 4.8($n=757$)	<0.01
Visuospatial function (mean ± SD)	6.1 ± 1.2	5.9 ± 1.3($n=755$)	<0.05
Reasoning (mean ± SD)	7.5 ± 4.4	6.6 ± 4.6($n=754$)	<0.01
GDS score (mean ± SD)	3.1 ± 2.9	4.2 ± 34.5($n=839$)	0.39

GDS, Geriatric Depression Scale.

GDS scores of 6 or more were diagnosed as having depression (MDE) as defined by the PAS subscale. Consequently, the remaining 123 individuals who did not qualify for the diagnosis of MDE, but scored 6 or higher on the GDS, were defined as DSC. On the other hand, 12 of 591 (2.0%) subjects that scored less than 6 were diagnosed as having depression (MDE) according to the PAS subscale. In total, 36 participants had a diagnosis of MDE.

Demographics and clinical data for ApoE4 carriers and ApoE4 non-carriers

The comparison between ApoE4 carriers and non-carriers revealed that only MCI prevalence was significantly different ($\chi^2 = 7.25$, $p < 0.01$) between the two groups (Table 3). Table 3 shows that ApoE4 was not associated with mood state.

Association of ApoE4 and depression (depressive symptoms cases and major depressive episode)

As shown in Table 4-A, sex (OR = 2.53, 95%CI = 1.33–4.79, $p < 0.01$), MCI (1.95, 1.21–3.14, $p < 0.01$), years of education (0.87, 0.79–0.95, $p < 0.01$), and N-ADL scores (0.75, 0.63–0.89, $p < 0.01$) correlated with prevalence of DSC. There were no significant risk factors for MDE (Table 4-B). Again, ApoE4 contributed to neither DSC nor MDE.

Discussion

In this study, we attempted to determine the relationship between ApoE4 and depression by considering the presence of MCI and subdividing depression into DSC and MDE. Our methodology differed from that of the previous studies noted in the Introduction. For the screening, we used five tests that measure five different cognitive domains; subsequently, we assessed general cognitive function and mood state by using a face-to-face structured interview. Finally, we made the diagnosis of MCI as well as dementia. After excluding the individuals with dementia, we examined the association between ApoE4 and depression (DSC and MDE).

As a result, our study revealed that MCI, years of education, N-ADL scores, and male gender were risk factors for DSC, whereas there were no risk factors for MDE. Thus, we conclude that ApoE4 contributed to neither DSC nor MDE.

Lower education (Beekman *et al.*, 2001; Jang *et al.*, 2002; Azar *et al.*, 2005) and functional limitation (Blumstein *et al.*, 2004; Horowitz *et al.*, 2005; Jorm *et al.*, 2005) have been pointed out as risk factors by many of the previous studies. Although there are several previous studies (Strawbridge *et al.*, 2002; van der Wurff *et al.*, 2004; Heun and Hein, 2005) reporting the relationship between depressive disorder and female gender, this relationship has been controversial. Our analysis showed no association between gender and MDE; however, unexpectedly, male gender was associated with DSC. A possible explanation for

Table 3 Demographics and clinical data for ApoE4 carriers and ApoE4 non-carriers

Characteristics	ApoE4 non-carriers	ApoE4 carriers	*p*-Value
	n = 589	*n* = 149	
Age, years (mean ± SD)	73.5 ± 5.6	73.5 ± 5.6	0.97
Sex (male/female), *n* (%)	254 (43.1)/335 (56.9)	59 (39.6)/90 (60.4)	0.51
Years of education (mean ± SD)	10.0 ± 2.6	10.0 ± 2.8	0.92
MCI, *n* (%)	100 (17.0)	40 (26.8)	<0.01
N-ADL score (mean ± SD)	49.7 ± 1.4	49.8 ± 0.8	0.40
GDS score (mean ± SD)	3.2 ± 2.9	2.9 ± 2.9	0.24
Mood state	—	—	0.77
Mood Normal, *n* (%)	460 (78.1)	119 (79.9)	-
Depression (DSC), *n* (%)	99 (16.8)	24 (16.1)	-
Depression (MDE), *n* (%)	30 (5.1)	6 (4.0)	-
Habitual alcohol drinking, *n* (%)	200 (34.0)	49 (32.9)	0.92
Habitual smoking, *n* (%)	201 (34.1)	50 (33.6)	>0.99
Hypertension, *n* (%)	164 (27.8)	45 (30.2)	0.54
Diabetes, *n* (%)	30 (5.1)	9 (6.0)	0.68
Hyperlipidemia, *n* (%)	15 (2.5)	7 (4.7)	0.18
Cerebral vascular disease, *n* (%)	24 (4.1)	3 (2.0)	0.33

ApoE4, apolipoprotein E4 allele; MCI, mild cognitive impairment; GDS: Geriatric Depression Scale; N-ADL: Nishimura's activities of daily living; DSC, depressive symptoms cases; MDE, major depressive episode.

Table 4 Risk factors contributing to depression: (A) depressive symptoms case and (B) major depressive episode

Depression	Mood Normal	Adjusted OR (95%CI)	*p*-Value
A. Depressive symptoms case			
Age, years	–	0.97 (0.93–1.01)	0.12
Sex (male: 1/female: 0)	–	2.53 (1.33–4.79)	<0.01
Years of education	–	0.87 (0.79–0.95)	<0.01
ApoE4 carrier (yes: 1/no: 0)	–	0.82 (0.48–1.39)	0.46
MCI (yes: 1/no: 0)	–	1.95 (1.21–3.14)	<0.01
N-ADL score	–	0.75 (0.63–0.89)	<0.01
Habitual alcohol drinking (yes: 1/no: 0)	–	0.63 (0.36–1.08)	0.09
Habitual smoking (yes: 1/no: 0)	–	0.68 (0.38–1.23)	0.21
Hypertension (yes: 1/no: 0)	–	0.66 (0.41–1.06)	0.09
Diabetes (yes: 1/no: 0)	–	0.92 (0.34–2.51)	0.87
Hyperlipidemia (yes: 1/no: 0)	–	1.17 (0.33–4.23)	0.81
Cerebral vascular disease (yes: 1/no: 0)	–	0.40 (0.09–1.69)	0.21
B. Major depressive episode			
Age, years	–	0.96(0.89–1.04)	0.32
Sex (male: 1/female: 0)	–	1.45(0.45–4.62)	0.53
Years of education	–	0.99(0.86–1.15)	0.92
ApoE4 carrier (yes: 1/no: 0)	–	0.79(0.29–2.14)	0.64
MCI (yes: 1/no: 0)	–	0.69(0.22–2.13)	0.52
N-ADL score	–	0.81(0.65–1.02)	0.07
Habitual alcohol drinking (yes: 1/no: 0)	–	0.68(0.26–1.74)	0.41
Habitual smoking (yes: 1/no: 0)	–	1.22(0.43–3.43)	0.71
Hypertension (yes: 1/no: 0)	–	0.44(0.17–1.19)	0.11
Diabetes (yes: 1/no: 0)	–	0.51(0.06–4.02)	0.52
Hyperlipidemia (yes: 1/no: 0)	–	2.79(0.58–13.38)	0.20
Cerebral vascular disease (yes: 1/no: 0)	–	3.34(1.00–11.19)	0.05

OR, odds ratio; ApoE4, apolipoprotein E4 allele; MCI, mild cognitive impairment; N-ADL, Nishimura's activities of daily living.

the discrepancy is that our definition of DSC is different from that of depressive symptoms, which were used in the previous studies. Unlike some previous studies that might have included individuals with dementia and depression, we strictly excluded such persons. In our study, the proportion of women having the diagnosis of dementia (65.0%) was larger than that of men (35.0%). Thus, strict exclusion of the female dominant dementia group might have contributed to the male dominance for DSC. These factors might have contributed to the male preponderance.

Although no relationship was found between ApoE4 and depression (DSC and MDE) in the present study, several studies (Krishnan *et al.*, 1996; Rigaud *et al.*, 2001; Yen *et al.*, 2007) showed affirmative results. Looking at the discrepancy, we found that MCI was associated with DSC (Table 4-A), and the prevalence rate of MCI among ApoE4 carriers was higher than that of MCI among the non-carriers (Table 3). Thus, it is possible that MCI might be a confounding factor for the association between ApoE4 and DSC. Theoretically, it is possible that the proportion of individuals with MCI compared with the total number of cognitively normal participants might have contributed to the presence or the absence of the association between ApoE4 and depression in the previous studies. In other words, the affirmative studies (Krishnan *et al.*, 1996; Rigaud *et al.*, 2001; Yen *et al.*, 2007) might have included more individuals with the comorbidity of depression and MCI, whereas the negative ones (Harwood *et al.*, 1999; Ohara *et al.*, 1999; Bonger *et al.*, 2009; Surtees *et al.*, 2009) might have included fewer of these individuals.

A number of studies reported the relationship between depression and MCI, and their prevalence rates of depression with MCI varied from 16% to 63% (Lyketsos *et al.*, 2002; Chan *et al.*, 2003; Lopez *et al.*, 2003; Palmer *et al.*, 2007; Solfrizzi *et al.*, 2007; Artero *et al.*, 2008; Muangpaisan *et al.*, 2008). As reported elsewhere, the prevalence for the present study was 26.2% (Hidaka *et al.*, 2012). This finding appears to be compatible with the previous results. On the other hand, some recent studies have reported the association between ApoE4 and MCI (Ramakers *et al.*, 2008; van der Filer *et al.*, 2008). As shown in Table 3, ApoE4 was associated with MCI in our study. Thus, our assumptions might reflect those variations to a certain extent.

The present study has limitations. As shown in Table 2, there are some demographic and functional differences between the subjects who participated in the second phase and those who dropped out. In general, the former were younger and had better cognitive function than the latter, and these differences

might have affected the results. However, our study included a fairly large number of subjects compared with most of the previous studies that examined the relationship between ApoE4 and depression. In addition, we used only data from those who provided full data and underwent a structured interview. Although standard practice for MCI study is typically based on average of at least two tests per domain, we used only one test per domain. Self-report measures were used in our study; however, the results of the measures from the participants with diminished insight may be a poor measure of actual functioning. Taking the limitations described earlier into consideration, we should take attention in interpreting the results of this study.

In conclusion, the association of MCI with ApoE4 and DSC suggests that MCI is a confounder for the association between ApoE4 and DSC. ApoE4 contributed to neither DSC nor MDE.

Key points

- ApoE4 contributed to neither DSC nor MDE.
- The comparison between ApoE4 carriers and non-carriers revealed that only MCI prevalence was significantly different between the two groups.
- Sex, MCI, years of education, and N-ADL scores significantly correlated with prevalence of DSC.
- The association of MCI with ApoE4 and DSC suggested that MCI is a confounder for the association between ApoE4 and DSC.

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Conflict of interest

None declared.

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ORIGINAL RESEARCH

Automatic Voxel-Based Morphometry of Structural MRI by SPM8 plus Diffeomorphic Anatomic Registration Through Exponentiated Lie Algebra Improves the Diagnosis of Probable Alzheimer Disease

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BACKGROUND AND PURPOSE: The necessity for structural MRI is greater than ever to both diagnose AD in its early stage and objectively evaluate its progression. We propose a new VBM-based software program for automatic detection of early specific atrophy in AD.

MATERIALS AND METHODS: A target VOI was determined by group comparison of 30 patients with very mild AD and 40 age-matched healthy controls by using SPM. Then this target VOI was incorporated into a newly developed automated software program independently running on a Windows PC for VBM by using SPM8 plus DARTEL. ROC analysis was performed for discrimination of 116 other patients with AD with very mild stage (n = 45), mild stage (n = 30) and moderate-to-advanced stages (n = 41) from 40 other age-matched healthy controls by using a z score map in the target VOI.

RESULTS: Medial temporal structures involving the entire region of the entorhinal cortex, hippocampus, and amygdala showed significant atrophy in the patients with very mild AD and were determined as a target VOI. When we used the severity score of atrophy in this target VOI, 91.6%, 95.8%, and 98.2% accuracies were obtained in the very mild AD, mild AD, and moderate-to-severe AD groups, respectively. In the very mild AD group, a high specificity of 97.5% with a sensitivity of 86.4% was obtained, and age at onset of AD did not influence this accuracy.

CONCLUSIONS: This software program with application of SPM8 plus DARTEL to VBM provides a high performance for AD diagnosis by using MRI.

ABBREVIATIONS: AD = Alzheimer disease; DARTEL = diffeomorphic anatomical registration through exponentiated lie algebra; FWHM = full width at half maximum; MCI = mild cognitive impairment; MMSE = Mini-Mental State Examination; ROC = receiver operating characteristic analysis; SPM = statistical parametric mapping; VBM = voxel-based morphometry

Increases in the number of individuals with dementia, the highest proportion of whom are affected by AD, have made early diagnosis of AD a major research and clinical priority. Of several neuroimaging techniques that provide surrogate markers for the diagnosis of AD, structural MRI is the most commonly used because of its noninvasiveness and excellent spatial resolution with good tissue contrast.[1] In AD, the earliest tissue loss occurs in the medial temporal structures, particularly in the entorhinal cortex.[2] However, visual inspection is insufficient for objective evaluation of mild atrophy. Although manual tracing of these structures can quantify the absolute volume, it is time-consuming and requires special expertise in anatomic knowledge for tracers. Recently, computer-aided VBM[3] has been applied to detect early atrophic changes in AD. Although this technique cannot provide the absolute volume, it can provide statistical results in comparisons of patients with AD with healthy controls.[4] Moreover VBM has been reported to be a surrogate indicator of the full brain topographic representation of the neurodegenerative aspect of AD pathology.[5] Hirata et al[6] proposed an automated software program, a voxel-based specific regional analysis system for AD, for the diagnosis of AD by using this VBM technique. In the present study, we revised this software by introducing new techniques and validated its utility.

Materials and Methods

A total of 251 subjects were studied in 1 center. We retrospectively chose 146 patients (65 men and 81 women) with a clinical diagnosis of probable AD according to the National Institute of Neurologic and Communicative Disorders and Stroke and the Alzheimer Disease and Related Disorders Association criteria.[7] These patients were classified into 3 groups of very mild, mild, and moderate-to-advanced AD. The very mild AD group comprised 75 patients (37 men and 38 women) who ranged in age from 51 to 86 years with a mean of 71.2 ± 7.4 years.

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At the initial visit, they had no apparent loss in general cognitive, behavioral, or functional status and corresponded to the criteria of the amnestic type of MCI[8] or 0.5 in the Clinical Dementia Rating.[9] The MMSE score ranged from 24 to 29 (mean, 25.7 ± 1.5). During the subsequent follow-up period of 2–6 years, the subjects showed progressive cognitive decline and eventually fulfilled the diagnosis of probable AD. The mild and moderate-to-advanced AD groups comprised 30 patients (8 men and 22 women, 71.4 ± 6.8 years of age) and 41 patients (20 men and 21 women, 71.3 ± 7.7 years of age), respectively. The MMSE score ranged from 20 to 25 (mean, 21.4 ± 1.3) and from 6 to 19; (mean, 15.0 ± 3.5) for the mild and moderate-to-advanced AD groups, respectively. Eighty-one of these patients with AD (48 very mild, 11 mild, and 22 moderate-to-advanced) underwent follow-up MRI studies at an interval of 1–4 years for, at most, 6 years (mean, 3.3 ± 1.2 years), and most patients in the very mild and mild AD groups moved to a more advanced group during the follow-up period. Consequently, the total of MRI studies was 89, 57, and 123 for the very mild, mild, and moderate-to-advanced AD groups.

Eighty age-matched control subjects (37 men and 43 women) were healthy volunteers with no memory impairment or cognitive disorders. They ranged in age from 54 to 86 years with a mean of 70.4 ± 7.8 years. Their performance was within normal limits both on the Wechsler Memory Scale–Revised and the Wechsler Adult Intelligence Scale–Revised. Their MMSE scores ranged from 26 to 30 (mean, 29.1 ± 1.2). They did not differ in age or education from the patients with AD. Additionally, 25 healthy volunteers (15 men and 10 women; mean, 31.1 ± 7.8 years of age) participated in this study for creation of a customized template for spatial normalization in the statistical image analysis. The ethics committee approved this study, and all subjects provided informed consent to participate. None of them had asymptomatic cerebral infarction detected by T2-weighted MRI.

All subjects underwent an MRI study on a 1.5T Vision Plus imager (Siemens, Erlangen, Germany). One hundred forty 3D sections of a T1-weighted magnetization-prepared rapid acquisition of gradient echo sequence were obtained in a sagittal orientation as 1.2-mm thick sections (FOV = 23, TR = 9.7 ms, TE = 4 ms, flip angle = 12°, and TI = 300 ms, with no intersection gaps).

First, to define a target VOI for early diagnosis of AD, we performed a group comparison between 30 patients (14 men and 16 women; mean age, 73.8 ± 4.8 years) randomly chosen in the present very mild AD group and the present 40 healthy controls group (19 men and 21 women; mean age, 70.8 ± 8.5 years). Using the latest version of SPM8 (Wellcome Department of Imaging Neuroscience, London, United Kingdom), we segmented MRIs into gray matter, white matter, and CSF images by a unified tissue-segmentation procedure after image-intensity nonuniformity correction. These segmented gray matter images were then spatially normalized to the customized template in the standardized anatomic space by using DARTEL (Wellcome Department of Imaging Neuroscience).[10] The customized template for DARTEL was created from the aforementioned 25 healthy young subjects. To preserve gray matter volume within each voxel, we modulated the images by the Jacobean determinants derived from the spatial normalization by DARTEL and then smoothed them by using an 8-mm FWHM Gaussian kernel. To compare the present analysis by using SPM8 plus DARTEL with the previously reported analysis,[6] we also defined a target VOI by using an old SPM version, SPM2 (Wellcome Department of Imaging Neuroscience) between the same 2 groups. Group comparisons by SPM were assessed by using the family-wise error at a threshold of $P < .05$, corrected for multiple comparisons.

A stand-alone software program running on Windows for VBM analysis by SPM8 plus DARTEL was developed to discriminate patients with AD from healthy controls. First, MRIs were spatially normalized with only a 12-parameter affine transformation to the SPM template so as to correct for differences in brain size. These linearly transformed images were nonlinearly transformed and then modulated to the customized template for DARTEL, followed by smoothing by using an 8-mm FWHM kernel. Each processed gray matter image of the remaining 116 patients with AD and 40 healthy controls was compared with the mean and SD of gray matter images of the 40 healthy volunteers chosen in the group comparison by using voxel-by-voxel z score analysis with and without voxel normalization to global mean intensities (global normalization): z score = ([control mean] − [individual value]) / (control SD). These z score maps were displayed by overlay on tomographic sections and surface rendering of the standardized brain. This program registered the target VOI defined by the aforementioned group comparison. This software program takes 8 minutes 40 seconds for all procedures by using a 64-bit PC with a Core i7 central processing unit and 6-gigabytes memory (Intel, Santa Clara, California).

We determined 4 indicators for characterizing atrophy in a target VOI and in the whole brain: first, the severity of atrophy obtained from the averaged positive z score in the target VOI; second, the extent of a region showing significant atrophy in the target VOI—that is, the percentage rate of the coordinates with a z value exceeding the threshold value of 2 in the target VOI; third, the extent of a region showing significant atrophy in the whole brain—that is, the percentage rate of the coordinates with a z value exceeding the threshold value of 2 in the whole brain; and fourth, the ratio of the extent of a region showing significant atrophy in the target VOI to the extent of a region showing significant atrophy in the whole brain. The utility of these indicators for this discrimination of AD from healthy controls has been reported in previous MRI[6] and SPECT studies.[11]

These 4 indicators were obtained under 2 conditions, with or without global normalization. Using the values of the 4 indicators as the threshold, we determined ROC curves for discrimination of patients with AD from healthy volunteers by using JMP 7.0 (SAS Institute, Cary, North Carolina). The program calculates the area under the ROC curves, sensitivity, specificity, and accuracy. Moreover, the age effects of AD onset on these 4 indicators and the results of the ROC were investigated in the very mild AD group classified into 2 subgroups with an age threshold of 65: the early-onset subgroup (16 patients, 9 men and 7 women; mean age, 58.0 ± 4.6 years) and the late-onset subgroup (29 patients, 14 men and 15 women; mean age, 73.8 ± 4.4 years).

Results

The group comparison by SPM8 plus DARTEL demonstrated significant decline of gray matter volume in the left (Talairach coordinates −24,−10,−14, x,y,z; z = 7.37 and 6.95 without and with global normalization, respectively) and the right (24,10,14, x,y,z; z = 7.42 and 7.05 without and with global normalization, respectively) parahippocampal gyri in patients with very mild AD (Fig 1). These bilateral regions involve the entorhinal cortex, head to tail of the hippocampus, and amygdala and are delineated as a target VOI for AD. Group comparison by SPM2 showed significant decline of gray matter volume in the left (−18, −7, −16, x,y,z; z = 6.18) and right

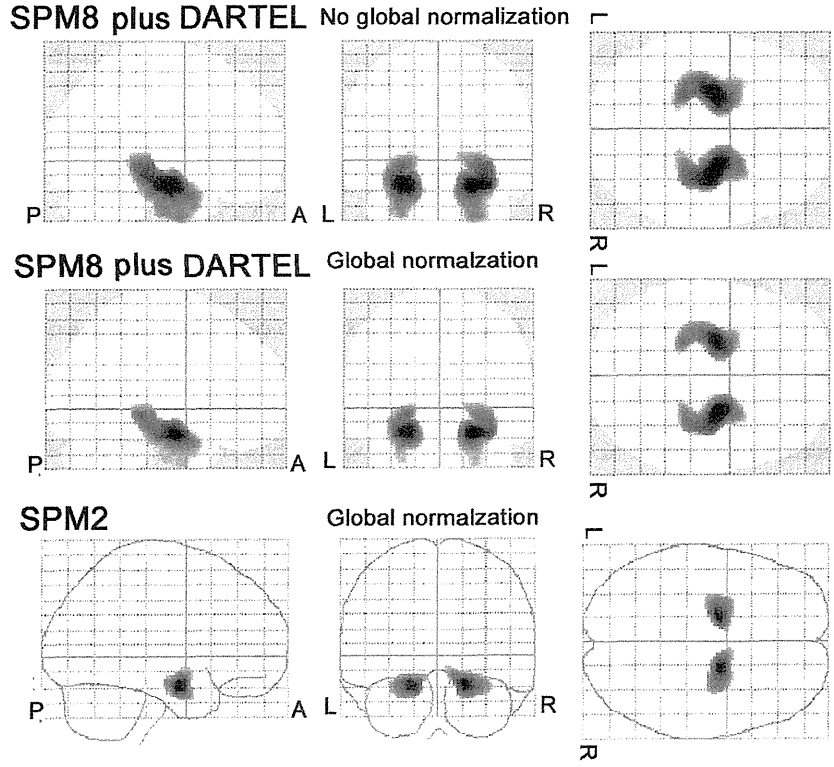

Fig 1. Group comparison of gray matter volume by SPM8 plus DARTEL and SPM2 between 30 patients with very mild AD and 40 healthy age-matched volunteers. The SPM8 plus DARTEL analysis demonstrates significant decline of gray matter volume in the bilateral medial temporal structures both with and without global normalization in patients with very mild AD. The cluster shape is very close to the anatomic configuration of the medial temporal structures involving the entorhinal cortex, amygdala, and hippocampal formation from head to tail. Although the SPM 2 analysis demonstrates a significant decline of gray matter volume in the bilateral medial temporal structures, the cluster is confined to the anterior parts of the medial temporal structures.

Table 1: Values of 4 indicators for characterizing atrophy[a]

		SPM8 plus DARTEL				SPM2			
		Target VOI			Whole-Brain	Target VOI			Whole-Brain
Group	Global Normalization	Severity	Extent (%)	Ratio	Extent (%)	Severity	Extent (%)	Ratio	Extent (%)
Healthy controls	−	0.7 ± 0.5	4.4 ± 9.8	0.8 ± 1.5	2.5 ± 4.7	NA	NA	NA	NA
	+	0.7 ± 0.3	2.0 ± 4.9	1.3 ± 2.8	1.4 ± 0.9	0.5 ± 0.3	1.8 ± 7.3	0.5 ± 1.8	2.6± 3.1
Very mild AD	−	1.8 ± 0.9[b]	39.0 ± 35.5[b]	9.9 ± 8.9[b]	5.4 ± 7.6[b]	NA	NA	NA	NA
	+	2.2 ± 0.9[b]	49.2 ± 30.2[b]	12.9 ± 7.8[b]	4.1 ± 2.5[b]	1.6 ± 1.0[b]	30.8 ± 32.1[b]	6.7 ± 7.8[b]	5.4 ± 3.7[b]
Mild AD	−	2.2 ± 0.7[b]	53.7 ± 29.8[b]	12.8 ± 8.8[b]	5.5 ± 5.1[b]	NA	NA	NA	NA
	+	2.7 ± 0.8[b]	63.7 ± 25.8[b]	15.4 ± 7.8[b]	4.3 ± 1.9[b]	2.1 ± 1.1[b]	42.0 ± 32.3[b]	9.6 ± 9.2[b]	5.4 ± 3.0[b]
Moderate-to-advanced AD	−	2.8 ± 1.0[b,c,d]	72.2 ± 26.5[b,c,d]	8.4 ± 7.2[b]	15.1 ± 14.0[b,c,d]	NA	NA	NA	NA
	+	3.0 ± 1.0[b,c]	68.7 ± 24.1[b,c]	11.7 ± 6.7[b,d]	7.1 ± 3.7[b,c,d]	2.6 ± 1.4[b,c]	56.3 ± 33.2[b,c]	7.6 ± 6.2[b]	9.0 ± 5.0[b,c,d]

Note:—NA indicates not applicable; +, presence; −, absence.
[a] Tukey honest significance test in each condition of global normalization.
[b] $P < .001$ versus healthy controls.
[c] $P < .001$ versus very mild AD group.
[d] $P < .001$ versus mild AD group.

(18, −5,15, x,y,z; $z = 5.86$) parahippocampal gyri (Fig 1). The cluster size was smaller in SPM2 than in SPM8 plus DARTEL.

The patients with AD showed significantly ($P < .001$, Tukey honest significance test) greater values than healthy controls in all 4 indicators in both SPM8 plus DARTEL and SPM2 analysis (Table 1). The mild AD group showed not significant but greater values of all 4 indicators than the very mild AD group. The moderate-to-advanced AD group showed significantly ($P < .001$) greater values of severity and extent for the target VOI and extent for the whole brain than the very mild AD group. In contrast, the ratio for the target VOI in the moderate-to-advanced AD group was lower than that in the mild AD group and almost equal to that in the very mild AD group. Global normalization in SPM8 plus DARTEL analysis elevated the severity and ratio for the target VOI and diminished the extent for the whole brain in all AD groups. The SPM2 analysis showed lower values in severity, extent, and ratio for a target VOI and greater values in extent for the whole brain than the SPM8 plus DARTEL analysis.

In the SPM8 plus DARTEL analysis, better ROC results were obtained in the condition with than without global normalization, particularly in specificity (On-line Table 1). Of the

Table 2: Values of 4 indicators in early- and late-onset subgroups in very mild AD

		SPM8 plus DARTEL				SPM2			
		Target VOI			Whole-Brain	Target VOI			Whole-Brain
Global Normalization	Onset	Severity	Extent (%)	Ratio	Extent (%)	Severity	Extent (%)	Ratio	Extent (%)
−	Early	1.5 ± 0.7	25.3 ± 31.1	3.8 ± 3.8	5.1 ± 4.9	NA	NA	NA	NA
−	Late	1.9 ± 0.9	43.8 ± 35.6	5.9 ± 8.4	11.6 ± 9.5	NA	NA	NA	NA
+	Early	1.9 ± 0.7	37.4 ± 26.2	9.5 ± 6.0	4.1 ± 1.7	1.4 ± 1.0	24.4 ± 34.6	4.7 ± 5.5	3.8 ± 2.4
+	Late	2.3 ± 0.9	53.6 ± 30.7	14.1 ± 8.1	4.1 ± 2.7	1.7 ± 1.0	33.2 ± 31.2	7.5 ± 8.5	6.0 ± 3.9

Note:—NA indicates not applicable; +, presence; −, absence.

4 indicators, the severity and extent for the target VOI with global normalization showed almost equal and high accuracy. Even in the very mild AD group, the severity showed a high accuracy of 91.6%, increasing to 95.8% in the mild AD group and 98.2% in the moderate-to-advanced AD group. SPM8 plus DARTEL showed better ROC results for all 4 indicators than SPM2.

Although the early-onset subgroup showed lower values of indicators for the target VOI than the late-onset subgroup (Table 2), global normalization elevated these indicators evenly in the early- and late-onset subgroups. These indicators were largely stable before and after global normalization in healthy controls. Consequently, ROC results in SPM8 plus DARTEL revealed equal accuracy after global normalization between these 2 subgroups (Online Table 2). In contrast, SPM2 analysis showed approximately 10% lower accuracy in the early-onset subgroup than that in the late-onset subgroup when using the severity score.

In each of 81 follow-up patients with AD, the severity score in a target VOI gradually increased from the baseline to follow-up studies. The annual increase of the severity score after global normalization was 0.27 ± 0.15.

Representative cross-sectional and longitudinal studies for SPM8 plus DARTEL analysis are demonstrated in Figs 2 and 3, respectively.

Discussion

Using severity as an indicator, we obtained a high sensitivity of 86.4% and extremely high specificity of 97.5%, resulting in an accuracy of 91.6% for discrimination of patients with very mild AD from healthy controls in the SPM8 plus DARTEL analysis. Extremely high specificity mainly contributed to this high accuracy. In the SPM2 analysis, ROC analysis presented 5% lower sensitivity and approximately 13% lower specificity compared with those in the SPM8 plus DARTEL analysis. Kawachi et al[12] reported 82.9% in both sensitivity and specificity in the patients with very mild AD in a similar VBM study by using an older SPM version, SPM99. This better specificity may result from application of the SPM8 plus DARTEL algorithm. This DARTEL algorithm can provide more precise spatial normalization to the template than the conventional algorithm.[10,13]

This improvement in the preciseness of the spatial normalization was confirmed by group comparison for determining a target VOI. The SPM8 plus DARTEL results showed significantly decreased volume with anatomically precise configura-

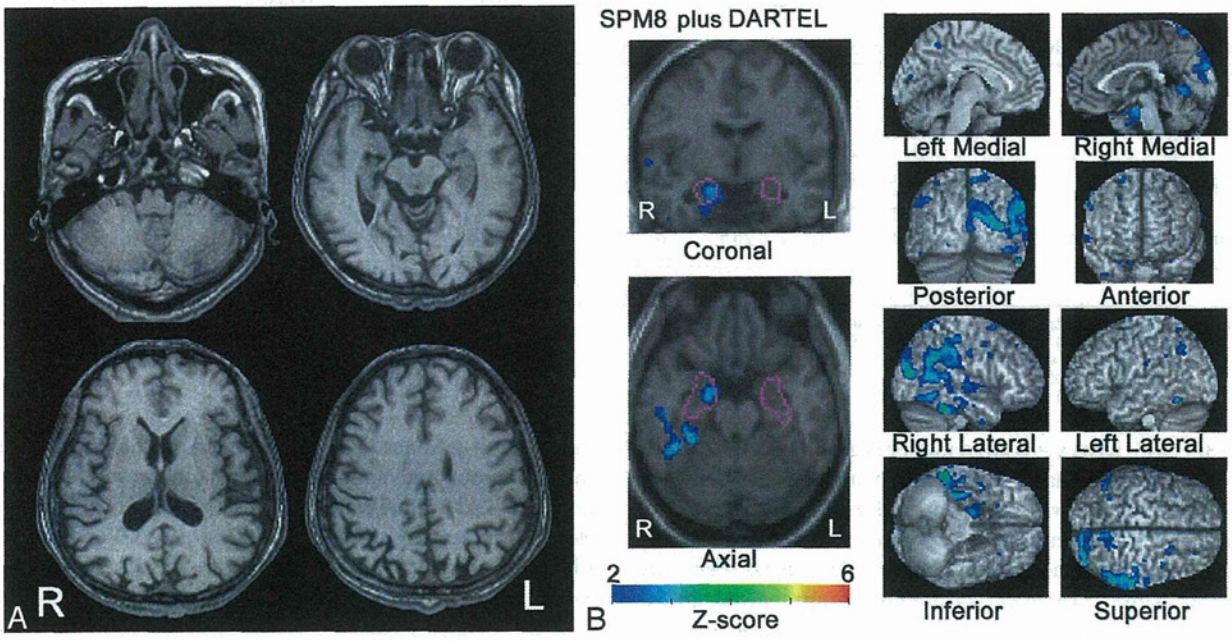

Fig 2. Cross-sectional VBM study by using SPM8 plus DARTEL. *A*, MR image of a 52-year-old woman with an MMSE score of 27. One year later the MMSE score declined to 19. *B*, SPM8 plus DARTEL analysis with global normalization reveals a significant decrease of gray matter volume in the right entorhinal area. Colored areas with *z* scores of >2 are overlaid as significantly atrophied regions on tomographic sections and cortical surface of the standardized MRI template. A target VOI in the medial temporal structures is demarcated with purple lines. The right temporoparietal cortex also shows extensive significant atrophy.

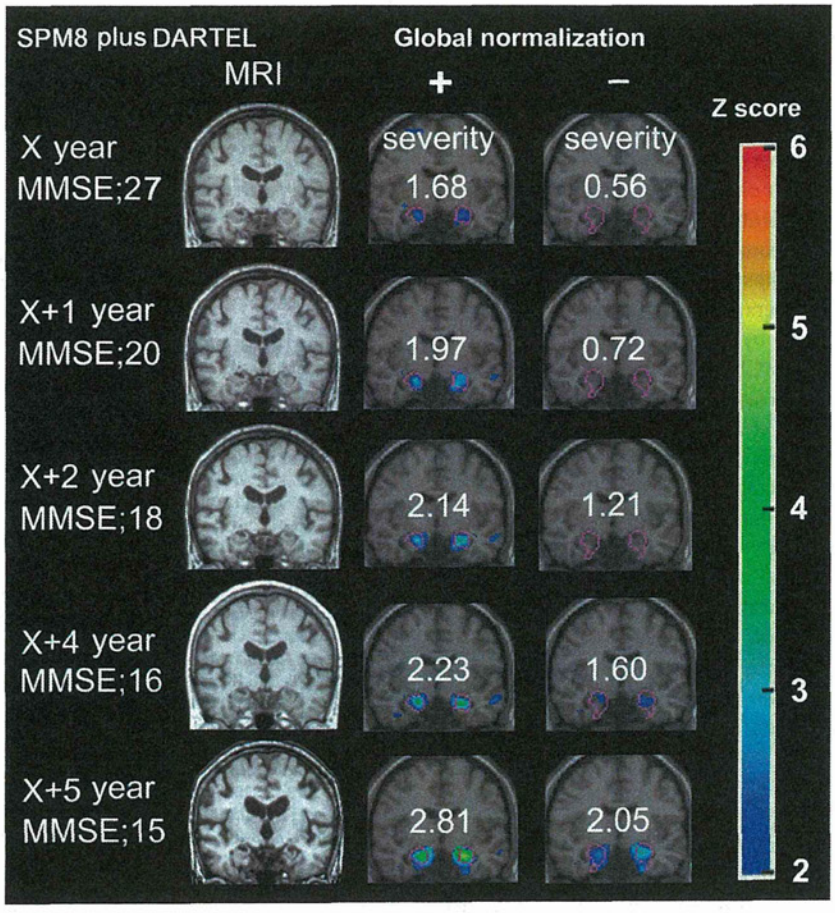

Fig 3. Longitudinal VBM studies by using SPM8 plus DARTEL. A 63-year-old woman with an MMSE score of 27 at the first visit was followed up for 6 years. One year later, the MMSE score decreased to 20 and gradually decreased thereafter. VBM analysis with global normalization reveals significant atrophy in the bilateral medial temporal areas even at the time of the initial study. Then the *z* score in a target VOI increased step by step with time. In contrast, analysis without global normalization does not demonstrate significant atrophy in the medial temporal areas for the first 3 years. Severity scores as an indicator for characterizing atrophy in the medial temporal structures are shown.

tion of the medial temporal structures involving the entorhinal cortex, amygdala, and total hippocampal formation. Takahashi et al[14] demonstrated almost identical results by using SPM8 plus DARTEL. The present SPM results by using conventional VBM by SPM2 showed decreased gray matter volume mainly in the anterior parts of the parahippocampal gyri with a less precise configuration. The severity score proved to be useful for longitudinal studies as well. The annual increase of this score may be indicative of disease progression.

The modulation in VBM allows comparison of the absolute amount of gray matter.[15] The step of global normalization allows correction of the absolute amount of gray matter for individual total brain volume. Comparison of discrimination performance demonstrated better results in the condition with than without global normalization. This difference in discrimination performance may arise from the well-known fact of selective atrophy in the medial temporal structures in AD.[1,4-6,16,17] Even if the absolute amount of gray matter of the medial temporal structures is decreased, a concomitant decrease in the total volume of gray matter would decrease specificity. The specificity in ROC analysis of the severity for the target VOI was 17% lower in the condition without than with global normalization in very mild AD. The degree of selective atrophy in the medial temporal areas can be assessed by the ratio as an indicator. In patients with AD, more than 10-fold selective atrophy was observed in the medial temporal areas compared with the whole brain in SPM8 plus DARTEL. Global normalization enhanced this ratio. Progression of neocortical atrophy would result in the decline of this ratio in advanced AD. This indicator may be useful for differentiation of AD from other neuropsychiatric diseases manifesting dementia.

The early-onset subgroup showed milder atrophy in the medial temporal structures than the late-onset subgroup. This is in line with several previous reports in which late-onset subgroups showed greater atrophy in the medial temporal structures than the early-onset subgroups.[16,17] However global normalization in SPM8 plus DARTEL extended the difference of indicators for a target VOI evenly in the early-onset and late-onset subgroups from indicators in healthy controls. This extension led to almost equal accuracy for discrimination of early-onset and late-onset very mild AD from healthy controls. This global normalization procedure may make it possible to use a common target VOI irrespective of age at onset of AD.

Thus the present study made it clear that the global normalization procedure in VBM by using SPM8 plus DARTEL has advantages in enhancing the discrimination power of diagnosing AD. However lower values of the extent for the whole brain after global normalization would underestimate neocor-

tical atrophy. The extent for the whole brain without global normalization may be useful for accurately evaluating the degree of neocortical atrophy.

This study is not without limitations. First, we should investigate whether this 1-site study is applicable to multicenter studies. Second, evaluation of the reproducibility of the present VBM technique may be necessary for longitudinal studies. Third, we investigated patients with amnestic MCI who all converted to AD. The outcome for any patient with MCI is uncertain because many subjects remain stable or even revert to a normal state, while others progress to dementia. Accordingly, the predictive study by using this VBM approach is much more important for MCI conversion to AD. Fourth, the single target VOI was used irrespective of age at onset of AD. A similar VBM study by Ishii et al[18] recommended the use of a target VOI involving not only medial temporal structures but also parietal and posterior cingulate cortices and precunei in early-onset AD. Although this software program presented the same accuracy between early- and late-onset very mild AD subgroups, we may have to investigate a more appropriate target VOI from a larger number of patients with early-onset AD. However, incorporation of 2 types of target VOIs for early- and late-onset AD into a software program may confound the program user in the selection of a target VOI in the case of follow-up studies on an approximately 65-year-old patient.

Conclusions

We proposed an automatic VBM software program of structural MRI for discrimination between patients with probable AD from the very-mild- to advanced stages and age-matched healthy controls. Application of the SPM8 plus DARTEL analysis to this software program provided a high accuracy of 91.6% for discrimination of patients with very mild AD from healthy controls by using a target VOI located in medial temporal structures. Equal accuracies were obtained in early-onset and late-onset very mild AD subgroups. This software program may be useful for early diagnosis and longitudinal evaluation of AD.

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Effect of plasma lipids, hypertension and APOE genotype on cognitive decline

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Abstract

We examined the combined effect of plasma lipids/hypertension and apolipoprotein E (APOE) genotype on cognitive function in elderly individuals. Plasma concentrations of high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglyceride (TG), total cholesterol (TC), APOE, and history of hypertension were evaluated in 622 community-dwelling individuals aged 65 years and older. We investigated the associations between plasma lipids/hypertension and cognitive function in apolipoprotein E4 allele (APOE4) carrier (E4+) and APOE4 noncarrier (E4−) groups using 3-year longitudinal data. At baseline and 3 years later, cognitive scores were correlated with plasma APOE levels in both E4− and E4+, and HDL level in E4−. The combination of hypertension and E4+, but not E4−, was associated with a significant deterioration in cognitive function during the 3-year follow-up. Our findings suggest that an interaction between APOE and HDL is facilitated by APOE4, and is possibly linked with a protective effect on cognitive decline in later life. The findings also indicate a synergistic effect of an APOE4 allele and hypertension on the acceleration of cognitive decline.

Keywords: Cognitive decline; APOE genotype; Hypertension; Apolipoprotein E (APOE); High-density lipoprotein (HDL); Low-density lipoprotein (LDL); Triglyceride (TG); Total cholesterol (TC)

1. Introduction

The presence of an apolipoprotein E4 allele (APOE4) increases the risk and reduces the age at onset of Alzheimer's disease (AD) in a dose-dependent manner (Rebeck et al., 1993; Saunders et al., 1993; Strittmatter et al., 1993). Additionally, APOE4 carriers have been reported to have higher rates of cognitive decline than noncarriers before the diagnosis of mild cognitive impairment (Caselli et al., 2007).

Apolipoprotein E (APOE) plays a significant role in cholesterol delivery to neurons and AD pathogenesis associated with amyloid-beta (Aβ) (Han, 2010; Iurescia et al., 2010; Pfrieger, 2003). The plasma level of APOE has been shown to depend upon the APOE genotype (Yasuno et al., 2011). In elderly individuals without dementia, the interactive effect of APOE and other plasma lipids on cognitive function has also been reported to vary depending upon the APOE genotype (Yasuno et al., 2011).

A complex synergism of APOE4 and cerebrovascular pathology in cognitive function of the elderly has been reported. Chronic hypertension, a major risk factor for cerebrovascular diseases, can exacerbate cerebral degenerative change (DeCarli et al., 1999; Petrovitch et al., 2000; Sparks et al., 1995) and was shown to be associated with tau

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pathology in APOE4 carriers (Kester et al., 2010). These findings appear compatible with the results of a longitudinal study showing an adverse effect of midlife hypertension on late-life cognitive function in APOE4 carriers (Peila et al., 2001).

The detrimental effect of APOE4 may be exacerbated by synergistic preventable risk factors such as plasma APOE/lipids and hypertension. With stratification by APOE allele status, we examined the effect of plasma APOE/lipids and hypertension on longitudinal change in the cognitive function of community-dwelling elderly using the data from a 3-year follow-up study.

2. Methods

2.1. Participants

Participants were recruited in the present study from the "Tone Project" in Tone town, Ibaraki, Japan (Miyamoto et al., 2009). The sampling procedures have been described elsewhere (Yasuno et al., 2011). After the assessment, a group of psychiatrists and neuropsychologists reviewed the data and reached a consensus regarding the presence or absence of psychiatric disease including dementia according to *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* (DSM-IV) criteria. We excluded the data from those with history of psychiatric diseases, and 1395 volunteers participated in the first baseline study between December 2001 and April 2002. Three years later, 622 of them who had no history of stroke during follow-up could be again evaluated between December 2004 and April 2005, and in this study, we used the results of those subjects tested twice.

At the initial examination, all of the eligible subjects provided written informed consent to their participation in the study. This study was approved by the ethics committee of Tsukuba University.

2.2. Plasma parameters

Blood samples were collected from the subjects at fasting visits at the initial examination. Plasma levels of low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglyceride (TG), and total cholesterol (TC) were measured using standard enzymatic methods on routine automated chemistry systems. Plasma APOE levels were determined by turbidimetric immunoassay. Genomic DNA was used for APOE typing. The APOE gene was amplified by the primer and amplification conditions described by Wenham and colleagues (Wenham et al., 1991). After amplification, the polymerase chain reaction (PCR) product was digested with the restriction enzyme HhaI, and subjected to electrophoresis in a 15% polyacrylamide gel.

2.3. Screening and structured interview

After blood sampling, all participants underwent a screening interview consisting of a structured questionnaire (questions on age, sex, education), a 15-item short version of the Geriatric Depression Scale (GDS) (Yesavage et al., 1982), and evaluation of their past medical histories of cardiovascular disease, diabetes mellitus, and hyperlipidemia.

2.4. Definition of hypertension requiring medication

Prior to the baseline examination, potential participants were instructed to bring all their prescribed drugs. At the baseline examination, participants were first asked whether they had a history of treated hypertension, and then medications were checked for antihypertensive therapies. Baseline blood pressure was measured. Using these data, a history of hypertension (HT) requiring medication was defined if any 1 of the following conditions was present: the history of hypertension with current use of antihypertensive medication, or systolic blood pressure (SBP)/diastolic blood pressure (DBP) $\geq$ 160/100 mm Hg at baseline.

2.5. Cognitive assessment

All participants underwent the same cognitive assessment at the baseline and 3-year examinations using a set of 4 tests to measure the following cognitive domains: attention, memory, language, and reasoning. We evaluated attention by using the Japanese version of a set-dependent activity (Sohlberg and Mateer, 1986), memory ability using the Category Cued Recall test (Grober et al., 1988), and language ability with a category fluency test (Solomon and Pendlebury, 1998). Abstract reasoning ability was evaluated with the Similarities subtest of the Wechsler Adult Intelligence Scale-Revised (WAIS-R) (Wechsler, 1981). The assessment procedures have been described elsewhere (Yasuno et al., 2011). A composite cognitive score was computed from the 4 scores using the first component of the scores of principal-component analysis (composite cognitive score = 0.853 $\times$ attention score + 0.809 $\times$ memory score + 0.856 $\times$ language score + 0.859 $\times$ reasoning score).

2.6. Statistical analysis of the effect of lipids/APOE

Subjects were divided into 2 APOE groups by E4 status with E4 noncarrier (E4–) (n = 509) (genotypes $\varepsilon2/\varepsilon3$, n = 52; $\varepsilon3/\varepsilon3$, n = 457) and E4 carrier (E4+) (n = 113) (genotypes $\varepsilon2/\varepsilon4$, n = 6; $\varepsilon3/\varepsilon4$, n = 99; $\varepsilon4/\varepsilon4$, n = 8) to test for the influence of genotype on the association between lipids and cognitive function.

Group differences in demographic characteristics were examined by unpaired t tests and Pearson χ^2 tests. To examine the influence of group differences on cognitive function, cognitive scores were compared between groups by analysis of covariance (ANCOVA), with age, sex, years of education, GDS score, cigarette smoking, and medical history of cardiovascular disease, diabetes mellitus, hyperlipidemia, and hypertension as covariates. The subjects in each category were divided into 3 strata according to the plasma concentrations of

lipids. To examine the influence of plasma lipids on cognitive function, composite cognitive scores of the 3 strata of plasma concentrations were compared in E4– and E4+ groups separately by ANCOVA, with age, sex, years of education, GDS score, cigarette smoking, and medical history of diseases as covariates. Follow-up t tests were performed to examine differences in cognitive score among the 3 strata according to the levels of lipids.

To examine the influence of plasma lipids on change in cognitive function during the 3-year follow-up, we performed repeated measures ANCOVA in the E4– and E4+ groups separately, with the level of lipids as between-subject variables, 2002 and 2005 composite cognitive scores as within-subject variables, and age, sex, years of education, GDS score, cigarette smoking, and the medical history of diseases as covariates.

To examine whether the levels of lipids were related to composite cognitive scores in the E4– and E4+ groups, we performed multiple regression analysis with composite cognitive score as dependent variable and plasma lipid levels as independent variables, after adjustment for the other factors of age, sex, years of education, GDS score, cigarette smoking, and medical history of diseases.

2.7. Statistical analysis of the effect of HT

Subjects were divided into 2 groups based on the presence/absence of HT requiring medication (HT+ and HT–) in each of the E4– and E4+ groups to test for the influence of genotype on the association between HT and cognitive function.

Group differences of HT+ and HT– in demographic characteristics were examined by unpaired t and Pearson χ^2 tests. To examine the influence of genotype and HT on the change in cognitive function during the 3-year follow-up, we performed repeated measures ANCOVA with genotype and HT as between-subject variables, 2002 and 2005 composite cognitive scores as within-subject variables, and age, sex, years of education, GDS score, cigarette smoking, and the medical history of diseases as covariates.

To examine the change in cognitive function during the 3-year follow-up, composite cognitive scores of the 2002 and 2005 examinations were compared in genotype/HT groups separately by repeated measures ANCOVA, with age, sex, years of education, GDS score, cigarette smoking, and medical history of diseases as covariates.

To examine the change in cognitive function between the groups with and without HT, composite cognitive scores in 2002 and 2005 were compared between HT+ and HT– groups by ANCOVA with age, sex, years of education, GDS score, cigarette smoking, and the medical history of diseases as covariates in the E4+ and E4– groups separately.

Multiple comparisons were adjusted by Bonferroni correction. All statistical tests were 2-tailed and reported at $\alpha < 0.05$. Effect sizes were calculated using partial η^2 to estimate and compare the effect of the level of lipids on cognitive score between groups of different sample size. $\eta < 0.01$ was regarded as no substantial effect. Statistical analysis of the data was performed using SPSS for Windows 19.0 (IBM, Japan Inc., Tokyo, Japan).

3. Results

The demographic data for the E4– and E4+ groups in the analysis of the effect of lipids/APOE on cognitive function are shown in Table 1. There were no group differences in demographic characteristics except for the cognitive score. Our finding of a higher cognitive score at 2002 and 2005 in the E4– group is consistent with previous studies (Small et al., 2004).

Tables 2 and 3 show the median plasma concentrations of lipids for the 3 strata according to the tertiles of plasma levels of lipids/APOE, and the mean cognitive scores of the

Table 1
Demographic characteristics in the analysis of the effect of lipids/lipoproteins

Characteristic	APOE E4– (n = 509)	APOE E4+ (n = 113)	t, χ^2, or F	p
Age, y[a]	73.0 ± 5.4	72.7 ± 4.8	$t_{620} = 0.5$	0.6
Male, n (%)[b]	226 (44)	43 (38)	$\chi^2_1 = 1.5$	0.2
Years of education[a]	10.1 ± 2.6	10.3 ± 2.9	$t_{620} = 0.3$	0.5
GDS score[a]	2.6 ± 2.5	2.1 ± 2.2	$t_{620} = 1.8$	0.1
Cigarette smoking, n (%)[b]	188 (37)	37 (33)	$\chi^2_1 = 0.7$	0.4
History of disease, n (%)[b]				
Cardiovascular disease	15 (2.9)	2 (1.7)	$\chi^2_1 = 0.5$	0.5
Diabetes mellitus	20 (3.9)	7 (6.1)	$\chi^2_1 = 1.1$	0.3
Hyperlipidemia	19 (3.7)	7 (6.1)	$\chi^2_1 = 1.4$	0.2
Hypertension	195 (38.3)	44 (38.9)	$\chi^2_1 = 0.02$	0.9
Cognitive score in 2002[c]	42.8 ± 11.7	40.3 ± 11.8	$F(1,611) = 4.1$	0.04
Cognitive score in 2005[c]	43.1 ± 13.8	39.0 ± 13.8	$F(1,611) = 8.3$	0.004

Data are mean ± SD after adjustment for covariates.

Key: APOE, apolipoprotein E; E4+, E4 allele carrier; E4–, E4 allele noncarrier; GDS, Geriatric Depression Scale.

[a] p value was calculated by unpaired 2-tailed t test.

[b] p value was calculated by Pearson χ^2 2-tailed test.

[c] p value was calculated by analysis of covariance (ANCOVA) with age, sex, and years of education, GDS score, cigarette smoking, and medical history of cardiovascular disease, diabetes mellitus, hyperlipidemia, and hypertension as covariates.

Table 2
Mean cognitive test score of each tertile groups of lipid levels in APOE E4– group[a]

	Plasma lipid/APOE level, tertiles, median (minimum-maximum)			ANCOVA (df = 2,498)			Group difference[b]
	Low	Middle	High	F	p	η^2	
HDL level (mg/dL)[c]	41.0 (25.0–47.0)	52.0 (48.0–60.0)	69.0 (61.0–138.0)				
Cognitive score in 2002	39.5 ± 11.7	42.9 ± 11.2	45.2 ± 11.7	9.3	< 0.001	0.04	a, b
Cognitive score in 2005	39.1 ± 13.5	43.9 ± 12.9	45.3 ± 13.5	9.3	< 0.001	0.04	a, b
APOE level (mg/dL)[d]	1.2 (0.5–1.7)	2.2 (1.8–3.0)	3.8 (3.1–10.5)				
Cognitive score in 2002	38.4 ± 11.5	42.8 ± 11.5	44.7 ± 11.6	11.3	< 0.001	0.04	a, b
Cognitive score in 2005	39.0 ± 13.4	43.0 ± 13.3	44.9 ± 13.4	7.3	0.001	0.03	a, b
LDL level (mg/dL)[e]	73.0 (25.6–87.0)	99.0 (88.0–116.0)	134.0 (117.0–350.0)				
Cognitive score in 2002	43.1 ± 11.8	42.9 ± 11.7	41.7 ± 11.9	0.7	0.5	0.003	
Cognitive score in 2005	42.9 ± 13.6	44.1 ± 13.4	41.6 ± 13.7	1.4	0.2	0.006	
TG level (mg/dL)[f]	85.0 (32.0–119.0)	148.0 (120.0–183.0)	258.0 (184.0–921.0)				
Cognitive score in 2002	42.0 ± 11.7	42.4 ± 11.7	43.4 ± 11.7	0.6	0.6	0.002	
Cognitive score in 2005	42.2 ± 13.4	42.9 ± 13.4	43.6 ± 13.4	0.4	0.7	0.002	
TC level (mg/dL)[g]	169 (95–190)	205 (191–220)	240 (221–360)				
Cognitive score in 2002	41.2 ± 12.0	42.3 ± 11.7	44.2 ± 12.0	2.5	0.1	0.01	
Cognitive score in 2005	41.2 ± 13.8	43.0 ± 13.4	44.4 ± 13.8	2.2	0.1	0.009	

Key: ANCOVA, analysis of covariance; APOE, apolipoprotein E; E4–, E4 allele noncarrier; GDS, Geriatric Depression Scale; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TC, total cholesterol; TG, triglyceride.

[a] Data are mean ± SD after adjustment for age, sex, years of education, GDS score, cigarette smoking, and medical history of cardiovascular disease, diabetes mellitus, and hypertension.

[b] Indicates significance at $p < 0.05$ after Bonferroni adjustment for multiple comparisons: a, low to middle concentration group comparison; b, low to high concentration group comparison.

[c-g] With repeated measures ANCOVA, no significant interaction between within-subject factor of 2002/2005 cognitive scores and between-subjects factor of tertiles of plasma lipids concentration (df = 2, 498; c, F = 1.4, p = 0.3; d, F = 0.1, p = 0.9; e, F = 1.5, p = 0.2; f, F = 0.06, p = 0.9; g, F = 0.2, p = 0.8).

E4– and E4+ groups at 2002 and 2005 according to the 3 strata of plasma concentrations of lipids/APOE.

ANCOVA analysis evaluating the influence of lipids level on cognitive function showed a significant influence of the HDL level on composite cognitive scores at both 2002 and 2005 in the E4– group (Table 2). Subjects with higher HDL concentrations had higher cognitive scores. The effect size of the influence of the plasma HDL level on cognitive

Table 3
Mean cognitive test score of each tertile groups of lipid levels in APOE E4+ group[a]

	Plasma lipid/APOE level, tertiles, median (minimum-maximum)			ANCOVA (df = 2,102)			Group difference[b]
	Low	Middle	High	F	p	η^2	
HDL level (mg/dL)[c]	41.0 (25.0–47.0)	52.0 (48.0–60.0)	69.0 (61.0–138.0)				
Cognitive score in 2002	39.8 ± 12.5	43.3 ±12.8	41.6 ± 12.7	0.7	0.5	0.01	
Cognitive score in 2005	40.0 ± 15.8	43.2 ±16.1	38.7 ± 16.0	0.7	0.5	0.01	
APOE level (mg/dL)[d]	1.2 (0.5–1.7)	2.2 (1.8–3.0)	3.8 (3.1–10.5)				
Cognitive score in 2002	40.4 ± 12.1	33.2 ± 12.1	50.6 ± 12.2	7.0	0.001	0.1	b, c
Cognitive score in 2005	39.6 ± 15.7	35.5 ± 15.7	45.7 ± 15.7	4.0	0.02	0.07	c
LDL level (mg/dL)[e]	73.0 (25.6–87.0)	99.0 (88.0–116.0)	134.0 (117.0–350.0)				
Cognitive score in 2002	42.6 ± 13.0	41.0 ± 12.6	40.8 ± 12.9	0.2	0.8	0.004	
Cognitive score in 2005	39.0 ± 12.1	42.1 ± 12.0	39.7 ± 12.3	0.4	0.7	0.007	
TG level (mg/dL)[f]	85.0 (32.0–119.0)	148.0 (120.0–183.0)	258.0 (184.0–921.0)				
Cognitive score in 2002	40.7 ± 12.5	40.3 ± 12.5	43.2 ± 12.8	0.5	0.6	0.009	
Cognitive score in 2005	38.9 ± 15.7	39.1 ± 15.6	43.0 ± 16.1	0.8	0.5	0.01	
TC level (mg/dL)[g]	169 (95–190)	205 (191–220)	240 (221–360)				
Cognitive score in 2002	38.8 ± 13.3	42.4 ± 12.6	42.0 ± 12.8	0.7	0.5	0.01	
Cognitive score in 2005	38.3 ± 16.8	41.5 ± 15.9	40.4 ± 161	0.3	0.8	0.005	

Key: ANCOVA, analysis of covariance; APOE, apolipoprotein E; E4+, E4 allele carrier; GDS, Geriatric Depression Scale; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TC, total cholesterol; TG, triglyceride.

[a] Data are mean ± SD after adjustment for age, sex, years of education, GDS score, and medical history of cardiovascular disease, diabetes mellitus, and hypertension.

[b] Indicates significance at $p < 0.05$ after Bonferroni adjustment for multiple comparisons: a, low to middle concentration group comparison; b, low to high concentration group comparison; c, middle to high concentration group comparison.

[c-g] With repeated measures ANCOVA, no significant interaction between within-subject factor of 2002/2005 cognitive scores and between-subjects factor of tertiles of plasma lipids concentration (df = 2, 102; c, F = 1.3, p = 0.3; d, F = 0.04, p = 1.0; e, F = 2.2, p = 0.1; f, F = 0.3, p = 0.7; g, F = 0.1, p = 0.9).

Table 4
Demographic characteristics of subjects in the analysis of the effect of hypertension

Characteristic	APOE E4– (n = 509)				APOE E4+ (n = 113)			
	HT–, n = 231	HT+, n = 278	t, χ^2, or F	p	HT–, n = 58	HT+, n = 55	t, χ^2, or F	p
Age[a]	72.4 ± 5.4	73.5 ± 5.3	t_{507} = 2.4	0.02	71.9 ± 4.2	73.6 ± 5.3	t_{111} = 2.0	0.05
Male, n (%)[b]	107 (46)	119 (43)	χ^2_1 = 0.6	0.4	18 (31)	25 (45)	χ^2_1 = 2.5	0.1
Systolic BP (mm Hg)[a]	136.7 ± 14.0	158.3 ± 20.8	t_{507} = 13.5	< 0.001	137.2 ± 13.8	159.4 ± 19.0	t_{111} = 7.1	< 0.001
Diastolic BP (mm Hg)[a]	80.3 ± 10.1	86.7 ± 12.3	t_{507} = 6.4	< 0.001	79.3 ± 9.1	85.2 ± 13.2	t_{111} = 2.8	0.007
Education, y[a]	10.2 ± 2.7	10.0 ± 2.6	t_{507} = 0.9	0.4	10.5 ± 3.1	10.0 ± 2.6	t_{111} = 0.5	0.6
GDS score[a]	2.5 ± 2.5	2.6 ± 2.5	t_{507} = 0.5	0.6	1.8 ± 1.9	2.4 ± 2.5	t_{111} = 1.5	0.1
Cigarette-smoking, n (%)	91 (40)	97 (35)	χ^2_1 = 1.1	0.3	16 (28)	21 (38)	χ^2_1 = 1.4	0.2
Disease, n (%)								
Cardiovascular disease[b]	6 (2.6)	9 (3.2)	χ^2_1 = 0.2	0.7	1 (1.7)	1 (1.8)	χ^2_1 = .001	0.9
Diabetes mellitus[b]	8 (3.5)	12 (4.3)	χ^2_1 = 0.2	0.6	4 (6.9)	3 (5.5)	χ^2_1 = 0.1	0.8
Hyperlipidemia[b]	10 (4.3)	9 (3.2)	χ^2_1 = 0.4	0.5	2 (3.4)	5 (9.1)	χ^2_1 = 1.6	0.2
Plasma lipids/APOE (mg/dL)								
TC[c]	204.7 ± 34.9	205.1 ± 34.9	F(1,500) = 0.02	0.9	214.6 ± 33.2	211.2 ± 33.2	F(1,104) = 0.3	0.6
HDL[c]	56.2 ± 15.2	53.7 ± 14.9	F(1,500) = 3.9	0.06	54.2 ± 14.4	55.7 ± 14.4	F(1,104) = 0.3	0.6
LDL[c]	100.8 ± 32.8	105.4 ± 32.7	F(1,500) = 2.5	0.1	105.7 ± 31.8	106.6 ± 31.8	F(1,104) = 0.03	0.9
TG[c]	167.3 ± 112.2	184.1 ± 112.2	F(1,500) = 2.8	0.1	193.0 ± 109.5	167.9 ± 109.6	F(1,104) = 1.5	0.2
APOE[c]	2.6 ± 1.4	2.5 ± 1.4	F(1,500) = 1.7	0.2	2.4 ± 1.3	2.2 ± 1.3	F(1,104) = 1.3	0.3

Data are mean ± SD after adjustment for covariates.
Key: ANCOVA, analysis of covariance; APOE, apolipoprotein E; BP, blood pressure; E4+, E4 allele carrier; E4–, E4 allele noncarrier; GDS, Geriatric Depression Scale; HDL, high-density lipoprotein; HT–, subjects with no hypertension requiring medication; HT+, subjects with hypertension requiring medication; LDL, low-density lipoprotein; TC, total cholesterol; TG, triglyceride.
[a] p value was calculated by unpaired 2-tailed t test.
[b] p value was calculated by Pearson χ^2 2-tailed test.
[c] p value was calculated by ANCOVA with age, sex, years of education, GDS score, cigarette smoking, and medical history of cardiovascular disease and diabetes mellitus as covariates.

score was more than 0.01. No such significant association was observed in the E4+ group (Table 3).

A significant main effect of the APOE level was found by ANCOVA on composite cognitive scores at 2002 and 2005 in both E4– and E4+ groups (Tables 2 and 3). Subjects with higher plasma APOE concentration had higher cognitive scores in both groups. The effect size of the association of the plasma APOE level on these cognitive scores was more than 0.01.

The results of ANCOVA analysis were supported by the multiple regression analysis evaluating whether the levels of HDL and APOE were related to cognitive scores. The plasma HDL level positively related to the composite cognitive score at 2002 ($\beta = 0.13, p < 0.001$) and at 2005 ($\beta = 0.14, p < 0.001$) in the E4– group. Plasma APOE level positively related to cognitive score at 2002 ($\beta = 0.12, p < 0.001$) and at 2005 ($\beta = 0.11, p = 0.001$) in the E4– group, and at 2002 ($\beta = 0.14, p = 0.05$) and at 2005 ($\beta = 0.13, p = 0.05$) in the E4+ group.

On the contrary, in the repeated measures ANCOVA analysis evaluating the influence of plasma lipids on the change of cognitive function during the 3-year follow-up, we found no significant interaction between the concentration of lipids and the 2002 and 2005 cognitive scores in all measured comparisons (Tables 2 and 3).

The demographic data of the HT–/HT+ in E4–/E4+ groups in the analysis of the effect of HT on cognitive function are shown in Table 4. There were no group differences in demographic characteristics in each genotype except age, and systolic and diastolic blood pressure in the E4– and E4+ groups.

Table 5 shows the change of the cognitive scores between 2002 and 2005 in the HT–/HT+ groups. With repeated measures ANCOVA, we found a significant interaction in the within-subject factor of 2002/2005 cognitive scores and the between-subject factor of the E4–/E4+ and HT–/HT+ groups [F(1,610) = 4.24. p = .04]. In the repeated measures ANCOVA analysis, we found a significant decrease in cognitive score during the 3 years of follow-up in the HT+/E4+ group (Table 5). When we added the plasma levels of APOE and HDL to the covariates in the above analysis, the results did not change.

The difference in cognitive scores between the HT– and HT+ groups in 2002 and 2005 is also shown in Table 5. In 2002, we found no significant difference in the cognitive scores between the HT– and HT+ groups in both the E4– and E4+ groups. Three years later, we found a significant difference in cognitive scores between the HT– and HT+ groups in the E4+ group, and subjects with HT had lower cognitive score.

Table 5
Mean cognitive test score in 2002 and 2005 in each groups of HT− and HT+/APOE E4− and APOE E4+

	Cognitive score		Repeated measures ANCOVA[a]			
	2002	2005	*df*	*F*	*p*	η^2
APOE E4−						
HT− (n = 231)	43.6 ± 11.0	43.7 ± 12.7	1, 222	0.05	0.8	0.000
HT+ (n = 278)	41.7 ± 11.8	42.1 ± 13.8	1, 269	0.8	0.4	0.003
ANCOVA[b]						
$F(1,499)$, p, η^2	0.05, 0.8, 0.000	0.06, 0.8, 0.000				
APOE E4+						
HT− (n = 58)	43.4 ± 13.8	44.3 ± 15.6	1, 49	0.8	0.4	0.01
HT+ (n = 55)	39.3 ± 10.9	36.1 ± 14.4	1, 46	5.3	0.03	0.1
ANCOVA[b]						
$F(1,103)$, p, η^2	0.4, 0.5, 0.004	3.9, 0.05, 0.04				

Key: ANCOVA, analysis of covariance; APOE, apolipoprotein E; E4+, E4 allele carrier; E4−, E4 allele noncarrier; GDS, Geriatric Depression Scale; HT−, subjects with no hypertension requiring medication; HT+, subjects with hypertension requiring medication.

[a] Repeated measures ANCOVA with 2002/2005 test years as within-subject factor with age, sex, years of education, GDS score, and medical history of cardiovascular disease, diabetes mellitus, and hyperlipidemia as covariates.

[b] ANCOVA with HT− and HT+ as between-subjects factor with age, sex, years of education, GDS score, and medical history of cardiovascular disease, diabetes mellitus, and hyperlipidemia as covariates.

4. Discussion

Each of the analyses using the data from the baseline and 3-year follow-up examinations revealed that cognitive scores were associated with the plasma APOE level in both E4− and E4+, and the HDL level in E4−. Another major finding was that the HT+/E4+ group showed a decline in cognitive score in the follow-up study. We will discuss these 2 findings.

APOE plays a significant role in response to neuronal injury by reducing inflammation, endothelial dysfunction, and lipid oxidation (Davignon et al., 1999). An antioxidant role of APOE in promoting the regression of atherosclerosis has also been reported (Tangirala et al., 2001). It is possible that a lower plasma APOE level impairs these normal physiological functions (Masliah et al., 1995). If this is the case, a lower plasma APOE level may lead to cognitive decline and the exacerbation of cerebral degenerative changes. On the other hand, APOE is thought to bind Aβ and promote its clearance and degradation, such that a lower APOE level may reduce the efficiency of Aβ clearance, and contribute to AD pathogenesis (Stratman et al., 2005).

Higher plasma levels of HDL were associated with better cognitive function in the E4− group. Low-level HDL is thought to be a risk factor for atherosclerotic diseases (Breteler et al., 1994; Kalaria, 2000), and it has been reported that HDL might prevent aggregation and polymerization of amyloid in the human brain (Koudinov et al., 1998; Olesen and Dagø, 2000). Anti-inflammatory properties of HDL could prevent inflammation from neurodegenerative processes (Cockerill et al., 2001).

Recent studies have presented evidence for the involvement of internalized triglyceride-rich lipoprotein (TRL)-derived APOE in the regulation of HDL metabolism (Heeren et al., 2003). The greater portion of TRL-derived APOE remains in peripheral recycling endosomes. This pool of APOE is then mobilized by HDL to be recycled back to the plasma membrane, followed by APOE resecretion and the subsequent formation of APOE-containing HDL. This recycling of APOE may prevent cognitive decline. We found no significant association between HDL and cognitive function in the E4+ group. A recent study has shown that HDL-induced recycling of TRL-derived apolipoprotein E4 allele (APOE4) is relatively inefficient (Heeren et al., 2004). Thus, in the E4+ group, the inefficiency might reduce the recycling of APOE and decrease the protective effect of HDL on cognitive decline.

As an intrinsic part of the cognitive score, we also performed the same analysis on the memory score. In that analysis, the primary outcome of the composite cognitive score is not entirely driven by memory score. The significance of the influence of APOE levels on memory scores was not shown in 2005 in E4− ($F(2,497) = 1.7$, $p = 0.18$) and in 2002 and 2005 in E4+ group ($F(2,102) = 2.5$, $p = 0.09$ in 2002; $F(2,102) = 2.3$, $p = 0.11$ in 2005). This means that APOE levels affect not only memory but also a wide range of other cognitive functions and it may support our consideration of the cerebrovascular contributions to the results.

Although no significant difference in cognitive function was found at baseline between the HT−/E4+ and HT+/E4+ groups, the latter showed decline in cognitive score in the follow-up study. These findings suggest that the combined effect of APOE4 and HT had a relatively small-magnitude effect on cognitive function prior to the baseline assessment, but affected cognitive function during the observation period. One possible explanation is that damage to cerebral function by the combined effect of these risk factors starts in midlife (Peila et al., 2001), but cerebral and cognitive reserve is enough to cope with detrimental effects in midlife. However, at some point in later life, the accumulated damage by the combined effect may exceed the brain reserve capacity and accelerate cognitive decline.

It is of interest that the HT+/E4+ group showed cognitive decline over as little as 3 years. Judging from the baseline demographic data, many of the APOE4 carriers were in their early to midseventies. According to the data from Corder et al., the mean age at onset of dementia decreased from 84 to 76, and then to 68 years in accordance with increasing numbers of APOE4 alleles in families with late-onset AD (Corder et al., 1993). Taking this and our findings together, it seems that the early to midseventies is a critical stage for APOE4 carriers, and especially for those with HT.

Chronic hypertension can induce atherosclerosis and capillary damage (Farkas and Luiten, 2001). The atherosclerotic conditions related to chronic hypertension are thought to induce cerebral hypoxia/ischemia, creating ischemia-related injuries that can ultimately lead to clinical and subclinical brain damage (Skoog et al., 1996). Furthermore, HT has been reported to be correlated with increased neuritic plaques and neurofibrillary tangles (DeCarli et al., 1999; Petrovitch et al., 2000; Sparks et al., 1995). On the contrary, APOE is considered to have a central role in response to neuronal injury, but its neuroprotective action is highly allele-specific: the apolipoprotein E3 allele (APOE3) seems to promote the repair process, whereas APOE4 seems to retard it both in vitro and in vivo (Pedersen et al., 2000). Further, the amount of Aβ and tau pathology has been reported to be greater in APOE4 carriers than in noncarriers (Leoni, 2011). Thus the impact of HT on brain damage would be expected to be much greater for E4+ carriers (Peila et al., 2001).

The present study has limitations. First, we have not quantified the longitudinal data for lipid and blood pressure prior to the baseline evaluation. Second, because we have no autopsies, an accurate pathological background of the cognitive decline among the E4+ carriers has not been determined. However, our longitudinal study is now continuing, and we hope that these limitations will be overcome in the future.

In conclusion, our findings suggest that a possible interaction between APOE and HDL may be linked to a protective effect on cognitive decline and that the interaction is affected by APOE4 allele in later life. Our present study also indicates a synergistic effect of APOE4 and HT on the cognitive decline during the 3-year follow-up. It is known that neuropathological cascades leading to cognitive impairment and AD start to develop before the manifestation of cognitive impairment. Therefore, use of antihypertensive medication while ensuring higher plasma APOE and HDL from an earlier stage of life may be useful for the maintenance of cognitive function in later life, and especially for APOE4 carriers.

Disclosure statement

The authors disclose no conflicts of interest.

Appropriate approval procedures were used concerning human subjects. At the initial examination, all of the eligible subjects provided written informed consent to their participation in the study. This study was approved by the ethics committee of Tsukuba University.

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RESEARCH ARTICLE

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Prevalence of depression and depressive symptoms among older Japanese people: comorbidity of mild cognitive impairment and depression

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Background: The aim of the study was to estimate the prevalence of *DSM-III-R* major depressive episodes (MDEs), depressive symptoms cases (DSCs) (defined as a score of ≥6 on the Geriatric Depression Scale but falling short of MDE), and coexisting mild cognitive impairment (MCI) among Japanese community-dwelling older people.

Methods: Prevalence was estimated based on screening evaluation, individual interviews, and door-to-door visits. MDE and DSC were diagnosed, and the cognitive status of the participants was determined to be dementia, MCI, or normal.

Results: A total of 1888 subjects of 2698 candidates (70.0%) participated. The prevalence of MDE and DSC were estimated to be 4.5% (95% CI, 3.4–6.0) and 11.5% (95% CI, 4.2–28.0), respectively. MCI was more prevalent in subjects with *depression* (26.2%) than those with normal mood (17.9%). Although no prototypical profile of cognitive dysfunction was revealed, multiple MCI was more prevalent in subjects with *depression* (12.2%) than subjects with normal mood (3.8%). Conversely, subjects with MCI (26.3%) were more likely to develop *depression* compared with those with normal cognitive function (18.0%).

Conclusions: The prevalence of *depression* in our subjects seems to be similar with that of previous studies. MCI was more prevalent in subjects with *depression* than those with normal mood. Individuals with *depression* showed no particular association with any of the four MCIs. Given that depression and MCI are often associated with each other and that MCI is a predictor for development of dementia, the risk of developing dementia in the depressed older people with coexisting MCI should be acknowledged.

Key words: community study; depression; mild cognitive impairment; older people; prevalence
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Introduction

A large number of studies have investigated the prevalence of depression in later life. Reviewing the relevant literature from 1993 onward, Djernes (2006) noted that methodological differences between the studies hinder consistent conclusions about geographical and cross-cultural variations in the prevalence of depression in populations of older White people.

During the last decade, a number of attempts have been made to detect a distinct state of abnormal cognition that does not amount to dementia but is