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Independent and Interactive Influences of the *APOE* Genotype and Beta-Amyloid Burden on Cognitive Function in Mild Cognitive Impairment

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This study aimed to investigate the independent and interactive influences of
apolipoprotein E (*APOE*) *ε4* and beta-amyloid (Aβ) on multiple cognitive domains in a large
group of cognitively normal (CN) individuals and patients with mild cognitive impairment
(MCI) and Alzheimer's disease (AD). Participants were included if clinical and cognitive
assessments, amyloid imaging, and *APOE* genotype were all available from the Alzheimer's
Disease Neuroimaging Initiative database (CN = 324, MCI = 502, AD = 182). Individuals
with one or two copies of *ε4* were designated as *APOE ε4* carriers (*ε4*+); individuals with no
ε4 were designated as *APOE ε4* non-carriers (*ε4*–). Based on mean florbetapir standard
uptake value ratios, participants were classified as Aβ burden-positive (Aβ+) or Aβ burden-
negative (Aβ–). In MCI, *APOE ε4* effects were predominantly observed on frontal executive
function, with *ε4*+ participants exhibiting poorer performances; Aβ positivity had no
influence on this effect. Aβ effects were observed on global cognition, memory, and
visuospatial ability, with Aβ+ participants exhibiting poorer performances. Measures of
frontal executive function were not influenced by Aβ. Interactive effects of *APOE ε4*+ and
Aβ were observed on global cognition and verbal recognition memory. Aβ, not *APOE ε4*+,
influenced clinical severity and functional status. The influences of *APOE ε4*+ and Aβ on
cognitive function were minimal in CN and AD. In conclusion, we provide further evidence
of both independent and interactive influences of *APOE ε4*+ and Aβ on cognitive function
in MCI, with *APOE ε4*+ and Aβ showing dissociable effects on executive and non-executive
functions, respectively.

Keywords: Alzheimer Disease; Mild Cognitive Impairment; *APOE ε4*+; Beta-amyloid
Burden; Neuropsychology

INTRODUCTION

Alzheimer's disease (AD) is characterized by progressive cognitive decline. Mild cognitive impairment (MCI) is known as an intermediate stage between healthy aging and clinical dementia. In particular, amnestic MCI (aMCI) is considered a prodromal stage of AD dementia (1-3); however, the characteristics of aMCI are both pathologically and clinically heterogeneous. Approximately 40% of individuals with MCI show very low levels of cerebral beta-amyloid (Aβ) deposition that are not sufficient to represent a prodromal stage of AD (4,5). There are various clinical phenotypes of MCI, and cognitive deficits can occur in single or multiple domains (2).

The apolipoprotein E (*APOE*) *ε4* allele is a major genetic risk factor for the development of late-onset AD dementia (6). Much

evidence supports the association between *APOE ε4* and cognitive decline in non-demented individuals (7,8). Nevertheless, there is disagreement regarding the specific cognitive domain affected by *APOE ε4* status. *APOE ε4* carriers show impairments compared to non-carriers in various cognitive domains, including episodic memory (8-10), executive function (7,11), language, and spatial ability (12).

Senile plaques containing Aβ are a hallmark of AD pathology. No associations between Aβ burden and cognitive function have been found in individuals with AD dementia (13,14). Some studies report a significant detrimental effect of Aβ on memory function in non-demented individuals (14,15), while others find no association (16,17). Most studies that assess non-memory cognitive domains report non-significant effects of Aβ (16), although some studies demonstrate a significant effect (15,17).

APOE ε4 is involved in Aβ binding and clearance during AD pathogenesis (18); therefore, an inextricable link between *APOE ε4* and Aβ burden likely plays a role in the process of cognitive decline in AD patients. Contradictions in previous studies mentioned above might be partially explained by a failure to consider both *APOE ε4* status and Aβ burden. Recent investigations of both factors report interactions in cross-sectional studies (15), suggesting that *APOE ε4* status modulates the effects of Aβ on cognition. However, it is also possible that both biomarkers independently influence cognitive function. *APOE ε4* is associated with a decline in executive function in subjects between the ages of 35 and 44 years who are unlikely to have significant Aβ burdens (7). Other studies have observed independent effects of the two factors (19,20). Understanding the influences of Aβ burden and *APOE ε4* on cognitive function, particularly during the MCI stage, could support early detection and intervention in AD dementia. However, it remains unclear whether these factors influence cognitive function independently or interactively and which cognitive domains are affected.

The majority of previous studies have evaluated *APOE ε4*- or Aβ-associated cognitive characteristics using brief cognitive measures. Some studies have used only the global cognitive or episodic memory tests (11,14) or the single executive function test (7). Executive function test was not included in some studies (19). More comprehensive neuropsychological measures that assess multiple cognitive domains should be utilized in order to clarify the specific cognitive domains affected by *APOE ε4* status and Aβ burden. Furthermore, although cognitive function consequently influences clinical severity and everyday function, the effects of *APOE ε4* status or Aβ burden on clinical severity or functional status are rarely examined.

We aimed to investigate the independent and interactive influences of *APOE ε4* status and Aβ burden on multiple cognitive domains in a large group of individuals with MCI and AD, as well as cognitively normal (CN) participants. We also examined the influences of these factors on clinical severity and functional status.

MATERIALS AND METHODS

Participants

Data were collected from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). For a detailed explanation and up-to-date information on ADNI, please see http://www.adni-info.org. We included participants from all phases of ADNI only if [18F] florbetapir positron emission tomography (PET) had been conducted within 3 months of clinical and cognitive assessment visits and *APOE* genotype was available. Initially, 1,030 subjects were selected. Subjects of the *APOE 2/4* genotype (n = 22) were excluded due to the unclear effects of these alleles. The final analysis included 324 CN el-

derly participants, 502 individuals with MCI, and 182 with AD dementia who had undergone clinical evaluations and florbetapir PET scans between April 2010 and December 2013 (Table 1). Detailed eligibility criteria for the diagnostic groups are described elsewhere (21). Briefly, CN subjects had a Clinical Dementia Rating (CDR) of 0 and Mini-Mental State Examination (MMSE) scores between 24 and 30, were non-depressed and non-demented, and had not been diagnosed with MCI. Subjects with MCI had a CDR of 0.5 and MMSE scores between 24 and 30, complained of objective memory loss but showed no impairment in other cognitive domains, demonstrated preserved activities of daily living, and were non-demented. AD dementia subjects had a CDR of 0.5 or 1.0 and MMSE scores between 20

Table 1. Demographic and clinical characteristics by patient groups

Characteristics	Patient groups		
	CN (n = 324)	MCI (n = 502)	AD (n = 182)
Age (SD), yr	74.6 (6.5)	72.5 (7.8)†	75.0 (7.8)*
Education (SD), yr	16.5 (2.6)	16.1 (2.7)	15.9 (2.7)
Female, n (%)	173 (53.4)	219 (43.6)	75 (41.2)
APOE ε4 carriers, n (%)	85 (26.2)	228 (45.4)	120 (65.9)
Positive Aβ status, n (%)	101 (31.2)	269 (53.6)	153 (84.1)
CDR-SOB	0.06 (0.24)	1.48 (0.62)†	4.95 (2.23)*,†
FAQ	0.35 (1.29)	2.68 (3.75)†	14.05 (7.04)*,†
Global cognition			
MMSE	28.99 (1.28)	28.05 (1.73)†	22.49 (3.20)*,†
ADAS-cog11	5.73 (3.09)	9.15 (4.39)†	21.12 (8.23)*,†
ADAS-cog13	9.06 (4.63)	14.75 (6.70)†	31.59 (9.72)*,†
MoCA	25.58 (2.53)	23.41 (3.15)†	17.01 (4.63)*,†
Memory			
ADNI_Mem	0.92 (0.53)	0.36 (0.55)†	-0.65 (0.54)*,†
RAVLT_imm	45.85 (10.43)	36.94 (11.04)†	22.13 (7.41)*,†
RAVLT_delayed	7.62 (4.03)	4.73 (4.08)†	0.71 (1.70)*,†
RAVLT_recog	12.73 (2.65)	11.35 (3.13)†	6.64 (3.96)*,†
LM_imm	14.64 (1.9)	9.78 (3.55)†	4.19 (2.78)*,†
LM_delayed	13.73 (3.33)	7.37 (3.42)†	1.59 (2.16)*,†
Frontal executive function			
ADNI_EF	0.81 (0.74)	0.34 (0.79)†	-0.77 (0.83)*,†
TMT A	33.77 (12.72)	39.00 (17.06)†	61.98 (35.80)*,†
TMT B	81.18 (38.46)	107.55 (60.27)†	189.38 (86.31)*,†
Animal fluency	20.98 (5.43)	17.92 (5.06)†	12.00 (5.01)*,†
BNT	28.04 (2.38)	26.40 (3.49)†	22.04 (6.25)*,†
Visuospatial ability			
Clock drawing	4.70 (0.54)	4.47 (0.81)†	3.38 (1.44)*,†
Clock copying	4.87 (0.35)	4.73 (0.59)†	4.34 (1.02)*,†

Data are presented as mean (standard deviation) or number (percentage). *Significant compared to MCI (*P* < 0.05); †Significant compared to CN (*P* < 0.05). CN, cognitively normal; MCI, mild cognitive impairment; AD, Alzheimer's disease; *APOE*, apolipoprotein E; Aβ, average florbetapir mean standard uptake value ratio of frontal, anterior cingulate, and parietal cortices and precuneus relative to the cerebellum; CDR-SOB, sum of boxes of the Clinical Dementia Rating scale; FAQ, Functional Assessment Questionnaire; MMSE, Mini-Mental State Examination; ADAS-cog11, Alzheimer's Disease Assessment Scale-cognitive subscale, consisting of 11 items; ADAS-cog13, Alzheimer's Disease Assessment Scale-cognitive subscale, consisting of 13 items; MoCA, Montreal Cognitive Assessment; ADNI_Mem, Alzheimer's Disease Neuroimaging Initiative composite score for memory; RAVLT_imm, Rey Auditory Verbal Learning test, immediate recall score; RAVLT_delayed, RAVLT, delayed recall score; RAVLT_recog, RAVLT, recognition score; LM_imm, Logical Memory test, immediate recall score; LM_delayed, LM, delayed recall score; ADNI_EF, Alzheimer's Disease Neuroimaging Initiative composite score for executive functioning; TMT, Trail Making Test; BNT, Boston Naming Test.

and 26 and met the National Institute of Neurological and Communicative Diseases and Stroke/Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria for probable AD (22). Participants with any significant neurological disease other than suspected incipient AD, such as Parkinson's disease, multi-infarct dementia, Huntington's disease, normal pressure hydrocephalus, brain tumor, progressive supranuclear palsy, seizure disorders, subdural hematoma, multiple sclerosis, or a history of significant head trauma, were excluded. In addition, participants with MRI evidence of brain infection, infarction or other focal lesions, multiple lacunes, or lacunes in a critical memory structure were also excluded.

Cognitive, clinical, and functional measures

We selected cognitive testing data from ADNI participants. Four tests were selected to evaluate global cognition, including the MMSE; the Alzheimer's Disease Assessment Scale-cognitive subscale, consisting of 11 (ADAS-cog11) and 13 items (ADAS-cog13); and the Montreal Cognitive Assessment (MoCA) (23). We included each of these global cognitive measures in the analysis because each measure has specific characteristics and clinical usefulness. For example, a delayed recall task and number cancellation item were added to the ADAS-cog13. The MoCA was designed for MCI screening. These two measures have additional executive function and attention components compared to the MMSE and ADAS-cog11. Six measures were selected to assess memory, including the Rey Auditory Verbal Learning Test (RAVLT) trials 1-5 total recall as immediate recall (RAVLT_imm), 30-minute delayed recall (RAVLT_delayed), and yes-no recognition (RAVLT_recog); Logical Memory immediate recall (LM_imm) and 30-minute delayed recall (LM_delayed) from the Wechsler Memory Scale-Revised; and the ADNI composite scores for memory (ADNI_Mem) (24). Four measures were selected to assess frontal executive function, including the Trail Making Test (TMT) parts A and B, Animal fluency, and ADNI composite scores for executive functioning (ADNI_EF) (25). The Boston Naming Test (BNT) was included as a measure of language. Clock drawing and copying tests were included to assess visuospatial ability.

We selected the CDR Sum of Boxes (CDR-SOB) score as a clinical measure. This scale is a useful tool for staging clinical severity. It evaluates six domains of cognitive and daily functioning, with possible scores ranging from 0 to 18. We included the Functional Assessment Questionnaire (FAQ) to assess everyday functioning. This tool assesses instrumental activities of daily living, with scores ranging from 0 to 30, and is useful for distinguishing MCI from very mild AD as well as for monitoring functional changes (26).

APOE genotyping

APOE genotyping was performed at the time of participant en-

rollment in the ADNI study. *APOE* genotypes were determined using standard polymerase chain reaction methods, which have been described previously (27). Individuals with one or two copies of *allele 4* were designated as *APOE ε4* carriers (*ε4*+); individuals with no allele 4 were designated as *APOE ε4* non-carriers (*ε4*–).

Florbetapir PET

We obtained the mean florbetapir standard uptake value ratio (SUVR) for each participant from the ADNI database. A detailed description of florbetapir PET acquisition and processing can be found on the ADNI website (http://adni.loni.usc.edu/wp-content/uploads/2010/05/ADNI2_PET_Tech_Manual_14201.pdf) or as previously published (14). Briefly, the subject's first florbetapir image was coregistered to their MR image and segmented into cortical regions (frontal, anterior/posterior cingulate, lateral parietal, and lateral temporal) using FreeSurfer (version 4.5.0). The mean florbetapir uptake from these gray matter regions was then extracted and normalized to uptake in the whole cerebellum. Participants were classified as Aβ burden-positive (Aβ+) or Aβ burden-negative (Aβ–) according to the SUVR cutoff of 1.11 for amyloid positivity (14).

Statistical analysis

Demographic and clinical data were compared between study groups using one-way analysis of variance (ANOVA) and χ^2 tests for continuous and categorical variables, respectively. Scores on neuropsychological, clinical, and functional measures were compared between groups using analysis of covariance (ANCOVA). To determine the main effects and interactive effects of *APOE ε4* and Aβ burden on these scores, a series of 2 × 2 ANCOVAs was performed. We corrected *P* values for multiple comparisons using false discovery rate (FDR) correction. Post hoc pairwise comparisons were performed using a general linear model. The effects of age, gender, and education were adjusted for all ANCOVAs and pairwise comparisons. Cohen's *d* was used to calculate the effect size between *ε4*+ and *ε4*– and between Aβ+ and Aβ– participants for each cognitive score. Statistical analyses were performed using SPSS (version 21.0) for Windows.

Ethics statement

Study procedures were approved by the institutional review boards of 55 research centers in the United States and Canada participating in ADNI. Written informed consent to share data for scientific research purposes was obtained from each participant.

RESULTS

Participant characteristics

The demographic and clinical characteristics of 1,008 subjects

are presented in Table 1. Participants with MCI were, on average, younger than CN and AD dementia participants, while participants with AD dementia had received fewer years of education than those in the CN and MCI groups ($P < 0.001$). The CN group included more women than the other two study groups (χ^2 [2, n = 1,008] = 9.90, $P = 0.067$). The frequencies of *APOE* ε4+ and Aβ+ statuses increased with increasing diagnostic severity (χ^2 [2, n = 1,008] = 77.43, $P < 0.001$ for *APOE* ε4; χ^2 [2, n = 1,008] = 131.76, $P < 0.001$ for Aβ). As expected, subsequent comparisons of cognitive test scores, clinical severity, and functional status revealed significant differences among groups after controlling for demographic variables. Post hoc pairwise comparisons showed multiple significant differences between groups (Table 1).

Effects of *APOE* ε4 status and Aβ positivity on cognitive function

There were no significant main effects or interactive effects of *APOE* ε4 status and Aβ positivity on any neuropsychological scores in the CN group, with the exception of LM_imm scores. The significant main effect of *APOE* ε4 status on LM_imm test scores ($F_{[1, 317]}$ = 6.84, FDR-corrected $P < 0.001$) indicated poorer performances by ε4+ compared to ε4– participants.

There were significant main effects of *APOE* ε4 status on scores on all measures of frontal executive function in the MCI group (Table 2). ADNI_EF scores were lower in ε4+ compared to ε4– participants. Significant main effects of *APOE* ε4 status were also observed on ADNI_Mem, LM_delayed, ADAS-cog13, and MoCA scores. In contrast, no significant effects of *APOE* ε4 status were found in the visuospatial and language domains. The magnitudes of the differences between ε4+ and ε4– participants, averaged for Aβ positivity, indicated that ε4+ participants showed poorer performances compared to ε4– participants on all measures of frontal executive function and on several measures of global cognitive and memory (Fig. 1). There were significant main effects of Aβ positivity on all tests of global cognition, memory, and visuospatial ability. Conversely, none of the measures of frontal executive function or language showed Aβ-related effects. The magnitude of the differences between Aβ+ and Aβ– participants, averaged for *APOE* ε4 status, indicated that Aβ+ participants showed poorer performances on global cognition, memory, and visuospatial tests compared to Aβ– participants (Fig. 1). Furthermore, pairwise comparisons between four subgroups (ε4–Aβ–, ε4–Aβ+, ε4+Aβ–, and ε4+Aβ+) of representative scores on the ADNI_EF and ADNI_Mem tests, which measure frontal executive function and memory, respectively, showed different patterns; *APOE* ε4 status and Aβ positivity predominantly affected scores on the ADNI_EF and ADNI_Mem tests, respectively (Fig. 2).

There were significant interactive effects of *APOE* ε4 status and Aβ positivity on ADAS-cog13 and RAVLT_recog scores in

Table 2. Effects of *APOE* ε4 and Aβ on neuropsychological performance and clinical characteristics in participants with MCI

Variables	Main effect		*APOE* ε4 × Aβ interaction
	APOE ε4	Aβ	
Global cognition			
MMSE	2.875	14.825*	1.246
ADAS-cog11	3.646	23.695*	3.424
ADAS-cog13	6.305*	31.948*	5.637*
MoCA	5.021*	5.424*	0.154
Memory			
ADNI_Mem	5.425*	26.353*	3.070
RAVLT_imm	2.981	19.013*	1.762
RAVLT_delayed	4.884	16.187*	2.430
RAVLT_recog	2.794	12.787*	9.112*
LM_imm	2.349	22.082*	1.211
LM_delayed	6.131*	24.250*	2.454
Executive/psychomotor speed			
ADNI_EF	12.437*	2.331	0.253
TMT A	5.118*	1.660	0.232
TMT B	7.039*	1.485	0.175
Animal fluency	10.165*	0.483	0.001
Language			
BNT	1.240	3.402	0.232
Visuospatial ability			
Clock Drawing	0.037	5.985*	1.155
Clock Copying	0.739	4.305	0.010
Clinical data			
CDR-SOB	0.146	9.560*	4.509
FAQ	0.228	10.458*	2.952

Data are presented as *F* values. *False discovery rate (FDR)-corrected $P < 0.05$, using 2 × 2 analyses of covariance (ANCOVA) with age, gender, and education as covariates. MCI, mild cognitive impairment; *APOE*, apolipoprotein E; Aβ, beta-amyloid; MMSE, Mini-Mental State Examination; ADAS-cog11, Alzheimer's Disease Assessment Scale-cognitive subscale, consisting of 11 items; ADAS-cog13, Alzheimer's Disease Assessment Scale-cognitive subscale, consisting of 13 items; MOCA, Montreal Cognitive Assessment; ADNI_Mem, composite score for memory using Alzheimer's Disease Neuroimaging Initiative; RAVLT_imm, Rey Auditory Verbal Learning Test, immediate recall score; RAVLT_delayed, RAVLT, delayed recall score; RAVLT_recog, RAVLT, recognition score; LM_imm, Logical Memory, immediate recall score; LM_delayed, LM, delayed recall score; ADNI_EF, Alzheimer's Disease Neuroimaging Initiative composite score for executive functioning; TMT, Trail Making Test; BNT, Boston Naming Test; CDR-SOB, sum of boxes of the Clinical Dementia Rating scale; FAQ, Functional Assessment Questionnaire.

the MCI group. For both measures, ε4-related poor performance was found only among Aβ+ participants (Fig. 3A and B, upper row). To elucidate the interaction between *APOE* ε4 status and Aβ positivity, post hoc pairwise comparisons among the ε4–Aβ–, ε4–Aβ+, ε4+Aβ–, and ε4+Aβ+ subgroups were performed after controlling for age, gender, and education. ε4+Aβ+ individuals showed significantly poorer performances compared to participants in the other subgroups on the ADAS-cog13 ($P < 0.001$ for each comparison). In addition, ε4–Aβ+ individuals showed poorer performances than ε4–Aβ– individuals ($P = 0.007$) but showed no significant difference compared to ε4+Aβ– individuals ($P = 0.067$). ε4+Aβ+ individuals showed significantly poorer performances compared to the other three subgroups on the RAVLT_recog test ($P < 0.001$ for each comparison); there were no significant differences between performances on this measure among the other three subgroups (Fig. 3A and B, lower row).
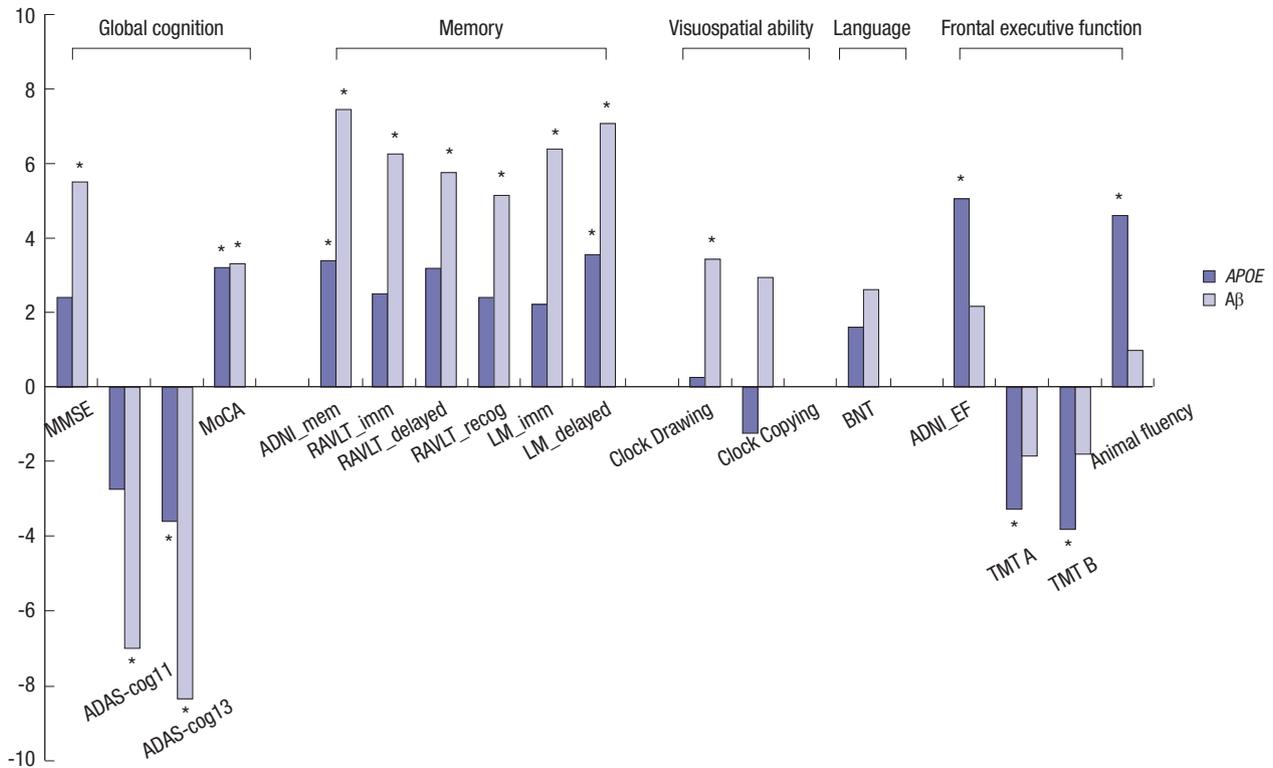
Fig. 1. Effect sizes of *APOE ε4* status and Aβ positivity on neuropsychological measures in participants with MCI. Effect sizes were calculated using Cohen's *d*. The magnitude of the differences in scores on each neuropsychological measure are presented according to apolipoprotein E (*APOE*) *ε4* status (*ε4* non-carriers and *ε4* carriers; gray bars) and beta-amyloid positivity (Aβ negative and positive; shaded bars). Lower scores on the ADAS-cog11, ADAS-cog13, TMT A, and TMT B indicate better performances. *False discovery rate (FDR)-corrected *P* < 0.05. MMSE, Mini-Mental State Examination; ADAS-cog11, Alzheimer's Disease Assessment Scale-cognitive subscale, consisting of 11 items; ADAS-cog13, Alzheimer's Disease Assessment Scale-cognitive subscale, consisting of 13 items; MoCA, Montreal Cognitive Assessment; ADNI_Mem, Alzheimer's Disease Neuroimaging Initiative composite score for memory; RAVLT_imm, Rey Auditory Verbal Learning Test, immediate recall score; RAVLT_delayed, RAVLT, delayed recall score; RAVLT_recog, RAVLT, recognition score; LM_imm, Logical Memory, immediate recall score; LM_delayed, LM, delayed recall score; ADNI_EF, Alzheimer's Disease Neuroimaging Initiative composite score for executive functioning; TMT, Trail Making Test; BNT, Boston Naming Test.
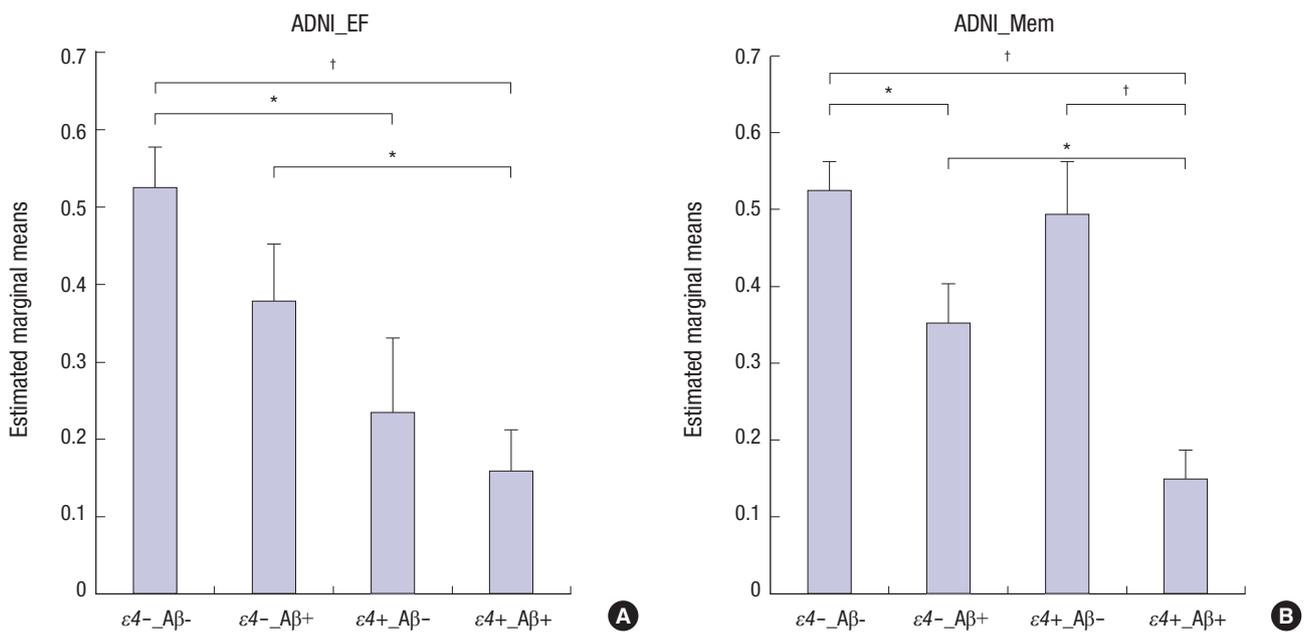

Fig. 2. Frontal executive and memory performances of four subgroups of participants with MCI. *P* < 0.01; †*P* < 0.001. *ε4*−, *APOE ε4* non-carriers; *ε4*+, *APOE ε4* carriers; Aβ−, beta-amyloid negative; Aβ+, beta-amyloid positive; ADNI_EF, Alzheimer's Disease Neuroimaging Initiative composite score for executive functioning; ADNI_Mem, Alzheimer's Disease Neuroimaging Initiative composite score for memory.
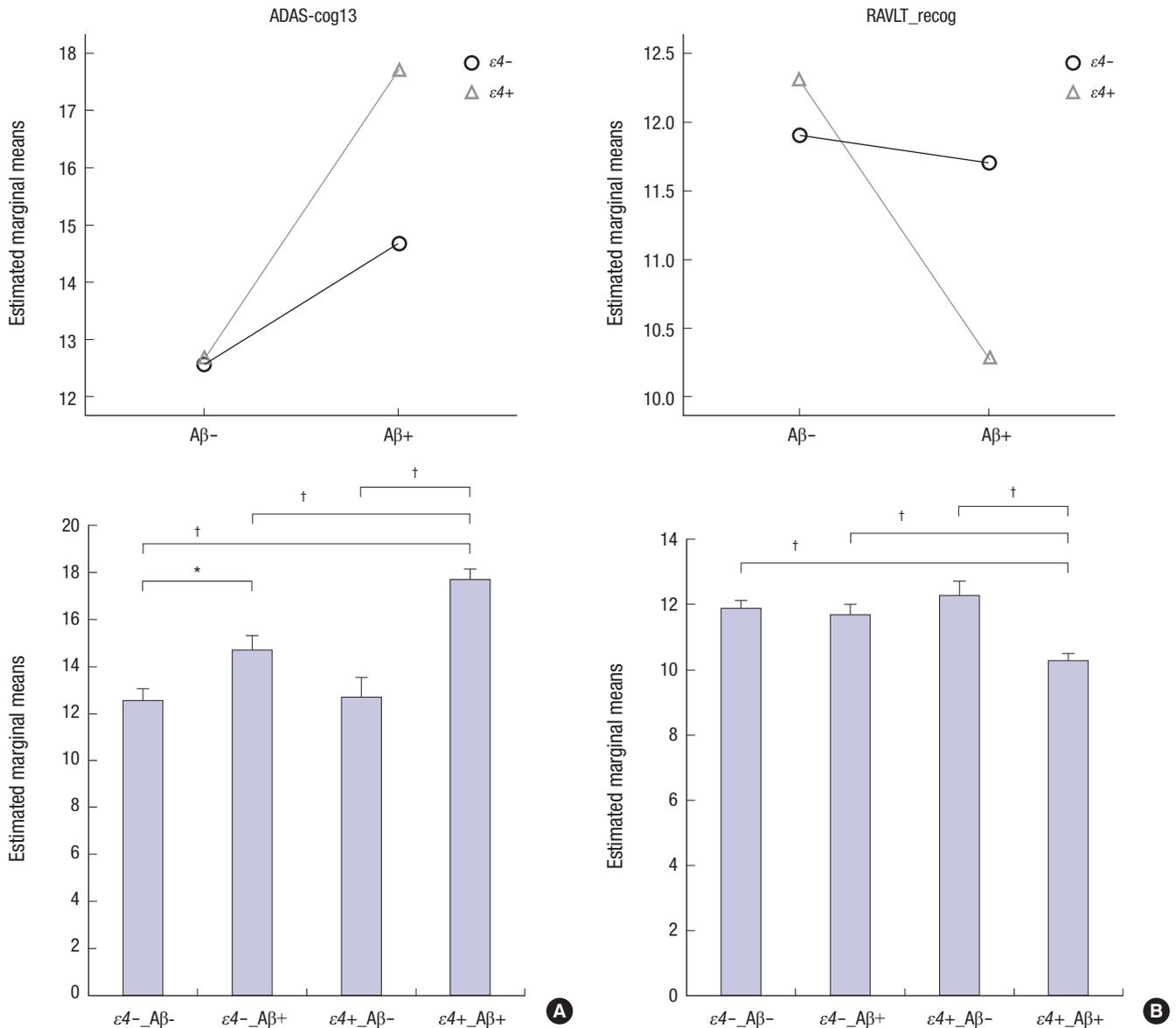
Fig. 3. Interactive effects of *APOE ε4* status and Aβ positivity on cognitive measures in participants with MCI. The upper row displays interactive effects of *APOE ε4* status and Aβ positivity on the ADAS-cog13 (**A**) and RAVLT_recog tests (**B**). The lower row displays four subgroups according to *APOE ε4* and Aβ status on the ADAS-cog13 (**A**) and RAV-LT_recog tests (**B**). *$P < 0.01$; †$P < 0.001$. *ε4−*, *APOE ε4* non-carriers, blue circle; *ε4+*, *APOE ε4* carriers, green triangle; Aβ−, beta-amyloid negative; Aβ+, beta-amyloid positive; ADAS-cog13, Alzheimer's Disease Assessment Scale-cognitive subscale, consisting of 13 items; RAVLT_recog, Rey Auditory Verbal Learning Test, recognition score.

No significant main effects or interactive effects of *APOE ε4* status and Aβ positivity on any neuropsychological measure were found in the AD dementia group.

Effects of *APOE ε4* status and Aβ positivity on clinical severity and functional status

No significant main effects or interactive effects of *APOE ε4* status and Aβ positivity on CDR-SOB or FAQ scores were found in the CN and AD dementia groups. In contrast, there was a significant main effect of Aβ positivity on both CDR-SOB and FAQ scores in the MCI group; however, there was no significant effect of *APOE ε4* status (Table 2).

DISCUSSION

Our data revealed four main findings. First, both *APOE ε4* status and Aβ positivity independently influenced cognitive function in participants with MCI. Specifically, there were *ε4*-related performance impairments in frontal executive function tests and other tests with frontal executive components. There were Aβ-related performance impairments in global cognition, memory, and visuospatial tests, but not in tests of frontal executive function. Second, there were interactive effects of the two factors on global cognition and verbal recognition memory in the MCI group, with *ε4+Aβ+* participants exhibiting the most significant

impairments. Third, Aβ positivity, but not *APOE ε4* status, influenced clinical severity and functional status in the MCI group. Lastly, the influences of *APOE ε4* status and Aβ positivity on cognitive function, clinical severity, and functional status were minimal in the CN and AD dementia groups.

Interestingly, there were dissociable influences of *APOE ε4* status and Aβ positivity on cognitive performances in the MCI group. *APOE ε4* status predominantly influenced scores on frontal executive function tests and other measures with frontal executive components (i.e., the ADAS-cog13, MoCA, and delayed free recall task). Aβ positivity had no significant influence on these effects. These results suggest that the contribution of *APOE ε4* to AD pathogenesis may be partially independent of its role in Aβ pathology. Furthermore, the predominantly affected cognitive domain may be frontal executive function. The frontal lobe is the neural substrate for executive function. A systemic review and meta-analysis study previously revealed a robust *APOE ε4*-related decrease in frontal lobe metabolism in non-demented subjects (28). Additionally, Chu et al. (11) recently reported that non-demented *APOE ε4* carriers showed impaired performances on frontal executive function measures, but not on memory tests. Previous reports assessing *APOE ε4*-related cognitive characteristics are inconsistent (29). The discrepancy among previous investigations may be attributable, at least in part, to failure to control for Aβ burden in their analyses. It is likely that Aβ burden is a confounding factor when assessing the relationship between *APOE ε4* status and cognitive function, particularly in the elderly population. Aβ deposition linearly increases with age, with a high number of Aβ+ individuals aged 60 and older (17). In agreement with this result, *APOE ε4* status affects performance on executive function tasks and frontal lobe thickness in younger subjects, in whom the accumulation of Aβ is unlikely to be a factor (7,30).

We found significant main effects of Aβ positivity on global cognition, memory, and visuospatial ability in participants with MCI, with Aβ+ individuals exhibiting poorer performances. However, measures of frontal executive function were not influenced by Aβ positivity. Although there are conflicting investigations assessing the effects of Aβ positivity (31,32), our results are consistent with observations that high Aβ burden is associated with poor cognitive function in subjects with MCI (14). Non-significant associations between Aβ positivity and frontal executive function are rarely reported; the majority of previous studies have mainly focused on episodic memory and lack detailed tests of frontal executive function (14,33). Thus, the influence of Aβ burden on frontal executive function has not been thoroughly examined. Consistent with our results, one recent follow-up study using comprehensive neuropsychological measures demonstrated that Aβ positivity did not affect frontal executive function in subjects with MCI but was associated with declines in other cognitive domains (20). Our subgroup analyses of representative scores on frontal executive function and memory tests showed that *APOE ε4* status and Aβ positivity predominantly affected frontal executive function and memory, respectively. These dissociable influences of *APOE ε4* status and Aβ positivity on executive and non-executive cognitive functions, respectively, in subjects with MCI could provide new insights into the mechanisms underlying AD-related cognitive decline.

We also found interactive effects of *APOE ε4* status and Aβ positivity on measures of global cognition and verbal recognition memory in the MCI group. Individuals positive for both *APOE ε4* and Aβ exhibited the most significant impairments in these tests, whereas no differences were found between individuals positive for only one of these factors. This result is in line with a previous study showing that the combination of *APOE ε4*+ status and Aβ burden is a significant risk factor for AD, though their independent effects may not be sufficient to cause AD (34). Our results suggest that the combination of *APOE ε4*+ genotype and Aβ burden is associated with greater detrimental effects on cognition than each single factor.

In the current study, the influences of *APOE ε4* status and Aβ positivity on cognitive characteristics were minimal in the CN group. *APOE ε4* influenced verbal immediate story recall; CN *ε4*+ subjects exhibited significantly lower performances compared to CN *ε4*− participants. Furthermore, Aβ positivity did not influence this effect. This result is consistent with previous studies showing adverse *APOE ε4* effects on verbal memory in a healthy normal elderly population, particularly in an episodic learning procedure similar to the test used in this study (8,35). Our findings also suggest that episodic memory, as measured by story recall, can be a sensitive tool to detect *APOE ε4*-related memory problems in the healthy normal elderly population. A meta-analysis of 77 studies reported that *ε4*+ individuals showed small but significant adverse effects not only on episodic memory, but also on global cognition, executive function, and perceptual speed (29). This discrepancy may be related to differences in study samples. Participants with MCI were not excluded in many of the studies included in the meta-analysis [e.g., (36)]. Although the meta-analysis included studies of cognitively intact subjects, it is likely that a considerable proportion of participants with MCI were also included. In contrast, MCI was not included in our CN group. We did not find any effect of Aβ positivity on cognitive performance. The relationship between Aβ burden and cognition in the healthy normal elderly is generally weak or absent (19,31). However, a recent meta-analysis investigating the relationship between Aβ and cognition reported that Aβ burden, as examined by amyloid imaging, was negatively associated with episodic memory (37). One possible reason for this discrepancy could be the study design (cross-sectional or longitudinal). The meta-analysis performed by Hedden et al. (37) included both cross-sectional and longitudinal analyses, whereas the current study is a cross-sectional analysis.

Effects of Aβ accumulation may be more apparent in longitudinal studies of cognitive decline rather than cross-sectional studies. A longitudinal study showed that, while effects of Aβ on cognitive function were insignificant in baseline evaluations, Aβ accumulation was significantly associated with declines in episodic memory after 18 months (19).

Neither *APOE ε4* status nor Aβ positivity influenced any cognitive measures in the AD dementia group. A lack of association between Aβ burden and cognition in AD dementia patients has been consistently reported (13,31). This implies that Aβ deposition is an early event that has plateaued at the point of clinical diagnosis of AD dementia (38). In line with previous studies (39,40), we failed to find *APOE ε4*-related differences in cognitive function in the AD dementia group. However, one study has demonstrated an *APOE ε4*-associated effect on memory and frontal executive function in AD dementia (10). The age of the participants may contribute to this inconsistency; Chang et al. (39) have hypothesized that the effect of *APOE ε4* on cognition may differ according to the mean age of the population being studied. Effects on cognitive function have been observed in elderly patients less than 75 years of age (10), whereas no significant effects have been found in studies of elderly patients over 80 years of age (39) or in our study, which included a wide range of ages (55 to 94 years).

We did not find any associations of *APOE ε4* status or Aβ positivity with clinical or functional status in the CN and AD dementia groups. However, Aβ positivity, but not *APOE ε4* status, was associated with poorer clinical and functional status in the MCI group, suggesting that a greater Aβ burden negatively influences both clinical severity and everyday functioning.

We propose several possible explanations for the strong effects of *APOE ε4* and Aβ burden observed only in the MCI group. First and most importantly, AD dementia has a very long preclinical period. *APOE ε4* and Aβ accumulation may negatively influence cognitive function during the prodromal stage (i.e., the MCI stage); however these effects may lessen during the clinical stage of AD dementia. Second, cognitive function in the MCI group was more heterogeneous than in the CN or AD dementia groups. CN subjects showed no cognitive impairments, raising the possibility of ceiling effects, whereas participants with AD showed significant cognitive impairments that may have been susceptible to floor effects. Third, the distribution of *APOE ε4* status and Aβ positivity within each group could influence the results. The CN and AD dementia groups included high percentages of *ε4*–Aβ– (56%) and *ε4*+Aβ+ individuals (64%), respectively, whereas the MCI group showed a relatively even distribution in the cells of positive or negative for both factors. Therefore, there may be insufficient variance between the two factors and cognitive scores to detect any effects in the CN and AD groups.

Some limitations and future directions should be discussed.

First, this study had a cross-sectional design, precluding an investigation of the effects of *APOE ε4* and Aβ on longitudinal cognitive decline in this population. To clarify the independent effects of these two factors, this issue should be addressed through further assessment of *APOE ε4*- or Aβ burden-related cognitive changes, particularly in the CN group. Second, cognitive performance is closely related to brain function and structure; however, dissociable influences of *APOE ε4* and Aβ burden on brain function were not examined in the current study. Future neuroimaging studies are needed to verify our results and to understand the precise role of the *APOE* gene in frontal executive function. Third, AD dementia diagnosis is made on a clinical rather than a pathological basis. Although the proportion of Aβ– AD dementia patients was small (16%), we cannot entirely exclude the possibility that participants with non-AD dementia were included in our study.

In conclusion, we provide further evidence that *APOE ε4* and Aβ burden play both independent and interactive roles in altering cognitive function in individuals with MCI. Dissociable, independent influences of *APOE ε4* and Aβ burden on executive and non-executive cognitive functions, respectively, were found in participants with MCI. Interactive effects of these two factors on global cognition and recognition memory were also observed.

ifornia, San Diego. ADNI data are disseminated by the Laboratory of Neuro Imaging at the University of Southern California.

DISCLOSURE

The authors have no potential conflicts of interest to disclose.

AUTHOR CONTRIBUTION

Conception and design: Seo EH, Choo IH. Analysis and interpretation of data: Seo EH, Choo IH. Writing and revision of the manuscript: Seo EH, Choo IH. Administrative, technical & material supports: Park SH, Kim SH, Kang SH. Critical revisions and approval of the manuscript: all authors.

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REFERENCES

1. Morris JC, Storandt M, Miller JP, McKeel DW, Price JL, Rubin EH, Berg L. *Mild cognitive impairment represents early-stage Alzheimer disease. Arch Neurol* 2001; 58: 397-405.

2. Petersen RC, Caracciolo B, Brayne C, Gauthier S, Jelic V, Fratiglioni L. *Mild cognitive impairment: a concept in evolution. J Intern Med* 2014; 275: 214-28.

3. Sohn BK, Yi D, Seo EH, Choe YM, Kim JW, Kim SG, Choi HJ, Byun MS, Jhoo JH, Woo JI, et al. *Comparison of regional gray matter atrophy, white matter alteration, and glucose metabolism as a predictor of the conversion to Alzheimer's disease in mild cognitive impairment. J Korean Med Sci* 2015; 30: 779-87.

4. Nordberg A, Carter SF, Rinne J, Drzezga A, Brooks DJ, Vandenberghe R, Perani D, Forsberg A, Långström B, Scheinin N, et al. *A European multicentre PET study of fibrillar amyloid in Alzheimer's disease. Eur J Nucl Med Mol Imaging* 2013; 40: 104-14.

5. Wolk DA, Price JC, Saxton JA, Snitz BE, James JA, Lopez OL, Aizenstein HJ, Cohen AD, Weissfeld LA, Mathis CA, et al. *Amyloid imaging in mild cognitive impairment subtypes. Ann Neurol* 2009; 65: 557-68.

6. Bertram L, McQueen MB, Mullin K, Blacker D, Tanzi RE. *Systematic meta-analyses of Alzheimer disease genetic association studies: the AlzGene database. Nat Genet* 2007; 39: 17-23.

7. Izaks GJ, Gansevoort RT, van der Knaap AM, Navis G, Dullaart RP, Slaets JP. *The association of APOE genotype with cognitive function in persons aged 35 years or older. PLoS One* 2011; 6: e27415.

8. Liu F, Pardo LM, Schuur M, Sanchez-Juan P, Isaacs A, Sleegers K, de Koning I, Zorkoltseva IV, Axenovich TI, Witteman JC, et al. *The apolipoprotein E gene and its age-specific effects on cognitive function. Neurobiol Aging* 2010; 31: 1831-3.

9. Kerchner GA, Berdnik D, Shen JC, Bernstein JD, Fenesy MC, Deutsch GK, Wyss-Coray T, Rutt BK. *APOE epsilon4 worsens hippocampal CA1 apical neuropil atrophy and episodic memory. Neurology* 2014; 82: 691-7.

10. Wolk DA, Dickerson BC, Weiner M, Aiello M, Aisen P, Albert MS, Alexander G, Anderson HS, Anderson K, Apostolova L, et al. *Apolipoprotein E (APOE) genotype has dissociable effects on memory and attentional-executive network function in Alzheimer's disease. Proc Natl Acad Sci U S A* 2010; 107: 10256-61.

11. Chu CS, Lu T, Tsai SJ, Hong CJ, Yeh HL, Yang AC, Liu ME. *APOE varepsilon4 polymorphism and cognitive deficit among the very old Chinese veteran men without dementia. Neurosci Lett* 2014; 576: 17-21.

12. Bretsky P, Guralnik JM, Launer L, Albert M, Seeman TE; MacArthur Studies of Successful Aging. *The role of APOE-epsilon4 in longitudinal cognitive decline: MacArthur Studies of Successful Aging. Neurology* 2003; 60: 1077-81.

13. Jack CR Jr, Lowe VJ, Weigand SD, Wiste HJ, Senjem ML, Knopman DS, Shiung MM, Gunter JL, Boeve BF, Kemp BJ, et al. *Serial PIB and MRI in normal, mild cognitive impairment and Alzheimer's disease: implications for sequence of pathological events in Alzheimer's disease. Brain* 2009; 132: 1355-65.

14. Landau SM, Mintun MA, Joshi AD, Koeppe RA, Petersen RC, Aisen PS, Weiner MW, Jagust WJ; Alzheimer's Disease Neuroimaging Initiative. *Amyloid deposition, hypometabolism, and longitudinal cognitive decline. Ann Neurol* 2012; 72: 578-86.

15. Kantarci K, Lowe V, Przybelski SA, Weigand SD, Senjem ML, Ivnik RJ, Preboske GM, Roberts R, Geda YE, Boeve BF, et al. *APOE modifies the association between Abeta load and cognition in cognitively normal older adults. Neurology* 2012; 78: 232-40.

16. Oh H, Mormino EC, Madison C, Hayenga A, Smiljic A, Jagust WJ. *beta-Amyloid affects frontal and posterior brain networks in normal aging. Neuroimage* 2011; 54: 1887-95.

17. Rodrigue KM, Kennedy KM, Devous MD Sr, Rieck JR, Hebrank AC, Diaz-Arrastia R, Mathews D, Park DC. β-Amyloid burden in healthy aging: regional distribution and cognitive consequences. Neurology 2012; 78: 387-95.

18. Dorey E, Chang N, Liu QY, Yang Z, Zhang W. *Apolipoprotein E, amyloid-beta, and neuroinflammation in Alzheimer's disease. Neurosci Bull* 2014; 30: 317-30.

19. Lim YY, Ellis KA, Pietrzak RH, Ames D, Darby D, Harrington K, Martins RN, Masters CL, Rowe C, Savage G, et al. *Stronger effect of amyloid load than APOE genotype on cognitive decline in healthy older adults. Neurology* 2012; 79: 1645-52.

20. Lim YY, Maruff P, Pietrzak RH, Ames D, Ellis KA, Harrington K, Lautenschlager NT, Szoeke C, Martins RN, Masters CL, et al. *Effect of amyloid on memory and non-memory decline from preclinical to clinical Alzheimer's disease. Brain* 2014; 137: 221-31.

21. Petersen RC, Aisen PS, Beckett LA, Donohue MC, Gamst AC, Harvey DJ, Jack CR Jr, Jagust WJ, Shaw LM, Toga AW, et al. *Alzheimer's Disease Neuroimaging Initiative (ADNI): clinical characterization. Neurology* 2010; 74: 201-9.

22. McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. *Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology* 1984; 34: 939-44.

23. Nasreddine ZS, Phillips NA, Bédirian V, Charbonneau S, Whitehead V,

Collin I, Cummings JL, Chertkow H. *The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. J Am Geriatr Soc* 2005; 53: 695-9.

24. Crane PK, Carle A, Gibbons LE, Insel P, Mackin RS, Gross A, Jones RN, Mukherjee S, Curtis SM, Harvey D, et al. *Development and assessment of a composite score for memory in the Alzheimer's Disease Neuroimaging Initiative (ADNI). Brain Imaging Behav* 2012; 6: 502-16.

25. Gibbons LE, Carle AC, Mackin RS, Harvey D, Mukherjee S, Insel P, Curtis SM, Mungas D, Crane PK; Alzheimer's Disease Neuroimaging Initiative. *A composite score for executive functioning, validated in Alzheimer's Disease Neuroimaging Initiative (ADNI) participants with baseline mild cognitive impairment. Brain Imaging Behav* 2012; 6: 517-27.

26. Teng E, Becker BW, Woo E, Knopman DS, Cummings JL, Lu PH. *Utility of the functional activities questionnaire for distinguishing mild cognitive impairment from very mild Alzheimer disease. Alzheimer Dis Assoc Disord* 2010; 24: 348-53.

27. Saykin AJ, Shen L, Foroud TM, Potkin SG, Swaminathan S, Kim S, Risacher SL, Nho K, Huentelman MJ, Craig DW, et al. *Alzheimer's Disease Neuroimaging Initiative biomarkers as quantitative phenotypes: genetics core aims, progress, and plans. Alzheimers Dement* 2010; 6: 265-73.

28. Liu Y, Yu JT, Wang HF, Han PR, Tan CC, Wang C, Meng XF, Risacher SL, Saykin AJ, Tan L. *APOE genotype and neuroimaging markers of Alzheimer's disease: systematic review and meta-analysis. J Neurol Neurosurg Psychiatry* 2015; 86: 127-34.

29. Wisdom NM, Callahan JL, Hawkins KA. *The effects of apolipoprotein E on non-impaired cognitive functioning: a meta-analysis. Neurobiol Aging* 2011; 32: 63-74.

30. Fennema-Notestine C, Panizzon MS, Thompson WR, Chen CH, Eyler LT, Fischl B, Franz CE, Grant MD, Jak AJ, Jernigan TL, et al. *Presence of ApoE epsilon4 allele associated with thinner frontal cortex in middle age. J Alzheimers Dis* 2011; 26 Suppl 3: 49-60.

31. Hampel H. *Amyloid-beta and cognition in aging and Alzheimer's disease: molecular and neurophysiological mechanisms. J Alzheimers Dis* 2013; 33 Suppl 1: S79-86.

32. Rodrigue KM, Kennedy KM, Park DC. *Beta-amyloid deposition and the aging brain. Neuropsychol Rev* 2009; 19: 436-50.

33. Lim YY, Maruff P, Pietrzak RH, Ellis KA, Darby D, Ames D, Harrington K, Martins RN, Masters CL, Szoeke C, et al. *Abeta and cognitive change: examining the preclinical and prodromal stages of Alzheimer's disease. Alzheimers Dement* 2014; 10: 743-751.e1.

34. Knopman DS. *beta-Amyloidosis and neurodegeneration in Alzheimer disease: who's on first? Neurology* 2014; 82: 1756-7.

35. Caselli RJ, Dueck AC, Osborne D, Sabbagh MN, Connor DJ, Ahern GL, Baxter LC, Rapcsak SZ, Shi J, Woodruff BK, et al. *Longitudinal modeling of age-related memory decline and the APOE epsilon4 effect. N Engl J Med* 2009; 361: 255-63.

36. Bookheimer SY, Strojwas MH, Cohen MS, Saunders AM, Pericak-Vance MA, Mazziotta JC, Small GW. *Patterns of brain activation in people at risk for Alzheimer's disease. N Engl J Med* 2000; 343: 450-6.

37. Hedden T, Oh H, Younger AP, Patel TA. *Meta-analysis of amyloid-cognition relations in cognitively normal older adults. Neurology* 2013; 80: 1341-8.

38. Jack CR Jr, Knopman DS, Jagust WJ, Shaw LM, Aisen PS, Weiner MW, Petersen RC, Trojanowski JQ. *Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. Lancet Neurol* 2010; 9: 119-28.

39. Chang YL, Fennema-Notestine C, Holland D, McEvoy LK, Stricker NH, Salmon DP, Dale AM, Bondi MW; Alzheimer's Disease Neuroimaging Initiative. *APOE interacts with age to modify rate of decline in cognitive and brain changes in Alzheimer's disease. Alzheimers Dement* 2014; 10: 336-48.

40. Kleiman T, Zdanys K, Black B, Rightmer T, Grey M, Garman K, Macavoy M, Gelernter J, van Dyck C. *Apolipoprotein E epsilon4 allele is unrelated to cognitive or functional decline in Alzheimer's disease: retrospective and prospective analysis. Dement Geriatr Cogn Disord* 2006; 22: 73-82.