distinguishable from normal cognitive decline associated with aging. Herein, the term *mild cognitive impairment* (MCI) is used to describe such transitional status. Currently, MCI as defined by Petersen *et al.* (1999) is the most frequently used definition of MCI (amnestic MCI). A mount of studies reported the prevalence of MCI, and their prevalence widely ranged (Panza *et al.*, 2005). Many of the studies reported 3%–5% prevalence for the amnestic MCI (Ritchie *et al.*, 2001; Hanninen *et al.*, 2002; Ganguli *et al.*, 2004).

A longitudinal study has shown that depressed mood was more common in individuals with MCI than those without cognitive deficits (Devanand *et al.*, 1996), and increasing attention has been paid to the relationship between depression and MCI (Steffens *et al.*, 2006; Panza *et al.*, 2010). Although there are several epidemiological studies that have reported the prevalence of depression among community-dwelling older people with MCI, little is known about the prevalence of MCI among those with depression (Panza *et al.*, 2010). In the present study, the prevalence of depression and its comorbidity with MCI was estimated in an older Japanese population.

Methods

The study was conducted in Tone Town of the Ibaraki prefecture, which is located approximately 40 km northeast of central Tokyo, consists of 22 districts, and has a population of about 20,000. The town was originally an agricultural area, but with mass migration into the town because of rapid economic growth over the past few decades, it became one of the typical Japanese commuter suburbs, consisting of residents of new town and old farming households.

As of 1 May 2001, 3083 inhabitants 65 years and older lived in Tone Town. (These 3083 inhabitants are hereafter referred to as the original candidates.) The proportion of aging (65 years and older) for the town was 15.6% at that time, whereas that for the overall Japan of the census day of 2000 was 17.2%. The employment structure ratio by industry in the town and the one in overall Japan were as follows: 5.1% and 5.4% for the primary industry; 28.9% and 20.5% for the secondary; and 65.2% and 64.3% for the tertiary. The previously mentioned data show that the proportion of the aging and the employment structure ratio of the town are very similar to the average in Japan. Therefore, the town, one of the typical Japanese suburb areas, would be considered a reflection of Japan at that time and an adequate sample.

The prevalence of depression was estimated using a three-phase design. Seven psychiatrists and eight psychologists, who were trained for this study by the authors, and public health nurses conducted the first phase (screening and clinical evaluation), the second phase (structured interview and cognitive assessment), and the third phase (door-to-door visits) of the study (Figure 1). The protocol of this study was approved by the ethics committee of the University of Tsukuba.

Phase 1

The first phase was conducted between December 2001 and April 2002. Before the baseline examination, invitation letters explaining the purpose and meaning of the project were sent to the original candidates. Local welfare commissioners, who are vested with promoting social welfare in each local area, were asked for their cooperation to increase the participation rate.

They called for the intended residents to participate in this research, regardless of their cognitive and mood status.

Individuals with whom a local welfare commissioner could not meet with and individuals whom no contact could be made, despite three telephone calls within the week prior to the initial examination, were excluded and termed as non-contacted individuals.

Each of the 22 districts was visited once a week, and group screenings were conducted. After giving their informed consent, all participants underwent a screening interview. In addition to the group screenings at the 22 districts, 44 individuals who were institutionalized in a long-term care facility were visited and examined using the same methods described in the following text.

Assessment procedures

Demographic and medical and psychiatric issues. The interview consisted of a structured questionnaire that assessed age, gender, education, self-reported previous medical and psychiatric diseases, current medication use, and a series of dementia risk factors including alcohol and tobacco consumption.

Mood status. This interview was followed by the 15-item short version of the Geriatric Depression Scale (GDS) for mood assessment. Those who scored ≥6 were considered to have depressive symptoms (Brink *et al.*, 1982).

Perceived cognitive difficulty. The participants were asked whether they had cognitive difficulties in

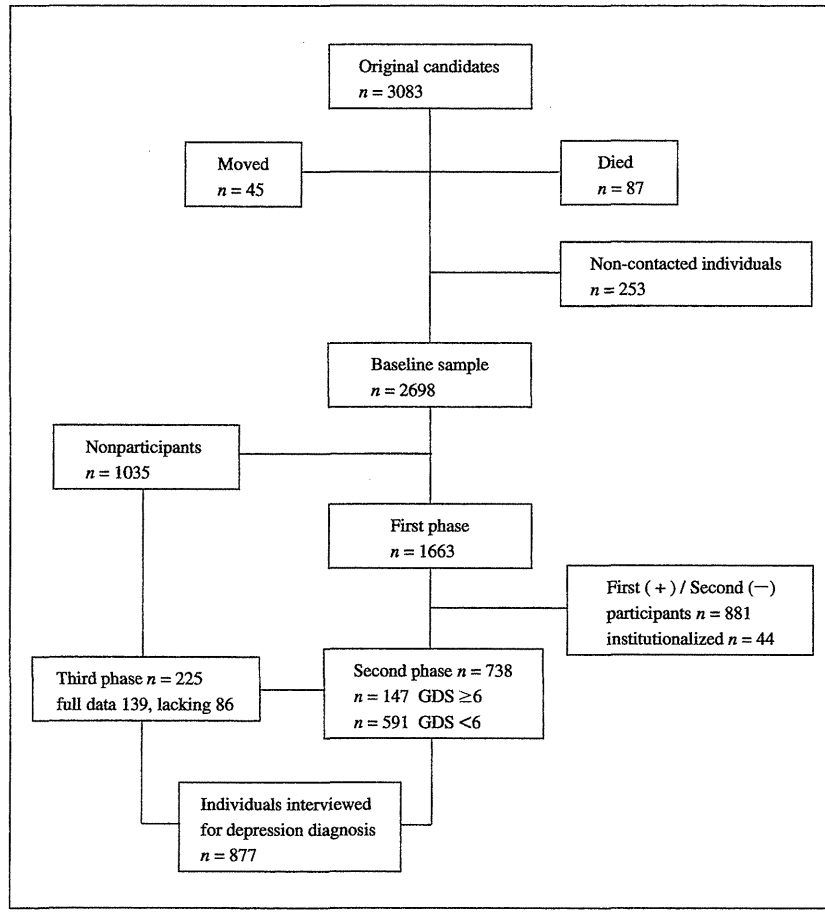

Figure 1 Flowchart for the diagnosis of depression.

general, as well as difficulties in specific areas according to the 19 items of the Détérioration Cognitive Observée questionnaire, which had been originally developed for an objective assessment of memory difficulty. The participants were considered to have cognitive complaints if they indicated that they had problems on ≥1 of the items (Ritchie and Fuhrer, 1992).

Assessment of activities of daily living. Basic activities of daily living (ADL) were measured using Nishimura's ADL (NADL) (Nishimura *et al.*, 1993), which determines the level of independence in five activities as follows: walking/transferring, going outside, dressing/bathing, feeding, and toileting. Respondents were considered to be functionally intact if they reported no difficulty on any of the five items of the NADL.

Neuropsychological assessment battery. After completing the interview, all the participants underwent a group assessment using a set of five tests (hereafter named 5-Cog), which measured the following cognitive domains: attention, memory, visuospatial function, language, and reasoning. Each of the tests has been reported to be valid and reliable. The details of the assessment battery have been reported (Sasaki *et al.*, 2009), and the battery is briefly described in the succeeding text.

Attention was evaluated using a Japanese version of a set dependency analysis (Sohlberg and Mateer, 1986). In order to assess memory ability, a category-cued recall test was used (Grober *et al.*, 1988). The clock drawing test, which requires subjects to draw the hands of a clock to depict the time at "ten after eleven" (Freedman *et al.*, 1994), was used for the assessment of visuospatial function. Language ability was examined using a category fluency test (Soloman and Pendlebury, 1998). To assess abstract reasoning ability, we used the similarity subset of the revised Wechsler Adult Intelligence Scale (Wechsler, 1981).

Test–retest reliability of the 5-Cog was confirmed using data from 38 randomly selected original participants collected at a mean interval of 64 days (SD = 28 days).

Consensus diagnosis of dementia. After each assessment, a group of psychiatrists and neuropsychologists reviewed the functional, medical, neurologic, psychiatric, and neuropsychological data and reached a consensus regarding the presence or absence of the *DSM-III-R* dementia (American Psychiatric Association, 1987). Only those subjects who were not diagnosed as having dementia were considered for a diagnosis of depression and/or MCI.

Mild cognitive impairment diagnostic criteria. Criteria for MCI were retrospectively applied among non-demented individuals after the consensus conference. Consistent with the standard criteria, for all subtypes of MCI, those considered for MCI were required to have (i) cognitive complaints (defined previously); (ii) objective impairment in at least one of five cognitive domains (memory, attention, language, visuospatial, and reasoning) based on the average scores on the neuropsychological measures within that domain and a cutoff of 1.5 SD using normative corrections for age, years of education, and gender; (iii) essentially preserved ADLs (defined previously); and (iv) no diagnosis of dementia at the consensus conference. The subtypes of MCI used were the following: amnestic MCI single (aMCIs), amnestic MCI multiple (aMCIm), non-amnestic MCI single (naMCIs), and non-amnestic MCI multiple (naMCIm) (Petersen and Morris, 2005). Classification into the four MCI subtypes was mutually exclusive.

Phase 2 (structured interview)

In order to make the final diagnoses of depression, phase 2 was conducted. For this phase, all individuals who had participated in the first phase were invited. As a result, 738 first-phase participants took part in the second phase. Of the 738 individuals, 147 scored 6 or greater on the GDS, and 591 scored less than 6. The participants were interviewed by seven psychiatrists and eight psychologists in a face-to-face setting between April and July 2002. The mean interval between the first and third phases was 62 days (SD = 36 days).

Interviews were conducted with the subjects using the Psychogeriatric Assessment Scale (PAS), which has been reported to provide a brief and comprehensive profile of an older individual's mental state (depression, cognitive impairment, and stroke) using a straightforward interview (Jorm *et al.*, 1995). It has been documented that the PAS has validity and reliability in general, and the depression subscale has excellent validity when judged against clinical diagnosis of the *DSM-III-R* major depressive episode (MDE) (American Psychiatric Association, 1987). According to the guidance of the PAS, a score of 4 and higher for the depression scale could identify approximately 80% of cases of depression.

Phase 3 (investigation of non-participants)

At the completion of the first phase, a total of 1035 non-participants were identified to have been contacted but had refused to participate, excluding the previously defined non-contacted individuals. For an accurate estimation of prevalence, a door-to-door survey for the non-participants was conducted. Between April and June 2002, 225 of the 1035 non-participants agreed to participate (hereafter referred to as delayed participants). A psychiatrist and a psychologist visited each delayed participant's home and conducted the same interview and tests that had been used in the first and second phases. The same diagnostic procedure described previously was also used for the delayed participants.

Diagnosis of depression

Participants who scored ≥4 on the PAS depression subscale were diagnosed as having MDE. Those who scored ≥6 on the GDS but fell short of MDE were defined as depressive symptoms cases (DSCs). Only those participants who were not diagnosed as having dementia were considered for a possible diagnosis of MDE or DSC. Hereafter, the term *depression* group is used to describe those participants with either MDE or DSC.

Statistical issues and analysis

The prevalence rates of MDE and DSC were estimated under missing at random assumption, that is, the rates were assumed to be equal between the participants who were interviewed and those who were not interviewed conditional on observed data, using the multiple imputation procedure with GDS as a predictor. Between the *depression* and the *non-depression* groups, we compared the demographics and the clinical data including several candidate risk factors for depression such as age, sex, years of

education, presence of apolipoprotein E 4, MCI, and so on (Djernes, 2006). Chi-squared test, *t*-test, and ANOVA were used for continuous and categorical variables, respectively. For analyses in which the expected frequency was less than five, Fisher's exact probability test was used. Statistical analysis was conducted using SAS package version 9.1 (SAS Institute Inc., Cary, NC, USA).

Results

General findings

Diagnostic procedure of *depression* is illustrated in Figure 1. As shown, 385 of the 3083 inhabitants died, moved, or were not contacted, yielding 2698 baseline candidate for the study. Among them, 1035 residents refused to participate in the first phase, but 225 of them participated in the third phase. Consequently, 1888 individuals (1663 for the first phase and 225 for the third phase) (70.0%) of 2698 baseline candidates were enrolled. Table 1 shows the demographic and clinical data for the 1888 participants. Of the 1888 participants, all of the 44 institutionalized people were diagnosed as having dementia. A total of 738 participants participated in the second phase, and the remaining 881 individuals took part in the first phase but not in the second or underwent the second phase but lacked any data (hereafter the combined persons are termed "first (+)/second (−) participants"). Of the 225 individuals who participated in the third phase, 86 were missing at least one data point, so the data from the remaining 139 individuals contributed to the prevalence estimation.

Between the 877 subjects individually interviewed for depression diagnosis and the remaining 1011 subjects, significant differences were found in the following: GDS score and age were lower, and the score of the five tests excluding the clock drawing test were higher for the interviewed subjects. These results indicate that the subjects were functionally better than those who were not interviewed for suspected depression.

Table 1 Demographic and clinical data for all participants

$n = 1888$	Mean ± SD
Age (years)	73.8 ± 6.0
Women, *n* (%)	969 (58.3)
Education (years)	9.9 ± 2.7
GDS score	2.9 ± 2.6
NADL score	49.6 ± 1.7
IADL score	5.1 ± 1.6
BMI	22.8 ± 3.2

GDS, Geriatric Depression Scale; NADL, Nishimura's activities of daily living; IADL, instrumental activities of daily living; BMI, body mass index.

Prevalence and characteristics of depression

Among the 738 second-phase participants (147 with GDS scores of ≥6 and 591 with GDS scores of <6), 24 of the 147 subjects with GDS scores of ≥6 were diagnosed as having MDE, and the remaining 123 subjects had a diagnosis of DSC. Twelve of the 591 subjects with GDS scores <6 were diagnosed as having MDE. Thus, 36 participants were diagnosed with MDE. On the other hand, among the 139 third-phase participants with full data, 3 were found to have MDE, and 10 were found to have DSC. In this diagnostic process, neither MDE nor DSC was diagnosed for the individuals with dementia.

In total, among the 877 interviewed subjects, 39 and 133 individuals were diagnosed with MDE and DSC, respectively. The prevalence of MDE and DSC for the target population were estimated to be 4.5% (95% CI, 3.4–6.0) and 11.5% (4.2–28.0), respectively.

Table 2 shows the demographic and clinical data for the 877 subjects in terms of the *depression* and normal mood groups. The prevalence of MCI was higher for the *depression* group, whereas ADL was better and education year was longer for the normal mood group.

Prevalence of coexisting depression and mild cognitive impairment

Different from the prevalence estimation study for the *depression*, for the purposes of accuracy in prediction of the prevalence of coexisting depression and MCI, the subjects of this portion of the study were confined to those who underwent the face-to-face interview for depression diagnosis. Among the 877 participants with full data (738 second-phase and 139 third-phase participants), the prevalence of the coexistence of the *depression* and the four MCI subtypes were estimated (Table 3). Using cutoff values of 1.5 SD for the diagnosis of MCI, 171 of the 877 participants (19.5%) were indicated to have MCI. The proportion of all subtypes of MCI combined was higher ($p < 0.01$) for the *depression* group (26.2%) than the normal mood group (17.9%). In addition, the prevalence of *depression* was significantly higher ($p < 0.01$) for the MCI group (26.3%) than the normal cognition group (18.0%). Taken together, the individuals with MCI were more likely to develop depressive symptoms, and vice versa.

Table 2 Demographic and clinical data for interviewed subjects

Characteristic	Non-depression	Depression (DSC or MDE)	*p*
	n=705	*n*=172	
Age (years)	73.5±5.6	73.4±5.4	NS
Women, *n* (%)	414 (58.7)	91 (52.9)	NS
Education (years)	10.1±2.7	9.6±2.3	$p<0.05$
NADL	49.6±1.4	49.1±2.7	$p<0.01$
MCI, *n* (%)	138 (16.7)	45 (26.2)	$p<0.01$
APOE4 carrier, *n* (%)	147 (20.9)	32 (18.6)	NS
Habitual alcohol drinking, *n* (%)	242 (34.3)	54 (31.4)	NS
Habitual smoking, *n* (%)	239 (33.9)	60 (34.9)	NS
Hypertension, *n* (%)	212 (30.0)	37 (21.5)	NS
Diabetes, *n* (%)	40 (5.7)	7 (4.1)	NS
Hyperlipidemia, *n* (%)	22 (3.1)	5 (2.9)	NS
Cerebral vascular disease, *n* (%)	24 (3.4)	7 (4.8)	NS

DSC, depressive symptoms case; MDE, major depressive episodes; NADL, Nishimura's activities of daily living; MCI, mild cognitive impairment; APOE4, apolipoprotein E type 4; NS, not significant.

Table 3 Coexistence of mild cognitive impairment and depression among the interviewed 877 subjects

Mood/cognition	Normal (80.5%)		Depressed pooled DSC+MDE (19.6%)		DSC (15.2%)		MDE (4.4%)	
	n=705 (100%)		*n*=172 (100%)		*n*=133 (100%)		*n*=39 (100%)	
Normal 706 (80.5%), NS	579	(82.1%)	127	(73.8%)	95	(71.4%)	32	(82.1%)
aMCIs 14 (1.6%), NS	10	(1.4%)	4	(2.3%)	4	(3.0%)	0	(0.0%)
aMCIm 25 (2.9%), NS	15	(2.1%)	10	(5.8%)	7	(5.3%)	3	(7.7%)
naMCIs 109 (12.4%), NS	89	(12.6%)	20	(11.6%)	18	(13.5%)	2	(5.1%)
naMCIm 23 (2.6%), NS	12	(1.7%)	11	(6.4%)	9	(6.8%)	2	(5.1%)
aMCIs+naMCIs 123 (14.0%), NS	99	(14.0%)	24	(13.9%)	22	(16.5%)	2	(5.1%)
aMCIm+naMCIm 48 (5.5%)*	27	(3.8%)	21	(12.2%)*	16	(12.0%)	5	(12.8%)

DSC, depressive symptom case; MDE, major depressive disorder; aMCIs, amnestic MCI single; aMCIm, amnestic MCI multiple; naMCIs, non-amnestic MCI single; naMCIm, non-amnestic MCI multiple; NS, not significant.
Statistical issues: comparison between normal mood group versus depressed pooled group.
*$p<0.01$.

It was also examined whether the *depression* group exhibited a prototypical profile of cognitive dysfunction. Using the generalized linear model, the difference in the proportion for each type of MCI between the normal mood and *depression* groups was examined. No significant differences were present in the prevalence of each of the four MCI types between the two groups. However, not MCI single (aMCIs+naMCIs) but MCI multiple (aMCIm+naMCIm) was more prevalent in the *depression* group (12.2%) than the normal group (3.8%).

Discussion

Prevalence of depression

Beekman *et al.* (1999) reviewed studies that dealt with the prevalence of depression in later life. According to the severity of cases, the reported weighted average of the prevalence of major and minor depressions was 1.8% (from 0.4% to 10.2%) and 9.8% (from 2.4% to 14.3%), respectively. To our knowledge, eight previous studies (Blazer and Williams, 1980; Kay *et al.*, 1985; Weissman *et al.*, 1985; Bland *et al.*, 1988; Kivela *et al.*, 1988; Komahashi *et al.*, 1994; Lobo *et al.*, 1995; Pahkala *et al.*, 1995) determined the prevalence of major depression based on the *DSM-III* or *DSM-III-R* criteria. The prevalence ranged from 0.4% to 3.7%. Because of the previously described many risk factors for depression besides ethnicity (Djernes, 2006), it is extremely difficult to make comparisons between the prevalence of the present study and the previous ones after controlling for the factors. However, a 4.5% prevalence rate of the *DSM-III-R* MDE in the present study appears to be similar to the results of the previous studies. As a category of depressive status other than MDE, we did not use dysthymia as listed in

the *DSM-III-R* but used our original definition of the DSC. The prevalence of DSC was estimated to be 11.5%. Regarding this issue, Djernes (2006) examined the prevalence of cases with depressive symptoms other than depressive disorders according to the *DSM* and International Classification of Diseases diagnostic criteria. Their cases of depressive symptoms were clinically diagnosed based on the presence of some depressive symptoms detected by rating scales, including the GDS. They reported that the prevalence of the cases among community-living older people widely ranges from 1.6% to 49%. However, more than half (12/22) of the studies showed the prevalence between 10% and 20%. Again, the risk factors aside, the results appear to be similar to our DSC prevalence.

As shown in Table 2, besides more prevalence of MCI, shorter education year and worse ADL for the *depression* groups were found. Both of them have been known as the risk factor for depression (Djernes, 2006). Although some studies reported cerebrovascular disease as the risk factor (Valvanne *et al.*, 1996; Schoevers *et al.*, 2006), our study did not find such result. Alcohol use has generally been regarded as a risk factor (Wilkins *et al.*, 2009); however, alcohol use was not higher for the *depression* group. The relationship between apolipoprotein E type 4 and depression has been controversial (Rigaud *et al.*, 2001; Bonger *et al.*, 2009), and we could not find the relationship.

Prevalence of coexisting depression and mild cognitive impairment

The present study showed high coexistence rate for *depression* and MCI (all subtypes of MCI combined). Regarding the epidemiology of depression among MCI individuals, several population-based studies (Chan *et al.*, 2003; Solfrizzi *et al.*, 2007; Geda *et al.*, 2008; Muangspaisan *et al.*, 2008) focused on the coexistence of exclusively amnestic MCI, and their results varied widely (prevalence of depression from 11.0% to 63.3%). Different from these studies, the cardiovascular health study (Lyketsos *et al.*, 2002) that determined the coexistence rate taking other types of MCI showed 26% coexistence of MCI (MCI amnestic type plus MCI multiple cognitive deficit type) and depressive symptoms. This result seems a little lower than our 36% prevalence for aMCIs plus aMCIm. The difference might be attributable to the difference in the methods between the two studies and the smaller sample size of our study.

This is the first study to report the prevalence of the four types of MCI among community-dwelling older people with depression. The present study also found higher prevalence of MCI among the subjects with *depression*. It is particularly interesting to understand whether individuals with *depression* show a certain prototypical profile of cognitive impairment. It has been said that older individuals with depression are likely to be worse in memory, attention, and executive function (Lockwood *et al.*, 2000; Butters *et al.* 2004; Rapp *et al.*, 2005), whereas those with Alzheimer's disease are likely to develop more severe amnesia (O'Brien *et al.*, 1994). However, in comparison with the normal mood group in the present study, individuals with *depression* showed no particular association with any of the four MCIs. It is possible that the *depression* group, especially the DSC group, was too heterogeneous to share cognitive impairment patterns, and that the number of the subjects was too small to show statistical significance. However, the prevalence of MCIm (aMCIm + naMCIm) was significantly higher for the *depression* group (12.2%) than the normal mood group (3.8%). A possible explanation for the result is that *depression* is apt to develop additional cognitive impairment in individuals with MCIs. Another explanation is that depression-related impairment in attention could simultaneously affect other cognitive domains.

The strength of the present study was that unlike most previous studies, the final diagnosis of MDE was performed on the basis of a face-to-face structured interview, and detailed cognitive assessments for the accurate examination of the relationship between depression and MCI were conducted. In terms of limitations, less than half of the first-phase participants underwent the individual interview. The resulting second-phase participants were superior in functions and demographics to the first (+)/second (−) participants. Thus, the prevalence of *depression* and coexisting conditions could have been underestimated.

In addition to higher prevalence of *depression* among individuals with MCI, this study was the first to report higher prevalence of MCI among community-dwelling depressed older people. Several researchers (Li *et al.*, 2001; Mondrego and Ferradez, 2004) have reported that the presence of depression promoted the conversion from MCI to dementia. Therefore, the present study suggests that attention should be paid to the risk of developing dementia for the older people with depression in general and the depressed older people with MCI in particular.

Conclusion

The prevalence of *depression* in our subjects seems to be similar with that of the previous studies. MCI was more prevalent in subjects with depression than those with normal mood. Individuals with *depression*

Key points

- The prevalence of major depressive disorder and DSC of the present study were 4.5% and 11.5%, which are similar to that of previous studies.
- Older subjects with depression were more likely to show MCI than those with normal mood.
- Although the older subjects with depression showed no prototypical profile of cognitive dysfunction, they were likely to show MCI multiple.
- Older subjects with MCI were more likely to develop depression than those with normal cognitive function.
- The risk of developing dementia in the depressed older people in general and those with coexisting MCI in particular should be acknowledged.

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 the development of dementia, the risk of developing dementia in the depressed older people, particularly in older people with coexisting MCI, should be acknowledged.

Author contributions

All of the authors contributed to the conception and design, and analysis and interpretation of data. Fumio Yamashita, Chiine Kodama, Chiaki Ikejima, Shin Hidaka, Megumi Sasaki, Satoshi Tanimukai, Katsuyoshi Mizukami, and Takashi Asada contributed to the collection of data. Toru Kinoshita, Shiro Tanaka, Hideto Takahashi, and Tatsuyuki Kakuma contributed to the statistical analysis. Shin Hidaka contributed to the drafting of the article, and Takashi Asada did the final approval of the version to be published.

Conflict of Interest

None declared.

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References

American Psychiatric Association. 1987. *Diagnostic and Statistical Manual of Mental Disorders*, 3rd edn-revised. American Psychiatric Association: Washington, DC.
Beekman ATF, Copeland JRM, Prince MJ. 1999. Review of community prevalence of depression in later life. *Br J Psychiatry* **174**: 307–311.
Bland RC, Newman SC, Orn H. 1988. Prevalence of psychiatric disorders in the elderly in Edmonton. *Acta Psychiatr Scand* **77**(Suppl. 338): 57–63.
Blazer D, Williams CD. 1980. Epidemiology of dysphoria and depression in an elderly population. *Am J Psychiatry* **137**: 439–444.
Bonger HR, Richie MB, de Vries HF, *et al.* 2009. Depression, cognition, apolipoprotein e genotype: latent class approach to identifying subtype. *Am J Geriatr Psychiatry* **17**: 344–352.
Brink TL, Yesavage JA, Lum O, *et al.* 1982. Screening tests for geriatric depression. *Clin Gerontol* **1**: 37–44.
Butters MA, Whyte EM, Nebes RD, *et al.* 2004. The nature and determinants of neuropsychological functioning in late-life depression. *Arch Gen Psychiatry* **61**: 587–595.
Chan DC, Kasper JD, Black BS, Rabins PV. 2003. Prevalence and correlates of behavioral and psychiatric symptoms in community-dwelling elders with dementia or mild cognitive impairment: the memory and medical care study. *Int J Geriatr Psychiatry* **18**: 174–182.
Devanand DP, Sano M, Tang MX, *et al.* 1996. Depressed mood and the incidence of Alzheimer's disease in elderly living in the community. *Arch Gen Psychiatry* **53**: 175–182.
Djernes JK. 2006. Prevalence and predictors of depression in populations of elderly: a review. *Acta Psychiatr Scand* **113**: 372–387.
Freedman M, Leach L, Kaplan E, *et al.* 1994. *Clock Drawing. A Neuropsychological Analysis.* Oxford University Press: New York.
Ganguli M, Dodge HH, Shen C, DeKosky ST. 2004. Mild cognitive impairment, amnestic type: an epidemiologic study. *Neurology* **63**: 115–121.
Geda YE, Roberts RO, Knopman DS, *et al.* 2008. Prevalence of neuropsychiatric symptoms in mild cognitive impairment and normal cognitive aging: population-based study. *Arch Gen Psychiatry* **65**: 1193–1198.
Grober E, Buschke H, Crystal H, Bang S, Dresner, R. 1988. Screening for dementia by memory testing. *Neurology* **38**: 900–903.
Hanninen T, Halilainen M, Tuomainen S, Vanhanen M, Soininen H. 2002, Prevalence of mild cognitive impairment: a population-based study in elderly subjects. *Acta Neurol Scand* **106**: 148–154.
Jorm AF, Mackinnon AJ, Henderson AS, *et al.* 1995. The Psychogeriatric Assessment Scales: a multi-dimensional alternative to categorical diagnoses of dementia and depression in the elderly. *Psychol Med* **25**: 447–460.
Kay DWK, Henderson AS Scott R, *et al.* 1985. Dementia and depression among the elderly living in the Hobart community: the effect of the diagnostic criteria on the prevalence rates. *Psychol Med* **15**: 771–788.
Kivela SL, Pahkala K, Laippala P. 1988. Prevalence of depression in an elderly population in Finland. *Acta Psychiatr Scand* **78**: 401–413.
Komahashi T, Ohmori K, Nakano T, *et al.* 1994. Epidemiological survey of dementia and depression among the aged living in the community in Japan. *Jpn J Psychiatry Neurol* **48**: 517–526.
Li YS, Meyer JS, Thornby J. 2001. Longitudinal follow-up of depressive symptoms among normal versus cognitively impaired subjects. *Int J Geriatr Psychiatry* **16**: 718–727.
Lobo A, Saz P, Marcos G, Día JL, De-la-Cámara C. 1995. The prevalence of dementia and depression in the elderly community in a southern European population. The Zaragosa study. *Arch Gen Psychiatry* **52**: 497–506.
Lockwood KA, Alexopoulos GS, Kakuma T, Van Gorp WG. 2000. Subtypes of cognitive impairment in depressed older adults. *J Geriatr Psychiatry* **8**: 201–208.
Lyketsos CG, Lopez OL, Jones B, *et al.* 2002. Prevalence of neuropsychiatric symptoms in dementia and mild cognitive impairment: results from the cardiovascular health study. *JAMA* **288**: 1475–1483.
Mondrego PJ, Ferradez J. 2004. Depression in patients with mild cognitive impairment increases the risk of developing dementia of the Alzheimer type: a prospective cohort study. *Arch Neurol* **61**: 1290–1293.
Muangspaisan W, Intalapaporn S, Assantachai P. 2008. Neuropsychiatric symptoms in the community-based patients with mild cognitive impairment and the influence or demographic factors. *Int J Geriatr Psychiatry* **23**: 699–703.
Nishimura T, Kobayashi T, Hariguchi S, *et al.* 1993. Scales for mental state and daily living activities for the elderly: clinical behavioral scales for assessing demented patients. *Int Psychogeriatr* **5**: 117–134.
O'Brien, Desmond P, Ames D, *et al.* 1994. The differentiation of depression from dementia by temporal lobe magnetic resonance imaging. *Psychol Med* **24**: 633–640.
Pahkala K, Kesti E, Köngäs-Saviaro P, Laippala P, Kivelä SL. 1995. Prevalence f depression in an aged population in Finland. *Soc Psychiatry Psychiatr Epidemiol* **30**: 99–106.

Panza F, D'Introno A, Colaccicco AM, *et al.* 2005. Current epidemiology of mild cognitive impairment and other predementia syndromes. *Am J Geriatr Psychiatry* **13**: 633–644

Panza F, Frisardi V, Capurso C, *et al.* 2010. Late-life depression, mild cognitive impairment, and dementia: possible continuum? *Am J Geriatr Pstychiatry* **18**: 98–116.

Petersen RC, Morris JC. 2005. Mild cognitive impairment as a clinical entity and treatment target. *Arch Neurol* **62**: 1160–1163.

Petersen RC, Smith GE, Warning SC, *et al.* 1999. Mild cognitive impairment: clinical characterization and outcome. *Arch Neurol* **56**: 303–308.

Rapp MA, Dahlman K, Sano M, *et al.* 2005. Neuropsychological differences between late-onset and recurrent geriatric major depression. *Am J Psychiatry* **162**: 691–698.

Rigaud AS, Traykov L, Caputo L, *et al.* 2001. Association of the apolipoprotein E epsilon4 allele with late-onset depression. *Neuroepidemiology* **20**: 268–272.

Ritchie K, Fuhrer R. 1992. A comparative study of the performance of screening tests for senile dementia using receiver operating characteristics analysis. *J Clin Epidemiol* **45**: 627–637.

Ritchie K, Artero S, Touchon J. 2001. Classification criteria for mild cognitive impairment: a population-based validation study. *Neurology* **56**: 37–42.

Sasaki M, Kodama C, Hidaka S, *et al.* 2009. Prevalence of four subtypes of mild cognitive impairment and APOE in a Japanese Community. *Int J Geriatr Psychiatry* **24**: 1119–1126.

Schoevers RA, Smit F, Deeg DJH, *et al.* 2006. Prevention of late-life depression in primary care: do we know where to begin? *Am J Psychiatry* **163**: 1611–1621.

Sohlberg M, Mateer CA. 1986. *Attention Process Training Manual.* Association for Neuropsychological Research and Development: Washington, DC.

Solfrizzi V, D'Introno A, Colacicco AM, *et al.* 2007. Incident occurrence of depressive symptoms among patients with mild cognitive impairment—the Italian longitudinal study on aging. *Dement Geriatr Cogn Disord* **24**: 55–64.

Soloman PR, Pendlebury WW. 1998. Recognition of Alzheimer's disease: the 7 minute screen. *Fam Med* **30**: 265–271.

Steffens DC, Otey E, Alexopoulos GS, *et al.* 2006. Perspectives on depression, mild cognitive impairment, and cognitive decline. *Arch Gen Psychiatry* **63**: 130–138.

Valvanne J, Juva K, Erkinjuntti T, Tilvis R. 1996. Major depression in the elderly: a population study in Helsinki. *Int Psychogeriatr* **8**: 437–443.

Wechsler D. 1981. *Wechsler Adult Intelligence Scale—Revised.* Psychological Corporation: Cleveland, OH.

Weissman MM, Myers JK, Tischler GL, *et al.* 1985. Psychiatric disorders (DSM-III) and cognitive impairment among the elderly in a U.S. urban community. *Acta Psychiatr Scand* **71**: 366–379.

Wilkins CH, Mathews J, Sheline Y. 2009. Late life depression with cognitive impairment: evaluation and treatment. *Clin Interv Aging* **4**: 51–57.

Association Between Cognitive Function and Plasma Lipids of the Elderly After Controlling for Apolipoprotein E Genotype

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Objective: *Although the relationship between cognitive function and plasma lipids has attracted attention, previous studies have shown conflicting results. One possible confounding factor is due to the influence of gene-related modulator. We investigated the relationship between cognitive function and lipid plasma levels of old age after controlling for apolipoprotein E* (APOE) *genotype.* **Methods:** *One thousand three hundred ninety-five subjects without dementia age 65 and older participated in this study. They were divided into two groups, with and without APOE4 [E4 (+) and E4 (−)]. Plasma concentrations of high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglyceride (TG), total cholesterol (TC) and apolipoprotein E (apoE) were measured. Associations between plasma concentrations of lipids and cognitive function were investigated for each group.* **Results:** *We found a positive association between cognitive scores and plasma apoE level in both E4 (−) and E4 (+) groups. A positive relationship was also observed between cognitive score and HDL level in the E4 (−) group, but not in the E4 (+) group. No substantial association between cognitive score and LDL, TG, and TC levels was found in either of the groups.* **Conclusions:** *Our findings suggest that plasma apoE have a positive influence on cognitive function in both E4 (−) and E4 (+) groups, whereas the positive influence of plasma HDL was shown only in E4 (−) group. The identification of the influences of* (APOE) *genotype and the intracellular linkage among apoE and HDL metabolism is hoped for new preventive and therapeutic strategies for cognitive change of elderly.* (Am J Geriatr Psychiatry 2012; 20:574–583)

Key Words: Apolipoprotein E, cognitive function, high-density lipoprotein, low-density lipoprotein, triglyceride, total cholesterol

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OBJECTIVE

Despite the conflicting results of previous studies, a possible relationship between cognitive function and plasma lipids has been attracting increasing attention. In one study of aged persons, a low plasma concentration of high-density lipoprotein (HDL) was related to cognitive impairment and dementia, whereas no association was found between low-density lipoprotein (LDL), triglycerides (TG) and Mini-Mental State Examination (MMSE) scores.[1] On the contrary, a 4-year longitudinal study of postmenopausal women reported that high-LDL levels were associated with concurrent cognitive impairment, whereas HDL and TG levels were not associated with cognition.[2] In younger middle-aged groups, HDL and TG concentrations were unassociated with memory performance, whereas higher plasma concentration of LDL was associated with better memory performance.[3] In a study of Alzheimer disease (AD) patients, no concurrent associations were found between HDL, LDL, or TG and MMSE.[4] In a systematic review of prospective studies of relationships between total cholesterol (TC) and dementia or cognitive decline, an association between high midlife TC and cognitive impairment was found, but there was only weak evidence for an association between TC and cognitive decline.[5]

One possible explanation for these contradictory results may lie in the age of the subjects when cognitive function and plasma lipids were assessed. The influence of gene-related modulator might be another confounding factor. Apolipoprotein E (apoE) is a polymorphic protein arising from 3 alleles ($\varepsilon 2/\varepsilon 3/\varepsilon 4$) at a single gene locus. Its three major isoforms, apoE2, apoE3, and apoE4, differ from one another only by single amino acid substitutions, yet these changes have profound functional consequences both at cellular and molecular levels.[6] The apolipoprotein E (*APOE*) gene plays a central and pervasive role in lipid metabolism.[7] Previous study supported the association between *APOE* gene polymorphisms and the vulnerability of the aging brain.[8] Cross-sectional studies have reported the association of E4 inheritance with poor global cognitive function, episodic memory, and executive function.[9,10] Thus, failure to control for *APOE* genotype may influence the result observed between cognitive function and plasma lipids. In one previous study, the different association of cholesterol on cognitive functioning was shown in oldest old (> = 85 years old) with and without *APOE4* allele, and the necessity of further examination of the role of *APOE* genotype is suggested.[11] The current study examines the relationship between cognitive function and plasma levels of lipids including HDL, LDL, TG, TC, and apoE in the community-dwelling elderly (> = 65 years old) with stratification by *APOE4* allele status.

METHODS

Participants

We recruited the participants in the present study from the "Tone Project" in Tone town, Ibaraki, Japan.[12] This town is located about 40 miles northeast of central Tokyo, and consists of both of newly-developed residential and agricultural areas. On November 30th, 2001, the town had 2,698 inhabitants age 65 and older (14.0% of the town population). On the basis of data from the national census, the age distribution in Tone town was almost identical to that of the whole of Japan. They were asked to participate in the project, and 1,888 of them were finally enrolled in the Tone Project between December 2001 and April 2002.

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 DSM-IV criteria. We excluded the data from those with psychiatric diseases (n = 123).

Two hundred eighty participants refused blood sampling because of fear or some other personal reason. Sixty-one participants had no blood sampling data because of error of blood sampling or of some measurement procedure. One hundred eighty-six participants did not complete the series of examinations of cognitive assessment because of fatigue, refusal, performance mistake, and so on. Among these participants, 157 participants had neither blood sampling nor cognitive assessment. After excluding data from those without blood data and/or incomplete data, we used the data from 1,395 subjects without dementia for the analysis.

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

Plasma Parameters

Blood samples were collected from the subjects at fasting visits. Plasma levels of LDL, HDL, TG, and 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.[13] After amplification, the PCR product was digested with the restriction enzyme *Hha* I, and subjected to electrophoresis in a 15% polyacrylamide gel.

Screening and Structured Interview

After blood sampling, all participants underwent a screening interview consisting of a structured questionnaire (questions on age, sex, education). This was followed by the 15-items short version of the Geriatric Depression Scale (GDS).[14] The participants were asked for their medical history of cardiovascular disease, diabetes mellitus, hyperlipidemia and hypertension for which they had received medical care. During the interview, we estimated visual acuity, hearing and speech ability of each subject.

Cognitive Assessment

After completing the interview, all the participants underwent group cognitive assessment using a set of four tests to measure these cognitive domains: attention, memory, language, and reasoning. We evaluated attention by using the Japanese version of a set-dependent activity.[15] The test assesses alternating attention, which refers to the capacity for mental flexibility that allows individuals to shift their focus of attention between tasks with different cognitive requirements. To assess memory ability, we used the Category Cued Recall test.[16] We examined language ability with a category fluency test.[17] The subjects were asked to generate as many examples as possible in 2 minutes from the semantic category "animals." To assess abstract reasoning ability, we employed the similarities subset of the Wechsler Adult Intelligence Scale-Revised (WAIS-R).[18]

This cognitive assessment was conducted in a group setting (maximum 50 participants) by an examiner using a projector. Before each of the four tests, the participants were given instructions by an examiner. All the participants were asked to record their answers on the answer sheet. Each screening was supervised by members of our research team, and they prevented communications among participants. If the responders had questions, the members answered them right away. The mean length of the four-test examination was 35 minutes. For proving the validity of the group-setting method, we examined the agreement of four tests scores between group setting and face-to-face method among 15 participants. For this purpose, the participants first underwent group-setting tests, and 35 days later they underwent face-to-face tests. Between the two trials, Pearson's correlation coefficient was above 0.70, and significance was $p < 0.01$ for all of the four tests. For participants with difficulty understanding tasks or with impaired hearing or vision (n = 261), we conducted the assessment by using the individual versions of the four tests in a face-to-face setting.

For delineating the cognitive composite score, a simple average score of the four individual scores is not enough, because the contribution of the individual scores to the composite scores should be considered. Evaluation of the results of the four tests revealed that the score for the four cognitive domains showed normal distribution and significant mutual correlation. Therefore, we attempted to convert the four scores into a composite cognitive score using the first component of the scores of principal component analysis (Eigenvalue 2.85, proportion 71%, N = 1395, Composite cognitive score = 0.853* attention score + 0.809* memory score + 0.856* language score + 0.859* reasoning score.)

Statistical Analysis

Subjects were divided into the two groups of E4(−)(n = 1118) (genotypes $\varepsilon2/\varepsilon2$ [n = 4], $\varepsilon2/\varepsilon3$ [n = 107], $\varepsilon3/\varepsilon3$ [n = 1007]) and E4(+) (n = 277) (genotypes $\varepsilon2/\varepsilon4$ [n = 18], $\varepsilon3/\varepsilon4$ [n = 240] and $\varepsilon4/\varepsilon4$ [N = 19]) 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*-test and Pearson χ^2 test. 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, and medical history of cardiovascular disease, diabetes mellitus, hyperlipidemia, and hypertension as covariates (Table 1). To examine group differences in the concentrations of lipids, ANCOVA was performed with age and sex as covariates (Table 2).

The subjects in each category were divided into three strata according to tertiles of the plasma concentrations of lipids. To examine the influence of plasma lipids and ApoE genotype on cognitive function, we performed ANCOVA with the three strata of the level of lipids and genotype as independent variables, the composite cognitive scores as dependent variables, and age, sex, years of education, GDS score, and the medical history of diseases as covariates (Tables 3–7).

Individual test scores and composite cognitive scores were compared in E4 (–) and E4 (+) groups separately among the three strata by ANCOVA, with age, sex, years of education, GDS score, and medical history of diseases as covariates. In addition, effect sizes were calculated using partial eta-squared (η^2) to estimate and compare the effect of the level of lipids on cognitive score between groups of different sample size η^2 0.01 was regarded as no substantial effect. Follow-up *t*-tests were performed to specify differences of cognitive score among the three strata according to the levels of lipids (Tables 3–7, Figure 1).

To examine whether the tertile of lipids/apoE level 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 the tertiles of lipids/apoE level as independent variables, after adjustment for other factors of age, sex, years of education, GDS score, and medical history of diseases.

Multiple comparisons were adjusted by Bonferroni correction. All statistical tests were two-tailed and reported at α <0.05. Statistical analysis of the data was performed using SPSS for Windows 16.0 (SPSS Japan, Inc., Tokyo, Japan).

RESULTS

The demographic data of the E4 (–) and E4 (+) groups are shown in Table 1. There were no group differences in demographic characteristics between the groups except the cognitive score. Our finding of a higher cognitive score of the E4(–) group is consistent with previous studies.[10] Table 2 shows the mean of the plasma concentrations of lipids for the E4 (–) and E4 (+) groups. There were group differences in the plasma concentrations of TC and apoE. The concentration of TC was lower and that of apoE was higher in the E4 (–) group.

Tables 3–7 show the median plasma concentrations of lipids for the three strata according to the tertiles of plasma levels of lipids and apoE. Individual test scores and composite cognitive scores of the E4 (–)

TABLE 1. Demographic Characteristics, Mean ± SD

Characteristic	ApoE4(–) (n = 1,118)	ApoE4(+) (n = 277)	df	*t*, χ^2 or *F*	p
Age, y[a]	73.6 ± 5.7	73.6 ± 5.8	1393	$t = 0.06$	0.95
Male, No (%)[b]	467 (42%)	108 (39%)	1	$\chi^2 = 0.71$	0.40
Education, y[a]	10.0 ± 2.6	10.0 ± 2.7	1393	$t = 0.20$	0.85
GDS score[a]	3.0 ± 2.7	2.7 ± 2.6	1393	$t = 1.63$	0.10
Cardiovascular disease, No (%)[b]	40 (3.6%)	11 (4.0%)	1	$\chi^2 = 0.10$	0.76
Diabetes mellitus, No (%)[b]	59 (5.3%)	13 (4.7%)	1	$\chi^2 = 0.16$	0.69
Hyperlipidemia, No (%)[b]	31 (2.8%)	13 (4.7%)	1	$\chi^2 = 2.68$	0.10
Hypertension, No (%)[b]	314 (28.4%)	70 (25.2%)	1	$\chi^2 = 0.88$	0.35
Composite cognitive score[c]	39.3 ± 12.0	37.1 ± 12.0	1, 1384	$F = 7.3$	0.005[d]

[a]The p value was calculated by unpaired two-tailed *t* test.

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

[c]The p value was calculated by analysis of covariance (ANCOVA) with age, sex, years of education, score of GDS and medical history of cardiovascular disease, diabetes mellitus, hyperlipidemia and hypertension as covariates. Data are mean ± SD after adjustment for covariates.

[d]p <0.05.

TABLE 2. Concentrations of Lipid and ApoE in *APOE4*(−) and *APOE4*(+) Groups[a]

Concentration	*APOE4*(−) (n = 1,118)	*APOE4*(+) (n = 277)	ANCOVA (df = 1, 1,390) *F*	p
HDL (mmol/L)	1.44 ± 0.38	1.41 ± 0.38	1.33	0.25
LDL (mmol/L)	2.66 ± 0.83	2.73 ± 0.83	1.97	0.16
TG (mmol/L)	1.93 ± 1.17	1.97 ± 1.17	0.31	0.58
TC (mmol/L)	5.32 ± 0.92	5.48 ± 0.92	6.76	0.01[b]
apoE (mg/dL)	2.66 ± 1.44	2.28 ± 1.45	15.8	<0.001[c]

[a]Data are mean ± SD after adjustment for age and sex.
[b]p <0.05.
[c]p <0.001.

and E4 (+) groups according to the three strata of plasma concentrations of lipids/apoE are also shown in these tables.

ANCOVA analysis of the influence of HDL level and genotype on composite cognitive scores revealed interaction between HDL level and genotype (Table 3). In the E4 (−) group, subjects with higher HDL concentration had higher cognitive score. The effect size of the plasma HDL level on cognitive score showed a substantial influence of the HDL level on three individual test scores and composite cognitive scores. Follow-up *t*-tests showed differences of these cognitive scores among the three strata. In the E4 (+) group, such an association was not observed (Table 3, Figure 1). In multiple regression analysis, plasma HDL level positively related to composite cognitive score (β = 0.12, p <0.001, df = 1108) in the E4 (−) group.

A significant main effect of the apoE level was found by ANCOVA analysis of the influence of the apoE level and genotype on composite cognitive scores (Table 4). Subjects with higher apoE concentration had higher cognitive score in all individual and composite cognitive scores in the E4 (−) group, and one individual and composite cognitive scores in the E4 (+) group. The effect size of the plasma apoE level on these cognitive score showed a substantial influence of the apoE level on two individual and composite cognitive scores in the E4 (−) group, and one individual and composite cognitive scores in the E4 (+) group. Follow-up *t*-tests showed differences of these cognitive scores among the three strata. (Table 4, Figure 1). In multiple regression analysis, plasma ApoE level positively related to composite cognitive scorein the group of E4 (−) (β = 0.13, p <0.001, df = 1108) and E4(+) (β = 0.12, p = 0.009, df = 267).

TABLE 3. Mean Cognitive Score of Each Tertile of HDL Level by *APOE4*(−) and *APOE4*(+) Groups[a,b]

Concentrations, median (min–max)		HDL concentration (mmol/L), tertiles: Low 1.09 (0.57–1.24)	Middle 1.37 (1.27–1.58)	High 1.81 (1.60–3.57)	ANCOVA[c] *F*	p	η^2	Between groups[d]
E4(−)	Attention	15.0 ± 11.1	15.8 ± 11.1	16.8 ± 11.8	6.74	<0.001[f]	0.012[g]	B
	Memory	9.7 ± 7.3	10.3 ± 7.3	11.3 ± 7.7	12.4	<0.001[f]	0.022[g]	B, C
	Language ability	12.6 ± 6.6	13.2 ± 6.6	13.3 ± 7.0	3.15	0.043[e]	0.006	
	Reasoning	6.5 ± 6.1	7.2 ± 6.2	8.0 ± 6.5	17.5	<0.001[f]	0.031[g]	A, B, C
	Composite score	37.0 ± 20.1	39.2 ± 20.1	41.7 ± 21.3	14.2	<0.001[f]	0.025[g]	A, B, C
E4(+)	Attention	14.4 ± 10.6	15.7 ± 11.3	14.5 ± 11.6	1.06	0.35	0.008	
	Memory	9.5 ± 8.4	10.6 ± 8.9	9.8 ± 9.2	1.10	0.34	0.008	
	Language ability	12.9 ± 7.0	12.8 ± 7.4	12.5 ± 7.6	0.21	0.81	0.002	
	Reasoning	6.4 ± 5.7	7.2 ± 6.0	7.0 ± 6.2	1.30	0.28	0.010	
	Composite score	36.6 ± 21.3	39.0 ± 22.5	37.1 ± 23.2	0.97	0.38	0.007	

[a]Data are mean ± SD after adjustment for age, sex, years of education, GDS score, and medical history ofcardiovascular disease, diabetes mellitus, hyperlipidemia, and hypertension as covariates.
[b]With ANCOVA analysis of the effect of the level of HDL and genotype on composite cognitive scores,main effect of HDL level, $F_{[2,1381]} = 4.55$. p = 0.01; main effect of genotype, $F_{[1,1381]} = 7.84$. p = 0.005;HDL level-by-genotype interaction, $F_{[2,1381]} = 3.25$. p = 0.04.
[c]df = 2,1107 for *APOE4*(−), 2, 266 for *APOE4*(+).
[d]Significance at p <0.016 (0.05/3) after Bonferroni adjustment for multiple comparisons: A, low to middle:B, low to high: C, middle to high concentration group comparison.
[e]p <0.05.
[f]p <0.001.
[g]η^2 >0.01

TABLE 4. Mean Cognitive Score of Each Tertile of apoE Level by *APOE4*(−) and *APOE4*(+) Groups[a,b]

Concentrations, median (min–max)		apoE concentration (mg/dL), tertiles: Low 1.3 (0.5–1.7)	Middle 2.3 (1.8–3.0)	High 4.0 (3.1–10.5)	ANCOVA[c]: F	p	η^2	Between groups[d]
E4(−)	Attention	14.3 ± 11.5	15.8 ± 11.1	17.2 ± 11.1	17.2	<0.001[f]	0.030[g]	A, B, C
	Memory	9.9 ± 7.7	10.5 ± 7.4	10.9 ± 7.4	4.90	0.008[e]	0.009	B
	Language ability	12.8 ± 6.9	12.8 ± 6.7	13.4 ± 6.6	3.61	0.027[e]	0.006	
	Reasoning	6.5 ± 6.5	7.2 ± 6.2	7.8 ± 6.2	11.9	<0.001[f]	0.021[g]	A, B
	Composite score	36.7 ± 21.0	39.0 ± 20.3	41.7 ± 20.3	15.7	<0.001[f]	0.028[g]	A, B, C
E4(+)	Attention	13.9 ± 10.0	15.3 ± 11.3	15.8 ± 12.4	2.24	0.11	0.017[g]	
	Memory	9.3 ± 7.9	10.3 ± 8.9	10.6 ± 9.8	1.68	0.19	0.012[g]	
	Language ability	12.3 ± 6.6	12.9 ± 7.4	13.2 ± 8.2	1.14	0.32	0.008	
	Reasoning	5.9 ± 5.3	7.5 ± 5.9	7.5 ± 6.5	7.72	<0.001[f]	0.055[g]	A, B
	Composite score	35.0 ± 19.9	38.9 ± 22.4	39.8 ± 24.7	3.91	0.021[e]	0.029[g]	B

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

[b]With ANCOVA analysis of the effect of the level of apoE and genotype on composite cognitive scores,main effect of apoE level, $F_{[2,1381]} = 11.3$. $p = 0.00001$; main effect of genotype, $F_{[1,1381]} = 4.96$. $p = 0.03$; apoE level-by-genotype interaction, $F_{[2,1381]} = 0.45$. $p = 0.64$.

[c]df = 2, 1107 for *APOE4*(−), 2, 266 for *APOE4*(+).

[d]Significance at $p < 0.016$ (0.05/3) after Bonferroni adjustment for multiple comparisons: A, low to middle:B, low to high: C, middle to high concentration group comparison.

[e]$p < 0.05$.

[f]$p < 0.001$.

[g]$\eta^2 > 0.01$.

TABLE 5. Mean Cognitive Score of Each Tertile of LDL Level by *APOE4*(−) and *APOE4*(+) Groups[a,b]

Concentrations, median (min–max)		LDL concentration (mmol/L), tertiles: Low 1.89 (0.47–2.28)	Middle 2.59 (2.30–2.97)	High 3.44 (3.00–9.05)	ANCOVA[c]: F	p	η^2	Between groups[d]
E4(−)	Attention	16.3 ± 11.0	15.8 ± 11.4	15.2 ± 11.7	2.37	0.09	0.004	
	Memory	10.6 ± 7.3	10.7 ± 7.5	10.0 ± 7.7	3.12	0.05	0.006	
	Language ability	13.3 ± 6.5	13.2 ± 6.7	12.5 ± 6.9	5.00	0.007[e]	0.009	B, C
	Reasoning	7.2 ± 6.1	7.3 ± 6.4	7.0 ± 6.5	0.76	0.47	0.001	
	Composite score	40.0 ± 20.0	39.8 ± 20.7	37.7 ± 21.2	4.03	0.02[e]	0.007	B
E4(+)	Attention	15.0 ± 11.9	14.9 ± 11.1	14.7 ± 10.8	0.05	0.95	0.0004	
	Memory	10.0 ± 9.4	10.1 ± 8.7	9.8 ± 8.5	0.14	0.87	0.001	
	Language ability	13.1 ± 7.8	12.9 ± 7.2	12.3 ± 7.0	0.92	0.40	0.007	
	Reasoning	7.1 ± 6.3	7.0 ± 5.9	6.5 ± 5.8	0.78	0.46	0.006	
	Composite score	38.2 ± 23.7	37.9 ± 22.1	36.6 ± 21.5	0.44	0.65	0.003	

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

[b]With ANCOVA analysis of the effect of the level of LDL and genotype on composite cognitive scores,main effect of LDL level, $F_{[2,1381]} = 2.70$. $p = 0.07$; main effect of genotype, $F_{[1,1381]} = 6.89$. $p = 0.009$;LDL level-by-genotype interaction, $F_{[2,1381]} = 0.05$. $p = 0.95$

[c]df = 2, 1107 for *APOE4*(−), 2, 266 for *APOE4*(+).

[d]Significance at $p < 0.016$ (0.05/3) after Bonferroni adjustment for multiple comparisons: A, low to middle:B, low to high: C, middle to high concentration group comparison.

[e]$p < 0.05$.

We found no main effect of LDL and its interaction with genotype by ANCOVA analysis of the influence of LDL level and genotype on composite cognitive scores (Table 5). We found an association between cognitive scores and the plasma concentration of LDL in the E4 (−) group in two individual and composite cognitive score. In multiple regression analysis plasma LDL level positively related to composite cognitive score ($\beta = -0.06$, $p < 0.001$, df = 1,108) in the E4 (−) group. However, the effect size of the plasma

TABLE 6. Mean Cognitive Score of Each Tertile of TG Level by *APOE4*(−) and *APOE4*(+) Groups[a,b]

Concentrations, median (min–max)		TG concentration (mmol/L), tertiles: Low 0.98 (0.34–1.31)	Middle 1.63 (1.32–2.03)	High 2.77 (2.04–10.4)	ANCOVA[c] F	p	η^2	Between groups[d]
E4(−)	Attention	15.4 ± 11.0	15.7 ± 11.4	16.4 ± 11.5	2.17	0.12	0.004	
	Memory	10.4 ± 7.3	10.5 ± 7.5	10.4 ± 7.6	0.08	0.92	0.0002	
	Language ability	13.0 ± 6.5	12.9 ± 6.7	13.1 ± 6.8	0.25	0.78	0.0004	
	Reasoning	7.2 ± 6.1	7.0 ± 6.4	7.4 ± 6.4	0.96	0.38	0.002	
	Composite score	38.7 ± 20.1	39.0 ± 20.8	39.9 ± 21.0	0.95	0.39	0.002	
E4(+)	Attention	14.0 ± 11.5	15.0 ± 11.5	15.5 ± 10.4	1.37	0.26	0.009	
	Memory	9.7 ± 9.1	10.6 ± 9.1	9.6 ± 8.2	1.08	0.34	0.008	
	Language ability	12.3 ± 7.5	13.1 ± 7.5	12.9 ± 6.8	0.80	0.45	0.006	
	Reasoning	6.3 ± 6.1	6.9 ± 6.1	7.3 ± 5.5	2.13	0.12	0.016[e]	
	Composite score	35.6 ± 22.9	38.5 ± 22.9	38.3 ± 20.7	1.35	0.26	0.009	

[a]Data are mean ± SD after adjustment for age, sex, years of education, GDS score, and medical history of cardiovascular disease, diabetes mellitus, hyperlipidemia, and hypertension as covariates.
[b]With ANCOVA analysis of the effect of the level of TG and genotype on composite cognitive scores, main effect of TG level, $F_{[2,1381]} = 1.58$. $p = 0.21$; main effect of genotype, $F_{[1,1381]} = 7.71$. $p = 0.006$; TG level-by-genotype interaction, $F_{[2,1381]} = 0.59$. $p = 0.56$.
[c]df = 2, 1107 for *APOE4*(−), 2, 266 for *APOE4*(+).
[d]Significance at $p < 0.016$ (0.05/3) after Bonferroni adjustment for multiple comparisons: A, low to middle: B, low to high: C, middle to high concentration group comparison.
[e]$\eta^2 > 0.01$.

TABLE 7. Mean Cognitive Score of Each Tertile of TC Levels by *APOE4*(−) and *APOE4*(+) Groups[a,b]

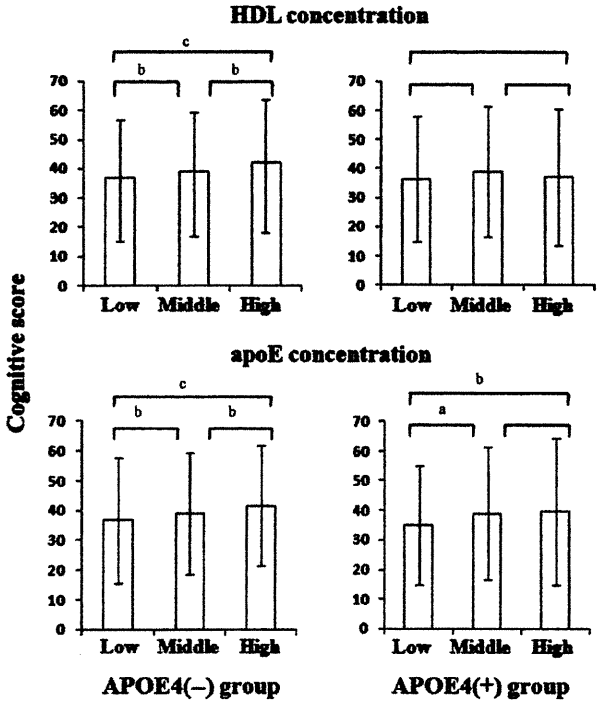
Concentrations, median (min–max)		TC concentration (mmol/L), tertiles: Low 4.34 (1.78–5.04)	Middle 5.33 (5.07–5.87)	High 6.28 (5.90–9.31)	ANCOVA[c] F	p	η^2	Between Groups[d]
E4(−)	Attention	15.2 ± 10.6	16.2 ± 11.1	16.0 ± 13.2	2.53	0.08	0.005	
	Memory	10.2 ± 7.0	10.7 ± 7.4	10.4 ± 8.7	0.97	0.38	0.002	
	Language ability	12.9 ± 6.3	13.1 ± 6.2	13.0 ± 6.6	0.10	0.91	0.0001	
	Reasoning	6.8 ± 5.9	7.4 ± 6.2	7.5 ± 7.4	3.84	0.02[e]	0.007	B
	Composite score	38.2 ± 19.3	40.0 ± 20.3	39.7 ± 24.1	2.51	0.08	0.005	
E4(+)	Attention	13.9 ± 11.5	14.8 ± 11.3	15.8 ± 11.5	1.75	0.18	0.013[f]	
	Memory	9.6 ± 9.2	10.3 ± 9.0	9.9 ± 9.1	0.43	0.65	0.003	
	Language ability	12.2 ± 7.5	13.4 ± 7.3	12.6 ± 7.5	1.98	0.14	0.015[f]	
	Reasoning	6.3 ± 6.1	7.0 ± 6.0	7.3 ± 6.1	1.51	0.22	0.011[f]	
	Composite score	35 ± 23.0	38.5 ± 22.5	38.6 ± 22.9	1.44	0.24	0.009	

[a]Data are mean ± SD after adjustment for age, sex, years of education, GDS score and medical history of cardiovascular disease, diabetes mellitus, hyperlipidemia, and hypertension as covariates.
[b]With ANCOVA analysis of the effect of the level of TG and genotype on composite cognitive scores, main effect of TC level, $F_{[2,1381]} = 2.95$. $p = 0.06$; main effect of genotype, $F_{[1,1381]} = 7.99$. $p = 0.005$; TC level-by-genotype interaction, $F_{[2,1381]} = 0.033$. $p = 0.97$
[c]df = 2,1107 for *APOE4*(−), 2, 266 for *APOE4*(+).
[d]Significance at $p < 0.016$ (0.05/3) after Bonferroni adjustment for multiple comparisons: A, low to middle: B, low to high: C, middle to high concentration group comparison.
[e]$p < 0.05$.
[f]$\eta^2 > 0.01$.

LDL level on these cognitive scores was small and less than 0.01, and we could not regard this influence of the LDL level as a substantially meaningful one on cognitive scores. In the E4 (+) group, significant association was not observed (Table 5).

By ANCOVA analysis of the influence of TG or TC level and genotype on composite cognitive scores, we found no main effect of TG/TC and their interaction with genotype (Tables 6 and 7). We found no association between cognitive scores and the concentrations

FIGURE 1. Mean cognitive scores of each of the tertiles of lipid measures by E4 (−) and E4 (+) groups



Two-sample *t*-tests were performed to specify differences in cognitive score among the three strata according to the levels of HDL and apoE. P values after Bonferroni adjustment for multiple comparisons are shown.
[a]p <0.033 (0.10/3), [b]p <0.016 (0.05/3), [c]p <0.00033 (0.001/3).

of TG in either of the groups (Table 6). Subjects with higher TC concentration had one higher individual cognitive score in the E4 (−) group, but the effect size was small and less than 0.01. In the E4 (+) group, significant association was not observed (Table 7). In multiple regression analysis, there was no relationship of TG or TC level to composite cognitive score in either of the groups.

CONCLUSIONS

This is the first study to examine the relationship between cognitive function and the plasma levels of lipids including LDL, HDL, TG, TC, and apoE of elderly adults from the general population under consideration of the influence of *APOE* genotypes. In our analysis, we found that higher plasma levels of HDL were associated with better cognitive function in the E4 (−) group. Subjects with higher plasma levels of apoE had higher cognitive scores in both E4 (−) and E4 (+) groups. The concentrations of these lipids had a substantial influence on cognitive scores.

A number of possible mechanisms may explain the observed association between HDL and cognitive function in the E4 (−) group. One plausible explanation may be found in the involvement of HDL in their cerebral vascular pathology. Particles of HDL are assumed to be linked with small-vessel disease through their role in the removal of excess cholesterol from the subendothelian space of cerebral microvessels.[19] In fact, reduced HDL levels have been observed in vascular dementia (VaD).[20] In addition, low-level HDL is thought to be a risk factor for atherosclerotic diseases, leading to ischemic lesions in the brain that contribute to the development of cognitive decline and dementia.[21,22] It has been reported that HDL might also prevent aggregation and polymerization of amyloid in human brain.[23,24] In addition, anti-inflammatory properties of HDL could prevent inflammation from neurodegenerative processes.[25] However, these factors should be carefully considered for explanation of our findings, as plasma and brain cholesterol are separated by the blood brain barrier (BBB), and intact BBB prevents cholesterol influx from the circulation into the brain. Brain cholesterol is almost entirely synthesized in situ.[26]

We found the difference in cognitive score between the E4 (+) and E4 (−) groups by the degree of HDL concentration. By ANCOVA analysis of the influence of HDL level and ApoE genotype on the cognitive score, we found the interaction between them. When we added the plasma apoE level as covariate to the ANCOVA analysis, the interaction between HDL level and genotype failed to reach significance. ($F_{[2,1381]} = 3.25$. $p = 0.04 \rightarrow F_{[2,1380]} = 2.59$. $p = 0.08$). This interaction between HDL level and genotype on cognitive function may suggest the presence of interaction between HDL and apoE in cognitive function of the elderly. Considering the positive relationship of the apoE level with cognitive function in our study, it is possible that HDL might prevent the progression of cognitive decline via its influence on apoE.

Recent studies presented evidence of the involvement of internalized triglyceride-rich lipoprotein

(TRL)-derived apoE in the regulation of cellular cholesterol transport and HDL metabolism.[27] The greater portion of TRL-derived apoE forms a complex with cholesterol and remains in peripheral recycling endosomes. This pool of TRL-derived 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 HDL-induced recycling of apoE is accompanied by cholesterol enrichment of HDL and cholesterol efflux, and it may maximize the removal of cholesterol from the periphery.[28] Thus, HDL may prevent the progression of atherosclerosis and cognitive decline via apoE recycling, thereby reducing cholesterol accumulation. Our finding of the positive association between plasma apoE level and cognitive function is in agreement with the earlier-described hypothesis of the influence of apoE recycling. Further, it has been reported that elevated levels of plasma apoE reduce inflammation, endothelial dysfunction and lipid oxidation within lesions.[29] An antioxidant role of apoE in promoting the regression of atherosclerosis has also been reported.[30]

However, the absence of an association of HDL and cognitive function in the E4 (+) group remains a question to be addressed. Recent study showed that HDL-induced recycling of TRL-derived apoE4 is impaired and is associated with decreased cholesterol efflux.[31] In agreement with this finding, previous studies showed that apoE4 is less efficient in comparison with apoE3 in promoting cholesterol efflux from the periphery.[32,33] In our data, we found a lower concentration of apoE in the E4 (+) than in the E4 (−) group, perhaps reflecting the impaired recycling of apoE4. Examining the correlation between the levels of HDL and apoE, we found a significant positive relationship in the E4 (−) group ($r = 0.28$, $p < 0.001$, $df = 1,116$), but not in the E4 (+) group ($r = 0.08$, $p = 0.15$, $df = 275$). This finding may reflect the impairment of HDL-induced recycling of apoE4. This impairment might reduce the preventive role of HDL on atherosclerosis and at least partly account for the lack of the association of the HDL level with cognitive function in the E4 (+) group.

The present study has limitations. Three hundred seventy participants had no blood sampling data and/or cognitive data, and these participants were excluded from our analysis. When we compared the demographic characteristic data of excluded (n = 370) and included subjects (n = 1395), significance was found in older age (excluded versus included subjects: 76.4 ± 7.6 versus 73.6 ± 5.8 years), shorter education (9.0 ± 2.7 versus 10.0 ± 2.7 years), higher GDS score (3.3 ± 3.2 versus 2.6 ± 2.9) and higher ratio of a medical history of CVD (7.7% versus 3.7%). There is a possibility that the excluded subjects produced some distortions in the results.

Two hundred sixty-one participants required a face-to-face testing procedure, whereas the other 1,134 participants had tests with the group-setting procedure. There is a possibility that this difference in testing procedure had some confounding effect, although composite cognitive scores were not different between the groups (group versus face-to-face testing; 38.6 ± 12.1 versus 39.8 ± 12.2, $F_{[1,1385]} = 1.86$. $p = 0.17$), and the results did not change when we added the difference of testing procedure as a confounding factor in all of the performed statistical analyses (data not shown).

The sample size of E4 (+) was only about a quarter of that of E4 (−), and it is possible that the insignificant result in the E4 (+) group is affected by its small sample size. However, effect size was less than 0.01 or near 0.01 in all of the influences of lipids on cognitive scores in the E4 (+) group except that of apoE. This means that there was no or nearly negligible influence of lipids (except apoE) on cognitive scores in the E4 (+) group.

In conclusion, our findings suggest that plasma apoE have a positive influence on cognitive function in both the E4 (−) and E4 (+) groups, whereas the positive influence of plasma HDL was shown only in the E4 (−) group. The interaction between HDL level and *APOE* genotype on cognitive function may suggest the possible interaction between HDL and apoE. High-density lipoprotein may prevent the progression of atherosclerosis and cognitive decline via apoE recycling, which reduces cholesterol accumulation. However, HDL-induced recycling of apoE4 may be impaired. Although further longitudinal study is needed for sufficient basis for conclusions, the identification of the influences of *APOE* genotype and the intracellular linkage among apoE, cellular cholesterol transport, and HDL metabolism is hoped for new preventive and therapeutic strategies for cognitive decline in the elderly.

References

1. van Exel E, de Craen AJ, Gussekloo J, et al: Association between high-density lipoprotein and cognitive impairment in the oldest old. Ann Neurol 2002; 51:716–721
2. Yaffe K, Barrett-Connor E, Lin F, et al: Serum lipoprotein levels, statin use, and cognitive function in older women. Arch Neurol 2002; 59:378–384
3. Henderson VW, Guthrie JR, Dennerstein L: Serum lipids and memory in a population based cohort of middle age women. J Neurol Neurosurg Psychiatry 2003; 74:1530–1535
4. Sabbagh M, Zahiri HR, Ceimo J, et al: Is there a characteristic lipid profile in Alzheimer's disease? J Alzheimers Dis 2004; 6:585–589
5. Anstey KJ, Lipnicki DM, Low LF: Cholesterol as a risk factor for dementia and cognitive decline: a systematic review of prospective studies with meta-analysis. Am J Geriatr Psychiatry 2008; 16: 343–354
6. Mahley RW, Rall SCJ: Apolipoprotein E: far more than a lipid transport protein. Annu Rev Genomics Hum Genet 2000; 1: 507–537
7. Pablos-Méndez A, Mayeux R, Ngai C, et al: Association of apo E polymorphism with plasma lipid levels in a multiethnic elderly population. Arterioscler Thromb Vasc Biol 1997; 17:3534–3541
8. Lee BK, Glass TA, Wand GS, et al: Apolipoprotein e genotype, cortisol, and cognitive function in community-dwelling older adults. Am J Psychiatry 2008; 165:1456–1464
9. Fillenbaum GG, Landerman LR, Blazer DG, et al: The relationship of *APOE* genotype to cognitive functioning in older African-American and Caucasian community residents. J Am Geriatr Soc 2001; 49:1148–1155
10. Small BJ, Rosnick CB, Fratiglioni L, et al: Apolipoprotein E and cognitive performance: a meta-analysis. Psychol Aging 2004; 19: 592–600
11. West R, Beeri MS, Schmeidler J, et al: Better memory functioning associated with higher total and low-density lipoprotein cholesterol levels in very elderly subjects without the apolipoprotein e4 allele. Am J Geriatr Psychiatry 2008; 16:781–785
12. Miyamoto M, Kodama C, Kinoshita T, et al: Dementia and mild cognitive impairment among non-responders to a community survey. J Cli Neurosci 2009; 16:270–276
13. Wenham PR, Price WH, Blandell G: Apolipoprotein E genotyping by one-stage PCR. Lancet 1991; 337:1158–1159
14. Yesavage JA, Brink TL, Rose TL, et al: Development and validation of a geriatric depression screening scale: a preliminary report. J Psychiatr Res 1982; 17:37–49
15. Sohlberg M, Mateer CA: Attention process training manual., Washington, Association for neuropsychological research & development, 1986
16. Grober E, Buschke H, Crystal H, et al: Screening for dementia by memory testing. Neurology 1988; 38:900–903
17. Solomon PR, Pendlebury WW: Recognition of Alzheimer's disease: the 7 Minute Screen. Fam Med 1998; 30:265–271
18. Wechsler D: WAIS-R : manual : Wechsler adult intelligence scale-revised., New York, Harcourt Brace Jovanovich for Psychological Corp, 1981
19. Zuliani G, Ble' A, Zanca R, et al: Lipoprotein profile in older patients with vascular dementia and Alzheimer's disease. BMC Geriatr 2001; 1:1–5
20. Muckle TJ, Roy JR: High-density lipoprotein cholesterol in differential diagnosis of senile dementia. Lancet 1985; 1:1191–1193
21. Breteler MM, van Swieten JC, Bots ML, et al: Cerebral white matter lesions, vascular risk factors, and cognitive function in a population-based study: the Rotterdam Study. Neurology 1994; 44:1246–1252
22. Kalaria RN: The role of cerebral ischemia in Alzheimer's disease. Neurobiol Aging 2000; 21:321–330
23. Koudinov AR, Berezov TT, Kumar A, et al: Alzheimer's amyloid beta interaction with normal human plasma high density lipoprotein: association with apolipoprotein and lipids. Clin Chim Acta 1998; 270:75–84
24. Olesen OF, Dagø L: High density lipoprotein inhibits assembly of amyloid beta-peptides into fibrils. Biochem Biophys Res Commun 2000; 270:62–66
25. Cockerill GW, Huehns TY, Weerasinghe A, et al: Elevation of plasma high-density lipoprotein concentration reduces interleukin-1-induced expression of E-selectin in an in vivo model of acute inflammation. Circulation 2001; 103: 108–112
26. Dietschy JM, Turley SD: Cholesterol metabolism in the brain. Curr Opin Lipidol 2001; 12:105–112
27. Heeren J, Grewal T, Laatsch A, et al: Recycling of apoprotein E is associated with cholesterol efflux and high density lipoprotein internalization. J Biol Chem 2003; 278:14370–14378
28. Heeren J, Beisiegel U, Grewal T: Apolipoprotein E recycling: implications for dyslipidemia and atherosclerosis. Arterioscler Thromb Vasc Biol 2006; 26:442–448.
29. Davignon J, Cohn JS, Mabile L, et al: Apolipoprotein E and atherosclerosis: insight from animal and human studies. Clin Chim Acta 1999; 286:115–143
30. Tangirala RK, Praticó D, FitzGerald GA, et al: Reduction of isoprostanes and regression of advanced atherosclerosis by apolipoprotein E. J Biol Chem 2001; 276:261–266
31. Heeren J, Grewal T, Laatsch A, et al: Impaired recycling of apolipoprotein E4 is associated with intracellular cholesterol accumulation. J Biol Chem 2004; 279:55483–55492
32. Huang Y, von Eckardstein A, Wu S, et al: Effects of the apolipoprotein E polymorphism on uptake and transfer of cell-derived cholesterol in plasma. J Clin Invest 1995; 96:2693–2701
33. Michikawa M, Fan QW, Isobe I, et al: Apolipoprotein E exhibits isoform-specific promotion of lipid efflux from astrocytes and neurons in culture. J Neurochem 2000; 74:1008–1016

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Anti-Aβ Drug Screening Platform Using Human iPS Cell-Derived Neurons for the Treatment of Alzheimer's Disease

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Abstract

Background: Alzheimer's disease (AD) is a neurodegenerative disorder that causes progressive memory and cognitive decline during middle to late adult life. The AD brain is characterized by deposition of amyloid β peptide (Aβ), which is produced from amyloid precursor protein by β- and γ-secretase (presenilin complex)-mediated sequential cleavage. Induced pluripotent stem (iPS) cells potentially provide an opportunity to generate a human cell-based model of AD that would be crucial for drug discovery as well as for investigating mechanisms of the disease.

Methodology/Principal Findings: We differentiated human iPS (hiPS) cells into neuronal cells expressing the forebrain marker, Foxg1, and the neocortical markers, Cux1, Satb2, Ctip2, and Tbr1. The iPS cell-derived neuronal cells also expressed amyloid precursor protein, β-secretase, and γ-secretase components, and were capable of secreting Aβ into the conditioned media. Aβ production was inhibited by β-secretase inhibitor, γ-secretase inhibitor (GSI), and an NSAID; however, there were different susceptibilities to all three drugs between early and late differentiation stages. At the early differentiation stage, GSI treatment caused a fast increase at lower dose (Aβ surge) and drastic decline of Aβ production.

Conclusions/Significance: These results indicate that the hiPS cell-derived neuronal cells express functional β- and γ-secretases involved in Aβ production; however, anti-Aβ drug screening using these hiPS cell-derived neuronal cells requires sufficient neuronal differentiation.

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Introduction

Alzheimer's disease (AD) is the most common cause of dementia in the elderly. It is characterized clinically by progressive declines in memory, executive function, and cognition. It is also characterized by pathological features, including the deposition of amyloid plaques and neurofibrillary tangles as well as neuronal and synaptic loss in particular areas of the brain [1]. Accumulation of amyloid β peptide (Aβ) is hypothesized to initiate the pathogenic cascade that eventually leads to AD. The amyloid hypothesis is based on an imbalance between the production and clearance of Aβ [2]. Aβ is produced by β- and γ-secretase-mediated sequential proteolysis of amyloid precursor protein (APP) and plays a central role in AD pathogenesis. Because β- and γ-secretases are directly involved in Aβ production, they are straightforward and attractive therapeutic targets for AD. A number of compounds that inhibit or modulate these secretase activities and Aβ levels *in vitro* and *in vivo* have to date been developed [3,4].

Development of a human, cell-based *in vitro* assay system is a basic requisite for drug discovery and for investigating mechanisms of the disease. Induced pluripotent stem (iPS) cells reprogrammed from somatic cells [5,6] provide an opportunity to easily generate and use patient-specific differentiated cells. Because previous AD assay systems using human cancer cell lines or primary rodent cell cultures did not perfectly present the human intracellular environment or components, human iPS (hiPS) cell-derived neuronal cells may enable the development of more efficient drugs, such as γ-secretase modulators, and the better elucidation of AD mechanisms. In this study, we successfully generated forebrain neurons from hiPS cells, and showed that Aβ production in

neuronal cells was detectable and inhibited by some typical secretase inhibitors and modulators. Thus, we provide a new platform for AD drug development, which might be applied to AD patient-specific iPS cell research.

Results

Differentiation of forebrain neurons from hiPS cells

Recently, forebrain neurons were successfully differentiated from mouse embryonic stem (ES) cells [7,8,9] and human ES and/or iPS cells [9,10,11]. The methods used for differentiation into spinal motor neurons and midbrain dopaminergic neurons required the morphogens retinoic acid (RA)/sonic hedgehog (SHH) and fibroblast growth factor 8 (FGF8)/SHH, respectively [11,12]. On the other hand, non-morphogens [10,11] or Lefty A and Dickkopf homolog 1 (Dkk1) [7,9] have been used for the induction of hiPS cells into forebrain neurons. Because amyloid plaques are observed in the cerebral cortex from the early stage of AD development [13], stem cells should be differentiated to at least forebrain neurons for *in vitro* assays in AD research.

We differentiated forebrain neurons from hiPS 253G4 cells, which were generated from human dermal fibroblasts using three reprogramming factors (Oct3/4, Sox2, and Klf4) [14], as described previously (Figure 1A) [12,15]. When neural stem cells were induced with Noggin and SB431542 for 17 days, we obtained cells that were positive for the neuroectodermal marker, Nestin (Figure 1B), as previously reported using human and monkey ES cells [15]. After culturing the cells with morphogen-free medium for days 17–24, Forkhead box G1 (Foxg1) expression was induced and Foxg1-positive cells were observed (Figure 1C, D) [11,15]. We also examined whether treatment with cyclopamine, an SHH inhibitor, increased the number of neurons presenting a glutamatergic phenotype as observed in mouse ES cells [8]. The expression level of vesicular glutamate transporter 1 (vGlut1), a glutamatergic marker, was not significantly increased by the addition of cyclopamine (final concentration 1 μM) from days 17 to 24 (data not shown). Therefore, we did not add cyclopamine in this period in subsequent experiments. At day 24, dissociated cells were reseeded on 24-well plates to further characterize the cells.

Next, we evaluated the hiPS cell-derived neuronal cells using four cortical layer-specific markers, T-brain-1 (Tbr1) and chicken ovalbumin upstream promoter transcription factor (COUP-TF)-interacting protein 2 (Ctip2) [9,10,11], and cut-like homeobox 1 (Cux1) and special AT-rich sequence-binding protein 2 (Satb2) [16]. Quantitative polymerase chain reaction (qPCR) revealed that expression levels of these markers were increased in a differentiation day-dependent manner (Figure 1E). At day 52, all four of these markers were visualized by immunocytochemistry (ICC) (Figure 1F). The percentages of marker-positive cells relative to the total number of cells were 62.2±2.9% for Tbr1, 11.9±3.0% for Ctip2, 82.6±5.0% for Cux1, and46.0±7.1% for Satb2. The population of each marker-positive cell was similar to that of data reported previously in human fetal brain around gestational week-20 [16]. In this experimental schedule, most cells expressed one or a few neocortical markers at day 52.

Characterization of hiPS cell-derived neuronal cells

Cells that were reseeded at day 24, were sparsely adhered to the culture plate and had proliferated and extended neurites in a time course-dependent manner as observed by the neuronal marker, class-III β-tubulin (Tuj1), and microtubule-associated protein 2 (MAP2) (Figure 2A). Tuj1 expression was almost saturated at day 45 (Figure 2B), but MAP2 and synapsin I expression were still increasing (Figure 2C, D). Synaptic development continued until day 52, and many synapsin I-positive puncta were detected by ICC at day 52 (Figure 2A). Expression of the glial marker, glial fibrillary acidic protein (GFAP), was highest at day 52 in this schedule (Figure 2E). This sequential expression pattern is similar to that reported recently in human pluripotent stem cell-derived neurons; the synapsin I-positive neuronal and GFAP-positive glial cultures at day 52 corresponded to the stage at which spontaneous neuronal activity was observed [17].

We then examined the neurotransmitter phenotypes of these differentiated neurons by evaluating the synthesizing enzymes for two typical cortical neurotransmitters, glutamate and γ-aminobutyric acid (GABA). Expression of the glutamatergic neuronal marker, phosphate-activated glutaminase (PAG) [18], and the GABAergic neuronal marker, glutamate decarboxylase (GAD), were observed by ICC at day 52 (Figure 2F). PAG- and GAD-positive neurons comprised 60±20% and 5±4% of total cells, respectively. Most of the Tuj1-positive neurons were also co-localized with the punctate signals of vGlut1 (Figure 2G). GABA-positive neurons comprised a similar population to the GAD-positive ones (Figure 2F, H). On the other hand, cholineacetyltransferase (ChAT) or vesicular acetylcholine transporter (VAChT)-positive cholinergic neurons were little observed at day 52, although their mRNA level increased with differentiation time (Figure S1). These data showed that a majority of differentiated neuronal cells possessed a glutamatergic phenotype in the present condition.

Differentiated neuronal cells express some components related to Aβ production

To evaluate their usefulness as an AD model, we measured the levels of Aβ secreted from the differentiated neuronal cells at days 38, 45, and 52. In the non-amyloidogenic pathway, α-secretase cleaves full-length APP (FL-APP) within the Aβ domain to the large soluble APP fragment (sAPPα) and APP-C terminal fragment α (CTFα) (Figure 3) [19]. In the amyloidogenic pathway, β-secretase, β-site APP cleaving enzyme 1 (BACE1), cleaves APP on the N-terminal side of the Aβ domain to soluble sAPPβ and APP-CTFβ (Figure 3). FL-APP and its cleavage products were increased in a time-course-dependent manner (Figure 3).

APP has three alternatively spliced isoforms: APP695, APP751, and APP770. APP695 is most abundantly expressed in neurons, whereas APP751 and APP770 show more ubiquitous expression patterns [20]. In cell lysates, we detected three separate APP variants on western blots. The estimated percentages of the neuron-dominant variant APP695 were 64.5±1.0%, 68.6±2.2%, and 69.6±2.1% at days 38, 45, and 52, respectively (Figures 3A and S2). The neuronal population at day 52 was approximately consistent with the sum of the percentages of the glutamatergic and GABAergic neurons mentioned above.

The aspartyl protease BACE1, the major β-secretase involved in cleaving APP, is a significant molecule for AD pathology because BACE1 protein levels and activity are increased in the brains of patients with the sporadic form of AD [21]. In our differentiated neurons, BACE1 protein levels were increased in a time course-dependent manner (Figure 4A, B), and we speculated that the upregulation of BACE1 protein levels may be due to a posttranscriptional mechanism [22]. BACE1 mRNA levels were slightly elevated with time (Figure 4B). These data may indicate that increased BACE1 protein levels were mainly induced by translational activation along with neuronal differentiation.

APP-CTFβ is cleaved to Aβ and APP intercellular domain (AICD) by γ-secretase (Figure 3). The γ-secretase complex consists of four core members, presenilin (PS; either PS1 or PS2), nicastrin, Pen-2, and Aph-1 [23]. PS1, nicastrin, and Pen-2 were detected by