a greater decrease in performance). Participants were considered to have memory complaints if they had problems on one or more of the items. This type of test does not involve direct cognitive examination of the older person but has the advantage of indicating degree of change from former level of functioning.

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

Neuropsychological assessment battery. After completing the interview, all participants underwent a group assessment using a set of five tests (5-Cog), which measured the following cognitive domains: attention, memory, visuospatial function, language, and reasoning. The validity and reliability of the tests and the details of the assessment battery have been described elsewhere (Miyamoto *et al.*, 2009; Sasaki *et al.*, 2009).

We evaluated attention by using a Japanese version of a set dependency activity (Sohlberg and Mateer, 1986). To assess memory, we used a category cued recall test (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 employed for the assessment of visuospatial function. We examined language ability by using a category fluency test (Soloman and Pendlebury, 1998). To assess abstract reasoning, we employed the similarity subset of the revised Wechsler Adult Intelligence Scale (Wechsler, 1981).

Test–retest reliability of the 5-Cog was confirmed (mean value of Pearson's correlation coefficient was 0.70, $p < 0.01$ for all five tests). We used data from 38 initial participants who were randomly selected; data were collected at a mean interval of 64 days (standard deviation (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 the absence of dementia by diagnosis of dementia according to the DSM-IV (American Psychiatric Association, 1994) criteria. Only those who were not diagnosed as having dementia were considered for a diagnosis of MCI.

Mild cognitive impairment diagnostic criteria. Criteria for MCI were retrospectively applied among individuals without dementia after the consensus conference. Consistent with standard criteria for all subtypes of MCI (Petersen and Morris, 2005), those with MCI were required to have the following: (i) a memory complaint (defined previously); (ii) objective impairment in at least one of five cognitive domains (memory, attention, language, visuospatial function, and reasoning) based on the average scores of the neuropsychological measures within that domain and 1.5 SDs cutoff using normative corrections for age, years of education, and sex; (iii) essentially preserved activities of daily living (defined earlier); and (4) no diagnosis of dementia at the consensus conference. We used the following subtypes of MCI: amnestic MCI single, amnestic MCI multiple, non-amnestic MCI single, and non-amnestic MCI multiple (Petersen and Morris, 2005). The classification into the four MCI subtypes was mutually exclusive.

Second phase (structured interview)

To make final diagnoses of depression, we conducted the second phase. For this phase, we invited all of the individuals who had participated in the first phase.

As a result, 738 first-phase participants who fulfilled the following criteria took part in the second phase: no diagnosis of dementia, acceptance of ApoE typing, and no missing data.

Except for the 44 institutionalized people, 881 individuals were not interviewed for the following reasons: diagnosis of dementia, refusal of ApoE typing, missing data, or refusal to participate in the second phase.

The participants of the first phase were interviewed by seven psychiatrists and eight psychologists in a face-to-face interview between April and July 2002. The mean interval between the first phase and the second phase was 62 days (SD = 36 days).

We conducted straightforward interviews with the subjects by using the Psychogeriatric Assessment Scale (PAS), which provides a brief but comprehensive profile of the older individual's mental state (depression, cognitive impairment, and stroke; Jorm *et al.*, 1995). There were three scales derived from an interview with the subject (depression, cognitive impairment, and stroke). In general, the validity and reliability of the PAS has been documented. The depression subscale, in particular, has excellent validity when judged against the clinical diagnosis of MDE based on DSM-III-R criteria (American Psychiatric Association, 1987). The guidelines of PAS

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.

Acknowledgement

Funding for this research was obtained from the Ministry of Health, Labor and Welfare (grant nos. 2001-dementia and fracture-003).

Conflict of interest

None declared.

References

American Psychiatric Association. 1987. *Diagnostic and Statistical Manual of Mental Disorders*, 3rd Ed-revised. American Psychiatric Association: Washington, DC.
American Psychiatric Association. 1994. *Diagnostic and Statistical Manual of Mental Disorders*, 4th Ed. American Psychiatric Association: Washington, DC.
Artero S, Ancelin ML, Porter F, *et al.* 2008. Risk profiles for mild cognitive impairment and progression to dementia are gender specific. *J Neurol Neurosurg Psychiatry* **79**: 979–984.
Azar AR, Murrell SA, Mast BT. 2005. Race and vascular depression risk in community-dwelling older adults. *Am J Geriatr Psychiatry* **13**: 329–332.
Beekman ATF, Deeg DJH, Geerlings SW, *et al.* 2001. Emergence and persistence of late life depression: a 3-year follow-up of the Longitudinal Aging Study Amsterdam. *J Affect Disord* **65**: 131–138.
Bisschop MI, Kriegsman DMW, Beekman ATF, *et al.* 2004. Chronic disease and depression: the modifying role of psychosocial resources. *Soc Sci Med* **59**: 721–733.
Blumstein T, Benyamini Y, Fuchs Z, *et al.* 2004. The effect of a communal lifestyle on depressive symptoms in late life. *J Aging Health* **16**: 151–174.
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.
Braam AW, Prince MJ, Beekman ATF, *et al.* 2005. Physical health and depressive symptoms in older Europeans. *Br J Psychiatry* **187**: 35–42.
Brink TL, Yesavage JA, Lum O, *et al.* 1982. Screening tests for geriatric depression. *Clin Gerontol* **1**: 37–44.
Chan DC, Kasper JD, Black BS, *et al.* 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.
Copeland JR, Chen R, Dewey ME, *et al.* 1999. Community-based case–control study of depression in older people. Case and sub-cases from the MRC-ALPHA Study. *Br J Psychiatry* **175**: 340–347.
Corder EH, Saunders AM, Strittmatter WJ, *et al.* 1993. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science* **261**: 921–923.
Freedman M, Leach L, Kaplan E, *et al.* 1994. *Clock Drawing: a Neuropsychological Analysis.* Oxford University Press: New York.
Gazmararian J, Baker D, Parker R, *et al.* 2000. A multivariate analysis of factors associated with depression: evaluating the role of health literacy as a potential contributor. *Arch Intern Med* **160**: 3307–3314.
Grober E, Buschke H, Crystal H, *et al.* 1988. Screening for dementia by memory testing. *Neurology* **38**: 900–903.
Harwood DG, Barker WW, Ownby RL, *et al.* 1999. Factors associated with depressive symptoms in non-demented community-dwelling elderly. *Int J Geriatr Psychiatry* **14**: 331–337.
Heun R, Hein S. 2005. Risk factors of major depression in the elderly. *Eur Psychiatry* **20**: 199–204.
Hidaka S, Ikejima C, Kodama C, *et al.* 2012. Prevalence of depression and depressive symptoms among older Japanese people: comorbidity of mild cognitive impairment and depression. *Int J Geriatr Psychiatry* **27**: 271–279.
Horowitz A, Reinhardt JP, Kennedy GJ. 2005. Major and subthreshold depression among older adults seeking vision rehabilitation services. *Am J Psychiatry* **13**: 180–187.
Jang Y, Haley WE, Small BJ, *et al.* 2002. The role of mastery and social resources in the associations between disability and depression in later life. *Gerontologist* **42**: 807–813.
Jorm AF, Anstey KJ, Christensen H, *et al.* 2005. MRI hyperintensities and depressive symptoms in a community sample of individuals 60–64 years old. *Am J Psychiatry* **162**: 699–705.
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.
Kraaij V, de Wilde EJ. 2001. Negative life events and depressive symptoms in the elderly: a life span perspective. *Aging Ment Health* **5**: 84–91.
Krishnan KR, Tupler LA, Ritchie JC Jr, *et al.* 1996. Apolipoprotein E-epsilon 4 frequency in geriatric depression. *Biol Psychiatry* **40**: 69–71.
Lopez OL, Jagust WJ, Dulberg C, *et al.* 2003. Risk factors for mild cognitive impairment in the Cardiovascular Health Study Cognitive Study: part 2. *Arch Neurol* **60**: 1394–1399.
Lyketsos CG, Lopez O, 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.
Miyamoto M, Kodama C, Kinoshita T, *et al.* 2009. Dementia and mild cognitive impairment among non-responders to a community survey. *J Clin Neurosci* **16**: 270–276.
Muangpaisan W, Intalapaporn S, Assantachai P. 2008. Neuropsychiatric symptoms in the community-based patients with mild cognitive impairment and the influence of 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.
Ohara K, Nagai M, Suzuki Y, *et al.* 1999. Apolipoprotein E epsilon 4 allele and Japanese late-onset depressive disorder. *Biol Psychiatry* **45**: 308–312.
Oldehinkel AJ, Ormel J, Brilman EI, *et al.* 2003. Psychosocial and vascular risk factors of depression in later life. *J Affect Disord* **74**: 237–246.

Palmer K, Berger AK, Monastero R, *et al.* 2007. Predictors of progression from mild cognitive impairment to Alzheimer disease. *Neurology* **68**: 1596–1602.
Petersen RC, Morris JC. 2005. Mild cognitive impairment as a clinical entity and treatment target. *Arch Neurol* **62**: 1160–1163.
Petersen RC, Smith GE, Waring SC, *et al.* 1999. Mild cognitive impairment: clinical characterization and outcome. *Arch Neurol* **56**: 303–308.
Ramakers IH, Visser PJ, Aalten P, *et al.* 2008. The association between APOE genotype and memory dysfunction in subjects with mild cognitive impairment is related to age and Alzheimer pathology. *Dement Geriatr Cogn Disord* **26**: 101–108.
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.
Sasaki M, Kodama C, Hidaka S, *et al.* 2009. Prevalence of four subtypes of mild cognitive and APOE in a Japanese community. *Int J Geriatr Psychiatry* **24**: 1119–1126.
Schoevers RA, Deeg DJ, van Tilburg W, *et al.* 2005. Depression and generalized anxiety disorder: co-occurrence and longitudinal patterns in elderly patients. *Am J Geriatr Psychiatry* **13**: 31–39.
Sohlberg M, Mateer CA. 1986. *Attention Process Training Manual.* Association for Neuropsychological Research & 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.
Strawbridge WJ, Deleger S, Roberts RE, *et al.* 2002. Physical activity reduces the risk of subsequent depression for older adults. *Am J Epidemiol* **156**: 328–334.
Surtees PG, Wainwright NW, Bowman R, *et al.* 2009. No association between APOE and major depressive disorder in a community sample of 17,507 adults. *J Psychiatr Res* **43**: 843–847.
van der Filer WM, Pijnenburg YA, Schoonenboom SN, *et al.* 2008. Distribution of APOE genotypes in a memory clinic cohort. *Dement Geriatr Cogn Disord* **25**: 433–438.
van der Wurff FB, Beekman ATF, Dijkshoorm H, *et al.* 2004. Prevalence and risk-factors for depression in elderly Turkish and Moroccan migrants in the Netherlands. *J Affect Disord* **83**: 33–41.
Wechsler D. 1981. *Wechsler Adult Intelligence Scale—Revised.* Psychological Corporation: Cleveland, OH.
Yen YC, Rebok GW, Gallo JJ, *et al.* 2007. ApoE4 allele is associated with late-life depression: a population-based study. *Am J Geriatr Psychiatry* **15**: 858–868.

Psychiatry Research: Neuroimaging 221 (2014) 149–154

Contents lists available at ScienceDirect

Psychiatry Research: Neuroimaging

journal homepage: www.elsevier.com/locate/psychresns

Microstructural changes of the nucleus accumbens due to increase of estradiol level during menstrual cycle contribute to recurrent manic episodes—A single case study

Kiwamu Matsuoka [a,c], Fumihiko Yasuno [a,b,*], Makoto Inoue [c], Akihide Yamamoto [b], Takashi Kudo [d], Soichiro Kitamura [a], Koji Okada [a], Kuniaki Kiuchi [a], Jun Kosaka [a], Hidehiro Iida [b], Toshifumi Kishimoto [a]

[a] Department of Psychiatry, Nara Medical University, 840 Shijocho, Kashihara, Nara 634-8522, Japan
[b] Department of Investigative Radiology, National Cerebral and Cardiovascular Center, Suita, Japan
[c] Department of Psychiatry, National Hospital Organization Yamato Mental Medical Center, Yamatokoriyama, Japan
[d] Department of Psychiatry, Osaka University Health Care Center, Toyonaka, Japan

ARTICLE INFO

Article history:
Received 30 May 2013
Received in revised form
18 November 2013
Accepted 20 November 2013
Available online 1 December 2013

Keywords:
Bipolar disorder
Menstrual cycle
Estradiol
Magnetic resonance imaging (MRI)
Diffusion tensor imaging (DTI)
Nucleus accumbens

ABSTRACT

We examined a rapid-cycling bipolar disorder patient who demonstrated manic episode regularly at around day 7 of the menstrual cycle. We hypothesize that gonadal hormones may induce a state-dependent change in cerebral microstructure and function. Following this hypothesis, the serum levels of estradiol and progesterone were analyzed and diffusion tensor imaging data were examined between the manic and euthymic states of the patient. Estradiol levels increased in the late follicular phase at manic state when compared to the luteal or early follicular phase at euthymic state. DTI results showed that the patient had increased fractional anisotropy values at manic state in the bilateral nucleus accumbens (NAc) and its connected areas, which is a major projection field of the mesolimbic dopamine (DA) system, perhaps reflecting microstructural changes due to neuronal activation related to manic episodes. According to these results, we consider that the mesolimbic DA system of this patient has hypersensitivity to estradiol, and elevation of the estradiol level increases the activity of the dopaminergic system, which in turn may contribute to recurrent manic episodes. Our findings provide a clue for understanding how fluctuations in gonadal hormone may amplify or ameliorate the symptomatology of psychiatric disorders related to the menstrual cycle.

1. Introduction

Affective fluctuations during the menstrual cycle have been studied (Akdeniz and Karadağ, 2006). A few previous case presentations of patients, including a report of a woman with bipolar disorder (BPD), showed experienced specific mood episodes in certain periods of the menstrual cycle, such as the premenstrual period (Kukopulos et al., 1985; D'Mello et al., 1993) and luteal phase (Becker et al., 2004). However, the mechanisms underlying the illness phases related to the menstrual cycle have not been investigated. In the present study we report a rapid-cycling bipolar disorder patient who regularly demonstrated manic episode starting in the follicular phase and continuing for about 2 weeks.

In our case of recurrent manic episodes related to the phases of the menstrual cycle, we hypothesize that fluctuations of gonadal hormones may induce a state-dependent change in cerebral microstructure and function that result in a recurrence of the manic symptoms. According to this hypothesis, the serum levels of estradiol and progesterone were analyzed at manic and euthymic states of the patient. In order to elucidate the regional microstructural changes related to manic symptoms, we performed exploratory voxel-based analysis and compared DTI images between the patient and healthy subjects. We expected that the patient would show manic state-dependent brain microstructural changes in the regions related to manic symptoms, which were affected by the fluctuations of gonadal hormones related to the phases of the menstrual cycle.

2. Materials and methods

2.1. Patient and healthy control subjects

The patient was a 32-year-old right-handed woman. She had no history of alcohol or illicit drug abuse. Since age 21, she had recurrent manic episodes every

* Corresponding author at: Department of Psychiatry, Nara Medical University, 840 Shijocho, Kashihara, Nara 634-8522, Japan.
Tel.: +81 744 22 3051; fax: +81 744 22 3854.
E-mail address: ejm86rp@yahoo.co.jp (F. Yasuno).

0925-4927/$ - see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.pscychresns.2013.11.006

month and numerous admissions to psychiatric units. At the age of 31, she was admitted to our psychiatric hospital. Since she was admitted to our psychiatric hospital, we observed her mood episodes for more than one year. During this period, in all of her menstrual cycles (MCs), the patient demonstrated manic episodes regularly beginning at around day 7 in the follicular phase. Her manic episodes continued for about two weeks, around ovulation, with euthymic state intervals. She had a natural 28-day menstrual cycle and did not take oral contraceptives at all during her recurrent manic/mood episodes. We diagnosed her as *rapid-cycling bipolar disorder based on the structured clinical interview for DSM-IV* axis I disorder (SCID) (First et al., 1997). We tried several mood-stabilizing medications, electroconvulsive therapy (ECT) treatments, but her manic episodes continued to recur.

To examine the mechanisms underlying the illness phases related to the menstrual cycle, the serum levels of estradiol and progesterone were analyzed twice: first at the euthymic state before the manic state (day 23 of MC), and second at the manic state (day 12 of MC). To replicate the relation of the gonadal hormones and the manic state, we performed a second analysis of gonadal hormones during another menstrual cycle [first at euthymic state (day 2 of MC), and second at manic state (day 12 of MC)].

For the purpose of investigating brain microstructural changes in manic episode compared to before and after manic episode, DTI was performed three times: first at the euthymic state before the manic state (day 23 of MC), second at the manic state (day 12 of MC), and third at the euthymic state following the previous manic state (day 2 of MC). We could not do further imaging analysis for replicating the results because of refusal by the patient. During the analyses of serum gonadal hormones and MRI scans, the patient was in a drug-free condition, taking no mood stabilizers or antipsychotic drugs.

Thirty-four healthy control subjects (11 female/23 male, age: 28.3 ± 6.4 years) were recruited from the local area by poster advertisement. Exclusion criteria for healthy subjects were a history or present diagnosis of any DSM-IV axis I diagnosis or any neurological illness. The patient and controls were subjected to a series of standardized, quantitative measurements of manic and depressive symptoms [Young Mania Rating Scale (YMRS) (Young et al., 1978), Montgomery Asberg Depression Rating Scale (MADRAS) score (Montgomery and Asberg, 1979), and Hamilton Rating Scale for Depression (HAMD-17) (Hamilton, 1960)] on the day of the MRI scan.

After complete description of the study, written informed consent was obtained from the patient and the healthy controls. The study was approved by the medical ethics committee of the National Cerebral and Cardiovascular Center in Japan.

2.2. Hormone assay

Blood was withdrawn via the median antebrachial vein. Sera were separated by centrifugation at 3200 rpm for 7 min and sent to Ikagaku CO., LTD (Kyoto, Japan), where the serum concentrations of estradiol and progesterone were measured by direct chemiluminescence, using Siemens ADVIA® Centaur™ Immunoassay System. For normal ranges of estradiol and progesterone for the menstrual phase, we used the laboratory data provided by Ikagaku CO., LTD determined from multiple subjects.

2.3. Data acquisition of MRI

All MRI examinations were performed by 3-Tesla whole-body scanner (Signa Excite HD V12M4; GE Healthcare, Milwaukee, WI, USA) with an 8-channel phased-array brain coil. Diffusion-weighted MR images were obtained with a locally modified single-shot echo-planar imaging (EPI) sequence by parallel acquisition at a reduction (ASSET) factor of 2, in the axial plane. Imaging parameters were as follows: TR=17 s; TE=72 ms; b=0, 1000 s/mm^2; acquisition matrix, 128×128; field of view (FOV), 256 mm; section thickness, 2.0 mm; no intersection gap; 74 sections. The reconstruction matrix was the same as the acquisition matrix, and 2 mm × 2 mm × 2 mm isotropic voxel data were obtained. Motion probing gradient (MPG) was applied in 55 directions, the number of images was 4144, and the acquisition time was 15 min and 52 s.

To reduce blurring and signal loss arising from field inhomogeneity, an automated high-order shimming method based on spiral acquisitions was used before acquiring the DTI scans. To correct for motion and distortion from eddy current and B0 inhomogeneity, FMRIB software (FMRIB Center, Department of Clinical Neurology, University of Oxford, Oxford, England; http://www.fmrib.ox.ac.uk/fsl/) was utilized. B0 field mapping data were also acquired with the echo time shift (of 2.237 ms) method based on two gradient echo sequences.

High-resolution three-dimensional T1-weighted images were acquired using a spoiled gradient-recalled sequence (TR=12.8 ms, TE=2.6 ms, flip angle=8°, FOV, 256 mm; 188 sections in the sagittal plane; acquisition matrix, 256×256; acquired resolution, $1 \times 1 \times 1$ mm^3). T2-weighted images were obtained using a fast-spin echo (TR=4800 ms; TE=101 ms; echo train length (ETL)=8; FOV=256 mm; 74 slices in the transverse plane; acquisition matrix, 160×160, acquired resolution, $1 \times 1 \times 2$ mm^3).

2.4. Imaging processing

FA images and three eigenvalues ($\lambda1$, $\lambda2$, and $\lambda3$) were generated from each individual using FMRIB software. First, brain tissue was extracted using the Brain Extraction Tool in FSL software. Diffusion-weighted images for each of the 55 directions were eddy-corrected, subsequent to which FA values were calculated at each voxel using the FSL FMRIB Diffusion Toolbox.

Image preprocessing and statistical analysis were carried out using SPM8 (Wellcome Department of Imaging Neuroscience, London, England). Each subject's echo planar image was spatially normalized to the Montreal Neurological Institute echo planar image template using parameters determined from the normalization of the image with a b value of 0 s/mm^2 and the echo planar image template in SPM8.

Normalized gray and white matter images were generated from each individual T1-weighted image using the VBM8 toolbox with SPM8 software (Ashburner and Friston, 2000).

Normalized images were spatially smoothed using an isotropic Gaussian filter (6-mm full-width at half-maximum).

2.5. Voxel-based analysis

Exploratory voxel-based analysis was performed using SPM8 software. FA and gray/white matter images were compared between the patient and healthy subjects with Jack-knife analysis. Statistical inferences were made with a voxel-level threshold of $p < 0.05$, after family-wise error correction for multiple comparisons, with a minimum cluster size of 50 voxels.

Spherical VOIs (3-mm radius) were determined from regions where the patient showed significantly higher or lower FA values than controls. The center of the spherical VOIs was determined from the MNI coordinate with peak t-value. The regional FA value was calculated by averaging values for all voxels within the spherical VOIs placed on the regions of FA images of controls and patient at euthymic and manic states. The same VOIs were applied to $\lambda1$, $\lambda2$ and $\lambda3$ images. $\lambda1$–$\lambda3$ Values were extracted, and mean diffusivity (MD) [$(\lambda1+\lambda2+\lambda3)/3$], axial ($\lambda1$) and radial diffusivity ($[\lambda2+\lambda3]/2$) were compared (Alexander et al., 2007).

To examine the effect of age on white matter integrity in our study, we examined the relationship between the regional FA values in the VOIs and age by Pearson's correlation analysis. To assess the effect of gender on white matter integrity in our study, we compared the regional FA values between male and female controls by t-test.

3. Results

3.1. Demographic and clinical data

Table 1 summarizes the demographic and clinical characteristics of the patient and controls. Manic and depressive symptoms were assessed on the day of the MRI session at euthymic and manic states. The patient showed manic symptoms at only manic states, and no manic or depressive symptoms at euthymic states. None of the control subjects showed manic or depressive symptoms at the examination.

3.2. Estradiol and progesterone levels in the patient's blood

As shown in Table 2, because of the normal menstrual cycle phase, estradiol levels increased in the late follicular phase at manic state when compared to the luteal or early follicular phase

Table 1
Demographic characteristics of the analysis of the patient and controls.

Characteristic	Patient	Controls (n=34)
Age, y	33	28.3 ± 6.4
Female, No (%)	–	11 (32%)
Young Mania Rating Scale	0 At euthymic state 42 at manic state	0 For all controls
MADRAS score	0 At both states	1.0 ± 1.7
HAM-D score	0 At both states	1.1 ± 1.6

MADRAS, Montgomery Asberg Depression Rating Scale; HAM-D, Hamilton Depression Rating Scale.

Table 2
Serum levels of estradiol and progesterone at euthymic and manic states.

Hormone	Euthymic state	Menstrual phase (day of cycle)	Manic state	Menstrual phase (day of cycle)
Estradiol (pg/mL) (normal range for menstrual phase)				
First test	87.4 (55.8–214.2)	Luteal (23)	512 (63.9–356.7)	Late follicular (12)
Second test	41 (19.5–144.2)	Early follicular (2)	206 (63.9–356.7)	Late follicular (12)
Progesterone (ng/mL) (normal range for menstrual phase)				
First test	15.2 (1.4–20.6)	Luteal (23)	< 0.2 (0.3–10.4)	Late follicular (12)
Second test	0.8 (< 1.2)	Early follicular (2)	0.2 (0.3–10.4)	Late follicular (12)

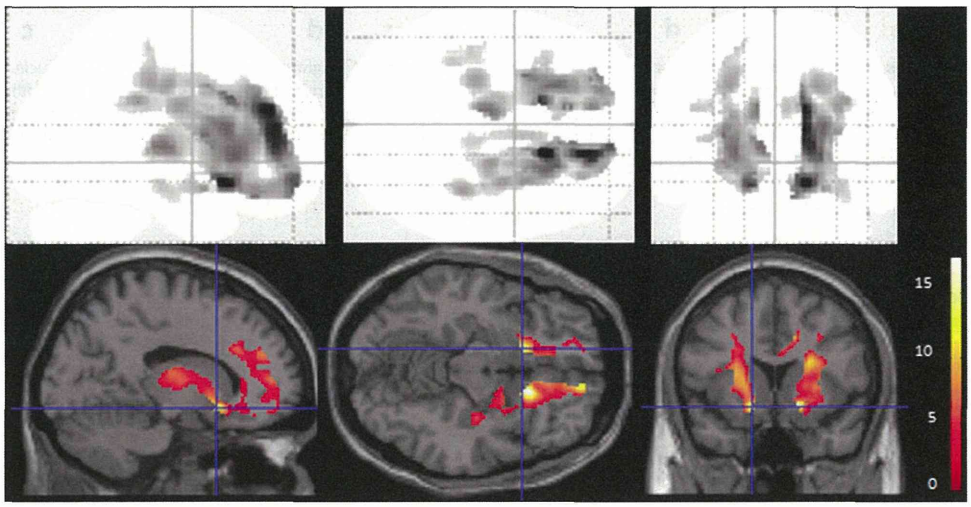

Fig. 1. Regions with significant differences in FA values between controls and the patient at manic state. Sagittal, coronal, and transverse brain views show voxels with significantly higher FA values in the patient at manic state when compared to controls. Detected areas shown in this figure exceed an uncorrected p value of 0.001 with 100 or more contiguous voxels. These statistical parametric mapping projections were then superimposed on representative sagittal ($x=-14$), coronal ($y=-12$), and transverse ($z=14$) magnetic resonance images.

Table 3
Regional change of FA values related to manic state of the patient.

Comparison	Region	MNI coordinates (x, y, z)	Voxels	t-Value	p-Value (FWE-corrected)
Patient < controls	None				
Patient > controls	Right nucleus accumbens	16, 18, −12	1458	17.84	< 0.001
	Left nucleus accumbens	−14, 14, −12	1053	14.25	< 0.001
	Left thalamus	−8, −16, 8	154	10.77	< 0.001

MNI: Montreal Neurological Institute; FWE-corrected: familywise error-corrected.

at euthymic state. The serum progesterone level was lower in the late follicular phase than those in the luteal and early follicular phase, although the difference in levels between the late and early follicular phase was small in the second test.

In the late follicular phase at manic state, one of the two tested estradiol levels was above the reference range and the two tested progesterone levels were below the reference range, although the deviations of the progesterone levels from the reference range were small. In the early follicular and luteal phase at euthymic state, the two tested estradiol and progesterone levels were within the reference range.

3.3. Comparisons of FA values between the patient and controls by voxel-based analysis

In the voxel-based analysis of FA values, the patient at manic state showed significantly higher FA values in the bilateral nucleus accumbens (NAc) and left thalamus. We found no significantly lower FA values in the patient. The areas of the higher FA values, shown in Fig.1, extended from NAc to the bilateral medial frontal region, anterior cingulate, thalamus, and right amygdala and hippocampus. We found no significant differences in FA values between the patient and healthy subjects at euthymic state. There were no significant differences in gray/white matter volumes between healthy subjects and the patient at manic and euthymic states.

3.4. Difference in patient FA values between manic and euthymic states

Spherical volumes of interest (VOIs) were placed on the bilateral NAc and left thalamus where the significant differences between the patient and controls were shown by voxel-based analysis (Table 3). The exact FA values of each area of the patient and controls at manic and euthymic states are shown in Fig. 2 and Table 4. The patient showed significantly increased FA values compared to controls (> mean + 2 S.D. of controls) only at manic state in these VOIs. We found no significant relationship between age and regional FA values in the control group by correlation

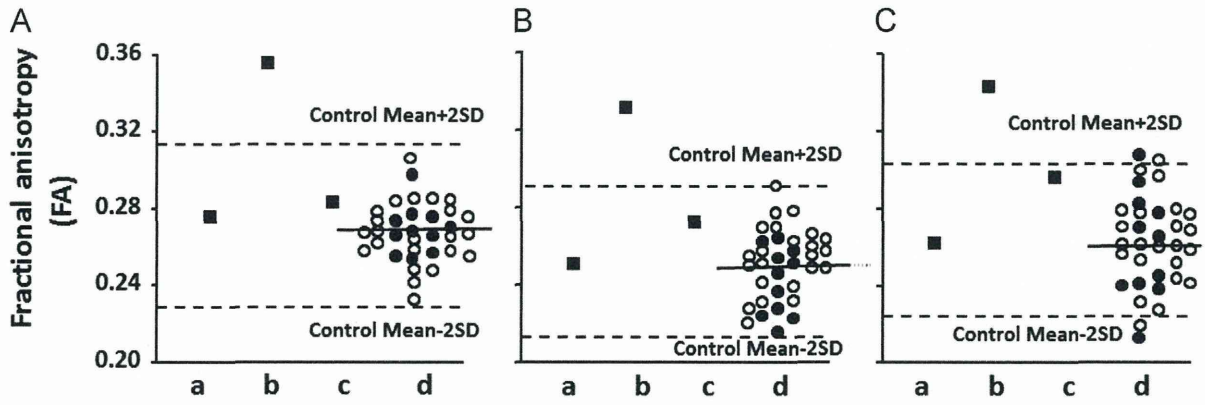

Fig. 2. Scatter plots of the FA values of the controls and patient with euthymic and manic states. (A) Right nucleus accumbens; (B) Left nucleus accumbens; and (C) Left thalamus: (a) Euthymic state (1st test); (b) Manic state (2nd test); (c) Euthymic state (3rd test) and (d) Controls ($n=34$). Filled squares: patient, filled circles: female controls, open circles: male controls. The FA values were derived from spherical VOIs (3-mm radius) placed on the regions with significant increases of FA values by voxel-based analysis (MNI coordinates are shown in Table 3). The exact FA values of each area of the patient and controls are also shown in Table 4. Significantly higher FA values of the patient than those of controls (> mean+2S.D. of controls) are shown only at manic state.

Table 4
FA values of the patient and controls.

	Mental state at MR imaging [menstrual phase (day of cycle)]	Right nucleus accumbens ($x, y, z=16, 18, -12$)	Left nucleus accumbens ($x, y, z=-14, 14, -12$)	Left thalamus ($x, y, z=-8, -16, 8$)
Patient	Euthymic state at 1st imaging [luteal (23)]	0.28	0.25	0.26
	Manic state at 2nd imaging [late follicular (12)]	0.36*	0.33*	0.34*
	Euthymic state at 3rd imaging [early follicular (2)]	0.29	0.27	0.30
Controls	(Mean ± S.D.)	0.27 ± 0.02	0.25 ± 0.02	0.26 ± 0.02

* Significantly different FA values of the patient from those of controls (< mean−2S.D. of controls, or > mean+2S.D. of controls).

Table 5
Values of mean diffusivity (MD), axial diffusivity (AD) and radial diffusivity (RD) ($\times 10^{-4}$) of the patient and controls.

	Mental state at MR imaging [Menstrual phase (day of cycle)]	Right nucleus accumbens ($x, y, z=16, 18, -12$)			Left nucleus accumbens ($x, y, z=-14, 14, -12$)			Left thalamus ($x, y, z=-8, -16, 8$)		
		MD	AD	RD	MD	AD	RD	MD	AD	RD
Patient	Euthymic state at 1st imaging [luteal (23)]	2.19	9.01	5.37	2.28	8.49	6.02	2.36	8.51	6.36
	Manic state at 2nd imaging [late follicular (12)]	2.39	10.5*	5.54	2.39	9.95*	5.80	2.62	10.6*	6.51
	Euthymic state at 3rd imaging [early follicular (2)]	2.37	9.66	5.83	2.31	9.02	5.91	2.27	8.86	5.79
Controls	(Mean ± S.D.)	2.27 ± 0.13	8.90 ± 0.51	5.78 ± 0.41	2.27 ± 0.11	8.78 ± 0.42	5.81 ± 0.37	2.49 ± 0.11	9.37 ± 0.43	6.53 ± 0.37

* Significantly different values of the patient from those of controls (< mean−2S.D. of controls, or > mean+2S.D. of controls).

analysis. Regional FA values in the control group showed no significant difference between males and females (male vs. female, 0.27 ± 0.02 vs. 0.27 ± 0.01 in the right NAc, 0.25 ± 0.02 vs. 0.24 ± 0.02 in the left NAc, 0.26 ± 0.02 vs. 0.26 ± 0.03 in the left thalamus). Table 5 shows the quantification of the differences in the values of MD and radial/axial diffusivity in these regions. The patient showed significantly increased axial diffusivity compared to controls (> mean+2 S.D. of controls) only at manic state.

4. Discussion

DTI results showed that the patient had increased FA values compared to controls during mania in the bilateral NAc and its connected areas. The increased FA level of the patient at manic state was associated with increased axial diffusivity (AD). Diffusion MRI has been used to visualize dynamic tissue changes associated with neuronal activation (Darquié et al., 2001; Le Bihan et al., 2006). The mechanisms underlying water molecule diffusion changes observed by MRI still have not been entirely clarified (Jones et al., 2013), but it has been suggested that such diffusion changes might result from the peculiar properties of water in biological tissues, and from their association with the biophysical events underlying neuronal activity (Le Bihan, 2007). Increased FA values at manic state may reflect the microstructural changes due to the neuronal activation associated with manic episodes as shown in our patient.

Bilateral NAc and its connected areas play an important role in reward-related neural networks mediating motivation and goal-directed behavior (Salamone et al., 2007). Manic state has been characterized as abnormal goal pursuit regulation with elevated levels of achievement motivation and drive (Abler et al., 2008), and our finding of the change in reward-related neural networks could explain the manic symptoms in our patient. NAc is also a major projection field of the mesolimbic dopamine (DA) system (Deutch and Cameron, 1992). It has been hypothesized that abnormalities in dopaminergic neurotransmission may be important to the etiology of BPD (Anand et al., 2011). This hypothesis is supported by pharmacological evidence: psycho-stimulants can

produce symptoms similar to manic state (Gerner et al., 1976; Swann et al., 2004), and commonly used treatments for BPD have actions on post-synaptic dopamine signaling (Berk et al., 2007; Cousins et al., 2009). The hyper-activation of the mesolimbic DA system may have contributed to the abnormality of the reward-related neural networks in our patient.

The patient demonstrated manic episodes regularly at around day 7 of her MC, and they continued for about 2 weeks, with euthymic state intervals. Estradiol levels increased in the late follicular phase at manic state when compared to those in the luteal or early follicular phase at euthymic state. The serum progesterone level was lower in the late follicular phase than those in the luteal and early follicular phase, but the difference in levels was small, almost negligible, in the second test. On the other hand, in the late follicular phase at manic state, one of the two tested estradiol levels was within normal range. The two tested progesterone levels were below the reference range, but their deviations from the normal range were extremely small. According to these results, it is reasonable to assume that the elevation in the estradiol level due to the normal menstrual cycle has a consistent role in triggering the onset of manic symptoms, but abnormal levels of estrogen/progesterone were not necessary for the onset of manic symptoms.

In healthy females, functional MRI studies showed greater reactivity to reward during the follicular phase compared to the luteal phase of the menstrual cycle. A positive correlation between estradiol and reward-related activation of the bilateral amygdalo-hippocampal complex was also reported (Dreher et al., 2007). The result of our study was thought to be at least partly in agreement with their report.

A previous study has shown that the basal concentration of dopamine in rats is dependent on the circulating levels of estrogen (Becker, 1999). Czoty et al. examined the menstrual cycle-related changes of DA D2 receptor in female cynomolgus monkeys by using micro-PET with [^{18}F] fluoroclebopride (Czoty et al., 2009), and they found lower D2 distribution volume ratios in the follicular phase, which may be related to increases in DA release produced by high levels of estrogen (Becker et al., 2001; Dazzi et al., 2007). Our finding of a rise in the serum estradiol level due to a normal menstrual cycle and assumed hyper-activity of the mesolimbic DA system in the late follicular phase at manic state is in line with these previous studies. When considering that healthy women do not show manic symptoms due to a normal menstrual cycle, it is reasonable to suppose that the mesolimbic DA system of this patient specifically has abnormal hypersensitivity to the increase in the estradiol level from a normal menstrual cycle.

There are several points in this study that should be taken into consideration. First, our study is a single-case study and the results cannot be interpreted generally. Future larger-group designs are needed. Second, age should be regarded as the cause of heterogeneity of controls (Lebel et al., 2008; Kumar et al., 2013). However, we regarded their effect on our DTI results as relatively little, as we found no significant relationship between age and regional FA values in the control group. Third, our control group included twice as many males as females. The gender-related changes of white matter integrity were shown in healthy subjects in a recent study (Kumar et al., 2013). Although we confirmed that there was no statistical difference in FA in the VOIs between males and females of our controls, the large proportion of males in a relatively small sample was still insufficient to definitively rule out any difference. Further, the women in the sample were not assessed with regard to the stage of their menstrual cycle at the time of scanning. With a hypothesis based upon differences in brain structure due to the menstrual cycle, such factors could significantly affect the interpretation of the results. Future analysis with a restricted female control group assessed in regard to the menstrual cycle will be necessary for forming definitive conclusions. Fourth, it was reported that treated women with BPD do not seem to have menstruation-related mood symptoms (Payne et al., 2007; Shivakumar et al., 2008; Sit et al., 2011). During our analysis, the patient remained free of mood stabilizers / antipsychotic drugs, so we cannot directly compare our results with the previous reports of treated women with BPD. Finally, we did not measure the DA level. To the best of our knowledge, there has been no report about variations of the DA level in different mood states of BPD. However, the results of the present study tend to encourage us toward further investigations concerning the relation between the DA level and manic episodes in the human menstrual cycle.

In conclusion, we speculate that the mesolimbic DA system of this patient specifically has abnormal hypersensitivity to the increase in the estradiol level. According to our results, it is reasonable to suppose that the rise of the serum estradiol level in accord with the normal menstrual cycle increased the activity of the dopaminergic system and abnormally activated the reward-related neural networks, which may have contributed to the recurrent manic episodes in the patient. We may have unearthed a clue toward understanding how fluctuations in gonadal hormone production may amplify or ameliorate the symptomatology of psychiatric disorders characterized by altered mood and emotional states related to the menstrual cycle.

Financial support

This research was supported by the Japan Society for the Promotion of Science, Grant-in-Aid for Scientific Research (C), 24591740.

Acknowledgment

We would like to thank members of the MRI facility staff of the Department of Investigative Radiology, National Cerebral and Cardiovascular Center in Japan, for carrying out the acquisition of MRI data and taking care of all subjects during the MRI procedures.

References

Abler, B., Greenhouse, I., Ongur, D., Walter, H., Heckers, S., 2008. Abnormal reward system activation in mania. Neuropsychopharmacology 33, 2217–2227.
Akdeniz, F., Karadağ, F., 2006. Does menstrual cycle affect mood disorders? Turkish Journal of Psychiatry 17, 296–304.
Alexander, A.L., Lee, J.E., Lazar, M., Field, A.S., 2007. Diffusion tensor imaging of the brain. Neurotherapeutics 4, 316–329.
Anand, A., Barkay, G., Dzemidzic, M., Albrecht, D., Karne, H., Zheng, Q.H., Hutchins, G.D., Normandin, M.D., Yoder, K.K., 2011. Striatal dopamine transporter availability in unmedicated bipolar disorder. Bipolar Disorders 13, 406–413.
Ashburner, J., Friston, K.J., 2000. Voxel-based morphometry-the methods. NeuroImage 11, 805–821.
Becker, J.B., 1999. Gender differences in dopaminergic function in striatum and nucleus accumbens. Pharmacology Biochemistry and Behavior 64, 803–812.
Becker, J.B., Molenda, H., Hummer, D.L., 2001. Gender differences in the behavioral responses to cocaine and amphetamine. Implications for mechanisms mediating gender differences in drug abuse. Annals of the New York Academy of Sciences 937, 172–187.
Becker, O.V., Rasgon, N.L., Marsh, W.K., Glenn, T., Ketter, T.A., 2004. Lamotrigine therapy in treatment-resistant menstrually-related rapid cycling bipolar disorder: a case report. Bipolar Disorders 6, 435–439.
Berk, M., Conus, P., Lucas, N., Hallam, K., Malhi, G.S., Dodd, S., Yatham, L.N., Yung, A., McGorry, P., 2007. Setting the stage: from prodrome to treatment resistance in bipolar disorder. Bipolar Disorders 9, 671–678.
Cousins, D.A., Butts, K., Young, A.H., 2009. The role of dopamine in bipolar disorder. Bipolar Disorders 11, 787–806.
Czoty, P.W., Riddick, N.V., Gage, H.D., Sandridge, M., Nader, S.H., Garg, S., Bounds, M., Garg, P.K., Nader, M.A., 2009. Effect of menstrual cycle phase on dopamine D2 receptor availability in female cynomolgus monkeys. Neuropsychopharmacology 34, 548–554.
D'Mello, D.A., Pinheiro, A.L., Lalinec-Michaud, M., 1993. Premenstrual mania: two case reports. Journal of Nervous and Mental Disease 181, 330–331.

Darquié, A., Poline, J.B., Poupon, C., Saint-Jalmes, H., Le Bihan, D., 2001. Transient decrease in water diffusion observed in human occipital cortex during visual stimulation. Proceedings of the National Academy of Sciences of the United States of America 98, 9391–9395.

Dazzi, L., Seu, E., Cherchi, G., Barbieri, P.P., Matzeu, A., Biggio, G., 2007. Estrous cycle-dependent changes in basal and ethanol-induced activity of cortical dopaminergic neurons in the rat. Neuropsychopharmacology 32, 892–901.

Deutch, A.Y., Cameron, D.S., 1992. Pharmacological characterization of dopamine systems in the nucleus accumbens core and shell. Neuroscience 46, 49–56.

Dreher, J.C., Schmidt, P.J., Kohn, P., Furman, D., Rubinow, D., Berman, K.F., 2007. Menstrual cycle phase modulates reward-related neural function in women. Proceedings of the National Academy of Sciences of the United States of America 104, 2462–2470.

First, M.B., Spitzer, R.L., Gibbon, M., Williams, J.B.W., 1997. Structured Clinical Interview for DSM-IV (SCID-I)—Research Version. Biometrics Research, New York.

Gerner, R.H., Post, R.M., Bunney, W.E.J., 1976. A dopaminergic mechanism in mania. American Journal of Psychiatry 133, 1177–1180.

Hamilton, M., 1960. A rating scale for depression. Journal of Neurology, Neurosurgery & Psychiatry 23, 56–62.

Jones, D.K., Knösche, T.R., Turner, R., 2013. White matter integrity, fiber count, and other fallacies: the do's and don'ts of diffusion MRI. Neuroimage 73, 239–254.

Kukopulos, A., Minnai, G., Muller-Oerlinghausen, B., 1985. The influence of mania and depression on the pharmacokinetics of lithium. Journal of Affective Disorders 8, 159–166.

Kumar, R., Chavez, A.S., Macey, P.M., Woo, M.A., Harper, R., 2013. Brain axial and radial diffusivity changes with age and gender in healthy adults. Brain Research 1512, 22–36.

Le Bihan, D., 2007. The 'wet mind': water and functional neuroimaging. Physics in Medicine and Biology 52, R57–R90.

Le Bihan, D., Urayama, S., Aso, T., Hanakawa, T., Fukuyama, H., 2006Direct and fast detection of neuronal activation in the human brain with diffusion MRIProceedings of the National Academy of Sciences of the United States of America1038263-8268.

Lebel, C., Walker, L., Leemans, A., Phillips, L., Beaulieu, C., 2008. Microstructural maturation of the human brain from childhood to adulthood. NeuroImage 40, 1044–1055.

Montgomery, S.A., Asberg, M., 1979. A new depression scale designed to be sensitive to change. British Journal of Psychiatry 134, 382–389.

Payne, J.L., Roy, P.S., Murphy-Eberenz, K., Weismann, M.M., Swartz, K.L., McInnis, M. G., Nwulia, E., Mondimore, F.M., MacKinnon, D.F., Miller, E.B., Nurnberger, J.I., Levinson, D.F., DePaulo, J.R.J., Potash, J.B., 2007. Reproductive cycle-associated mood symptoms in women with major depression and bipolar disorder. Journal of Affective Disorders 99, 221–229.

Salamone, J.D., Correa, M., Farrar, A., Mingote, S.M., 2007. Effort-related functions of nucleus accumbens dopamine and associated forebrain circuits. Psychopharmacology (Berlin) 191, 461–482.

Shivakumar, G., Bernstein, I.H., Suppes, T.S.F.B.N., Keck, P.E., McElroy, S.L., Altshuler, L.L., Frye, M.A., Nolen, W.A., Kupka, R.W., Grunze, H., Leverich, G.S., Mintz, J., Post, R.M., 2008. Are bipolar mood symptoms affected by the phase of the menstrual cycle? Journal of Women's Health (Larchmt) 17, 473–478.

Sit, D., Seltman, H., Wisner, K.L., 2011. Menstrual effects on mood symptoms in treated women with bipolar disorder. Bipolar Disorders 13, 310–317.

Swann, A.C., Dougherty, D.M., Pazzaglia, P.J., Pham, M., Moeller, F.G., 2004. Impulsivity: a link between bipolar disorder and substance abuse. Bipolar Disorders 6, 204–212.

Young, R.C., Biggs, J.T., Ziegler, V.E., Meyer, D.A., 1978. A rating scale for mania: reliability, validity and sensitivity. British Journal of Psychiatry 133, 429–435.

Chapter 4

EFFECT OF PLASMA LIPIDS AND APOE GENOTYPE ON COGNITIVE DECLINE

Fumihiko Yasuno[1,2,*] and Takashi Asada[1]

[1]Department of Neuropsychiatry, Institute of Clinical Medicine,
University of Tsukuba, Tsukuba, Japan
[2]Department of Neuropsychiatry, NMU Psychiatric Institute,
Nara Medical University, Nara, Japan

ABSTRACT

Although the presence of an APOE4 allele increases the risk of Alzheimer's disease (AD), there is no current evidence that the cognitive decline among those with an APOE4 allele can be prevented. Detrimental effects of APOE4 may be exacerbated by synergistic preventable risk factors, such as plasma lipids. We therefore examined the combined effect of plasma lipids and APOE genotype on cognitive function in elderly individuals. Plasma concentrations of high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglyceride (TG), total cholesterol (TC), apolipoprotein E (apoE) were evaluated in 622 community-dwelling individuals aged 65 years and older. We investigated the associations between plasma lipids and cognitive function in APOE4 carrier (E4+) and APOE4 non-carrier (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-. Our findings suggest that an interaction between apoE and HDL is affected by APOE4, and is possibly linked with a protective effect on cognitive decline in later life. Preservation of higher plasma apoE and HDL from early life is proposed as a possible strategy for maintaining cognitive function in later life, especially for APOE4-positive individuals.

* Fumihiko Yasuno, M.D., Ph.D., Department of Neuropsychiatry, NMU Psychiatry Institute, Nara Medical University, 840 Shijocho, Kashihara, Nara, 634-8522, Japan. Tel.: +81-744-22-3051; fax: +81-744-22-3854. E-mail address: yasunof@naramed-u.ac.jp

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

INTRODUCTION

The presence of an apolipoprotein E4 allele (APOE4) increases the risk and reduces the age at onset of Alzheimer 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 non-carriers 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, in press a,b). 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, in press, a, b).

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

METHODS

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 , in press, a, b). 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 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 we used the results of those subjects tested twice. This study uses 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.

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 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), 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.

Definition of Hypertension

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 anti-hypertensive therapies. Baseline blood pressure was measured. Using these data, a history of hypertension requiring medication (hereafter termed HT) was defined if any one of the following conditions was present: the history of hypertension with current use of anti-hypertensive medication, or SBP/DBP $\geqq$ 160/100 mmHg at baseline.

Cognitive Assessment

All participants underwent the same cognitive assessment at the baseline and three-year examinations using a set of four 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, in press a, b). A composite cognitive score was computed from the four scores using the first component of the scores of principal component analysis (Composite cognitive score=0.853*attention score + 0.809*memory score + 0.856*language score + 0.859*reasoning score).

Statistical Analysis of the Effect of Lipids/apoE

Subjects were divided into two APOE groups by E4 status with E4-(n=509) [genotypesε2/ε3 (n=52), ε3/ε3 (n=457)] and E4+ (n=113) [genotypes ε2/ε4 (n=6), ε3/ε4 (n=99) and ε4/ε4 (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 $\chi 2$ 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 three strata according to the plasma concentrations of lipids. To examine the influence of plasma lipids on cognitive function, composite cognitive scores of the three 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 three strata according to the levels of lipids.

To examine the influence of plasma lipids on change in cognitive function during the three-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.

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 eta-squared (η^2) to estimate and compare the effect of the level of lipids on cognitive score between groups of different sample size. $\eta^2<0.01$ was regarded as no substantial effect. Statistical analysis of the data was performed using SPSS for Windows 19.0 (IBM Japan Inc., Tokyo, Japan).

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 2-3 show the median plasma concentrations of lipids for the three strata according to the tertiles of plasma levels of lipids/apoE, and the mean cognitive scores of the E4- and

E4+ groups at 2002 and 2005 according to the three 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).

Table 1. Demographic characteristics in the analysis of the effect oflipids/lipoproteins

Characteristic	ApoE4- (n =509)	ApoE4+ (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, No (%) [b]	226 (44%)	43 (38%)	χ^2_1=1.5	0.2
Years of education, y [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, No (%) [b]	188 (37%)	37 (33%)	χ^2_1=0.7	0.4
History of disease, No (%)				
Cardiovascular disease [b]	15 (2.9%)	2 (1.7%)	χ^2_1=0.5	0.5
Diabetes mellitus [b]	20 (3.9%)	7 (6.1%)	χ^2_1=1.1	0.3
Hyperlipidemia [b]	19 (3.7%)	7 (6.1%)	χ^2_1=1.4	0.2
Hypertension [b]	195 (38.3%)	44 (38.9%)	χ^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

[a] P value was calculated by unpaired two-tailed t test.

[b] P value was calculated by Pearson χ^2 two-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.

Data are mean±sd after adjustment for covariates.

Subjects with higher HDL concentrations had higher cognitive scores. The effect size of the influence of the plasma HDL level on cognitive 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 of the E4- and E4+ group (Tables 2-3). 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 three-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 2-3).

Table 2. Mean cognitive test score of each tertile groups of lipid levels in ApoE4- group [a]

	Plasma lipid/apoE level, tertiles median (min-max)			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	

[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 $p < .05$ 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, 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).

Table 3. Mean cognitive test score of each tertile groups of lipid levels in ApoE4+ group [a]

	Plasma lipid/apoE level, tertiles median (min-max)			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	

[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 $p < .05$ 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).