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Apolipoprotein E ε4 Modulates Cognitive Profiles, Hippocampal Volume, and Resting-State Functional Connectivity in Alzheimer's Disease

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Abstract. The apolipoprotein E ε4 (*APOE* ε4) allele is a well-established genetic risk factor for Alzheimer's disease (AD). Numerous studies have suggested that the modulation of *APOE* ε4 affects cognition and brain structure and function in healthy populations, particularly in the hippocampus, a key area associated with AD pathology. However, the effect of *APOE* ε4 allele on cognitive performance, hippocampal structural morphology, and specifically on functional characteristics in patients with AD remains poorly understood. Here, we employed a neuropsychological battery test and multi-modal structural MRI and resting-state functional MRI dataset to systematically investigate cognitive performance, hippocampal structural volume, and functional properties (including local low-frequency oscillating amplitude, intra-regional functional synchrony, and inter-regional functional connectivity) in 16 *APOE* ε4-carriers and 26 non-carriers at early stages of AD. Compared to non-carriers, *APOE* ε4-carriers exhibited poorer performance on recognition performance, but performed better on the late item generation of the verbal fluency task (associated with executive function). Structural imaging analysis revealed that *APOE* ε4-carriers exhibited smaller left hippocampal volumes compared to non-carriers, and the result remains significant after correcting for effects of brain size. Functional imaging analysis revealed that *APOE* ε4-carriers exhibited decreased amplitude of low-frequency fluctuations in the left hippocampus, non-significant changes in intra-regional synchronization within the hippocampus and decreased hippocampal functional connectivity predominantly in components of the default-mode network including the medial frontal and parietal cortices and the lateral temporal cortical regions. Taken together, our results showed *APOE* genotypic effects on the cognitive profile and hippocampal structural and functional characteristics in patients at early stages of AD, thus providing empirical evidence for the modulation of the *APOE* genotype on disease phenotype.

Keywords: Apolipoprotein E, default-mode, functional connectivity, hippocampus, resting-state functional MRI

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INTRODUCTION

The apolipoprotein E ε4 (*APOE* ε4) allele is a well-established genetic risk factor for the development of Alzheimer's disease (AD) [1, 2]. The presence of a single ε4 allele increases the lifetime risk of AD by 2-3-fold relative to the ε3 allele, and two ε4 alleles increase the lifetime risk of AD by 12-fold [3].

Neuropsychological studies have reported that the *APOE* genotype is associated with cognitive performance in different domains in AD patients. Specifically, memory is more impaired in *APOE* ε4-carriers [4–10], while executive function, naming, and mental speed are more impaired in *APOE* ε4 non-carriers [4, 6, 9–11]. However, several groups did not find significant differences in cognitive performance between AD patients with and without the *APOE* ε4 allele [12]. Furthermore, structural imaging studies in AD revealed atrophy of medial temporal lobe (the earliest site of AD pathology), specifically in the hippocampus [13, 14]. Several studies have also reported more rapid hippocampal atrophy in *APOE* ε4-carriers compared with non-carriers in AD patients [15–18]; however, other studies did not find significant differences in hippocampal atrophy [19, 20]. Thus, modulation of *APOE* genetic variants on both cognitive performance and hippocampal volume in AD remains controversial.

In addition to hippocampal structural abnormalities, increasing evidence also suggests AD-related disruption in hippocampal functional characteristics, e.g., local neural fluctuations [21], intra-regional synchrony [22, 23], and inter-regional connectivity [24–26]. These functional features can be well-characterized by resting-state functional MRI (R-fMRI), a promising non-invasive and easy applicable imaging technique used to examine the brain's intrinsic or spontaneous activity [27, 28]. Previously, Biswal et al. [27] demonstrated that the spontaneous low frequency (0.01–0.1 Hz) fluctuations (LFFs) are physiologically meaningful. A previous study suggested that the LFFs of R-fMRI have the underlying electrophysiological mechanism as the task-induced fMRI BOLD signal [29]. Thus, the amplitude of LFF (ALFF) may indicate the amplitude of regional spontaneous neuronal activity [27]. Moreover, a number of R-fMRI studies have utilized the ALFF metric to study functional alterations in the hippocampus in patients with AD and/or mild cognitive impairment [21, 26, 30–34]. Cross-correlation coefficient of spontaneous low-frequency (COSLOF) is an index that measures mean connectivity within a brain region. Technically speaking, the COSLOF is similar to regional homogeneity [35], a widely used measurement to study local synchronization of brain activity among spatially adjacent voxels. Li et al. [22] were the first to employ the COSLOF index to measure functional integrity within the hippocampus and demonstrated that COSLOF within the hippocampus could be used as a quantitative marker in diagnosing AD. Recently, several R-fMRI studies have demonstrated modulation of the *APOE* genotype on hippocampal resting-state functional connectivity (RSFC) in healthy adults [36–39]. However, no R-fMRI studies have reported whether the *APOE* genotype modulates the intrinsic functional architecture of the hippocampus in AD.

This study aimed to systematically determine the *APOE* genotype effects on cognitive performance and hippocampal structure and functioning in AD. Thus, we collected psychometric, structural MRI, and R-fMRI data from 16 AD patients with at least one *APOE* ε4 allele and 26 age-, gender-, education-, and severity-matched AD patients without the *APOE* ε4 allele. On the basis of these studies, we hypothesized that the *APOE* genotype modulates the clinical phenotype of AD including memory performance, hippocampal volume, and hippocampal functional characteristics (including local and connectivity features).

MATERIALS AND METHODS

Participants

Patients with AD were prospectively recruited to establish a case registry at the Dementia Care and Research Center, Peking University Institute of Mental Health. Upon enrollment, a detailed clinical examination, neuropsychological battery test, laboratory tests, and multi-modal brain MRI examinations were performed on every participant. Participants in the registry were also invited to receive follow-up examinations every 6 months. For the purpose of the present study, we selected participants (registered between June 2007 through September 2009) who had a baseline diagnosis of AD and had completed an MRI examination before initiation of nootropic medication ($n = 74$). These patients were all Chinese Han and right-handed. At baseline, the patients had a clinical dementia rating (CDR) global score of 0.5, 1, or 2 [40]. A clinical diagnosis of AD was made according to the criteria for dementia cited in the International Classification of Diseases, 10th Revision (ICD-10) [41] and the criteria for probable AD of the National Institute of Neurological and Communicative Disorders

and the Stroke/Alzheimer Disease and Related Disorders Association (NINCDS-ADRDA) [42]. Participants were excluded if they presented structural abnormalities that could result in dementia, such as cortical infarction, tumor, or subdural hematoma, or they had concurrent illness other than dementia that interfered with cognitive function at the time of the MRI examination. The selected AD patients were further screened for the *APOE* genotype and classified as either *APOE* ε4 positive/carriers (genotype of ε3/ε4 or ε4/ε4) or *APOE* ε4 negative/non-carriers (genotype of ε3/ε3). Subjects carrying the *APOE* ε2 allele were excluded due to its potential protective effect based on epidemiological surveys [43]. After a final visual inspection of the MR images, a total of 42 subjects from the initial 74 AD patients were selected for the current study, including 16 *APOE* ε4-carriers (13 ε3/ε4 and 3 ε4/ε4) and 26 non-carriers. Of the 42 very mild to moderate AD patients, 23 patients had a CDR global score of 0.5, 15 patients had a CDR global score of 1, and 4 patients had a CDR global score of 2. There were no differences in the distribution of CDR global scores between the groups ($p = 0.831$). Informed consent was obtained from each participant and this study protocol was approved by the institutional review board of Peking University Institute of Mental Health. Further detailed clinical and demographic data of all AD patients are presented in Table 1.

APOE genotyping

DNA was isolated from 10 ml EDTA with blood QIAamp® DNA Blood Mini Kit (Qiagen Inc., Hilden, Germany) according to standard procedures. *APOE* genotyping was performed as previously described [44]. Genotype scorers (XW and HL) were blind to

Table 1
Demographics, clinical, and cognitive characteristics of the participants

	APOE ε4-carriers ($n = 16$)	*APOE* ε4 non-carriers ($n = 26$)	p-value
Age (years)	79.3 (5.1)	76.5 (5. 3)	0.104
Gender (M/F)	4/12	9/17	0.513
Education (years)	13.6 (2.8)	13.1 (4.2)	0.711
Illness duration (years)[a]	3.5 (1.8)	2.3 (1.7)	0.065
CDR-SB	5.1 (2. 5)	4.3 (2.0)	0.252
CASI	76.9 (11.0)	79.9 (7.5)	0.187
COMT			
immediate object memory – trial 1[b]	3.6 (1.2)	4.2 (1.5)	0.827
immediate object memory – trial 2[b]	5 (1.3)	5.4 (1. 9)	0.918
immediate object memory – trial 3[b]	4.9 (1.2)	6.0 (1.8)	0.090
immediate object memory – mean	4.7 (1.1)	5.2 (1.6)	0.210
free delayed recall – 5 min[b]	2.0 (1.8)	3.3 (2.6)	0.108
recognition – 5 min[b]	15.9 (2.2)	18.3 (1.8)	0.001**
free delayed recall – 30 min[b]	1.6 (1.8)	3.1 (3.0)	0.129
recognition – 30 min[b]	16.0 (3.4)	18.7 (1.5)	0.012*
Body part naming	10.0 (0)	10.0 (0)	0.999
Auditory comprehension[b]	22.9 (2.1)	23.8 (1.0)	0.288
Read time	4.8 (1.6)	5.0 (1.4)	0.502
Set time	5.4 (2.7)	5.4 (2.41)	0.814
Verbal fluency[b]	12.9 (2.9)	11.5 (2.7)	0.096
verbal fluency (0–15 s)[b]	5.8 (2.7)	7.5 (2.6)	0.051
verbal fluency (16–30 s)[b]	3.5 (2.8)	2.7 (1.9)	0.255
verbal fluency (31–45 s)[b]	1.7 (1.3)	1.6 (1.9)	0.894
verbal fluency (46–60 s)[b]	1.7 (0.8)	0.8 (0.9)	0.002**
Picture completion[b]	6 (2.4)	5.6 (2.3)	0.269
Digit span[b]	14 (4.8)	13.6 (3.3)	0.701
digit span – forward[b]	8.6 (2.4)	8.2 (2.1)	0.629
digit span – backward[b]	5.4 (2.8)	5.8 (3.0)	0.767
CERAD drawing	9.4 (2.0)	9.7 (1.8)	0.162
Trail-Making Test A – time (s)[b]	139.2 (80.5)	97.5 (48.1)	0.091
Trail-Making Test A– errors[b]	1.0 (1.6)	0.5 (0.9)	0.297

Data were presented as the mean (SD). *P*-values were obtained using the two-tailed Chi-square test for gender and two-tailed two-sample *t*-tests for other factors. *$p < 0.05$; **$p < 0.01$. [a]Data were missing for eight patients; [b]Data were missing for three patients. CDR-SB, Clinical Dementia Rating, sum of box; CASI, cognitive ability screening instrument; COMT, common object memory test; CERAD, Consortium to Establish a Registry for Alzheimer's Disease.

the identity of the samples. Eighteen samples were further evaluated using this sequencing technique, and the results were consistent with the *APOE* genotyping results obtained using the PCR-RFLP method, thus verifying our approach.

Neuropsychological assessment

Considering that the Mini-Mental State Examination (MMSE) can be affected by different linguistic, educational, cultural, and socioeconomic backgrounds, this measure was only used as a screening tool to evaluate the patients' suitability for inclusion/exclusion in the present study. The AD patients in this study had MMSE scores of >16. The overall cognitive functioning was evaluated using the Cognitive Abilities Screening Instrument, Chinese version 2.0 (CASI C-2.0) [45, 46]. All subjects were administered the cross-cultural neuropsychological test battery [47], including the CASI C-2.0 [45, 46], Common Objects Memory Test (COMT) [47, 48], body part naming, the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) verbal category fluency, auditory comprehension, read and set time, CERAD drawing [49], digit span (forward and backward), picture completion, and Trail-Making Test A. Given that previous studies have suggested a greater memory deficit in ε4-carriers [4, 5] and more impaired executive functions in ε4 non-carriers [4, 6], we particularly focused on the COMT test for episodic memory and the CERAD verbal category fluency for animal names, which is considered a measure of language and executive function.

COMT was developed as a culture fair measure of recent memory specifically for the cross-cultural neuropsychological test battery [47, 48]. This test was administered using standardized procedures as previously described [47, 48]. Briefly, the subject is shown a set of ten 3×5" color photographs of common objects (e.g., button, chair, clock) across three learning trials and the subjects were required to immediately recall as many as possible during each trail. After the third trial, the examinee is engaged with a brief distracter task (e.g., CERAD figure drawing) for 3 to 5 min and then asked again to recall the items. The 5-min delayed recall is immediately followed by a recognition test in which ten original objects are interspersed with ten distracters. The subject is asked to indicate with a simple "Yes" or "No" whether an item was seen in the original three learning tests. The distracter objects are similar to the original objects in terms of visual complexity and without distinctive details. Long-term retention of the original objects was assessed after a 30-min delay using tests of recall and recognition, with a different set of ten distracters. Responses during three learning with immediate free recall trials and those during the subsequent two delayed recall and recognition trials were used to assess the performance of recent memory.

The verbal fluency test for animal naming was also used to measure executive function. The examinees were asked to name "all the animals you can think of in one minute." The examinees received credit for naming general categories as well as specific examples. Repeated responses were counted only once. The total words generated are the most commonly used score in verbal fluency tests. However, this approach did not provide information about the mechanisms underlying a poor test performance. Several studies suggest the performance of semantic category fluency as a function of time, which might provide additional insights into cognitive processes [50–54]. Thus, we investigated the level of performance in every 15-s phase during a 60-s verbal fluency task to see if performance could be different in *APOE* ε4 carriers and non-carriers. Finally, the CDR sum of box (CDR-SB) was used for global evaluation of dementia [40].

Image acquisition

All MRI scans were performed on a 3.0 Tesla MR system (Siemens Magnetom Trio A Tim system, Germany) using a standard head coil. During the entire scanning procedure, cushions and headphones were used to reduce subject motion and scanner noise. A T1-weighted three-dimensional volumetric magnetization-prepared rapidly acquired gradient-echo (3D-MPRAGE) sequence was used to acquire high-resolution anatomical images. The parameters were as follows: repetition time (TR) = 2530 ms; echo time (TE) = 3.44 ms; time inversion (TI) = 1100 ms; slice number = 192; slice thickness = 1.0 mm; gap = 0 mm; matrix = 256 × 256; and field of view (FOV) = 256×256 mm^2. The scan time of this sequence was approximately 6 min. The R-fMRI data were collected using an echo-planar imaging (EPI) sequence: axial slices, TR = 2000 ms; TE = 30 ms; flip angle = 90°; slice number = 30; slice thickness = 4.0 mm; gap = 0.8 mm; matrix = 64 × 64; and FOV = 220 × 220 mm^2. During the functional image acquisition, the participants were instructed to keep their eyes closed, to relax their minds and to remain motionless as much as possible, but to not fall asleep. The R-fMRI scan lasted for 420 s in total.

Structural imaging analysis

Determination of the region of interest (ROI) and hippocampal volume

The borders of the bilateral hippocampi were manually traced sequentially on each slice of individual T1-weighted 3D MR images from posterior to anterior using previously defined boundaries [55], including the hippocampus proper, dentate gyrus, subicular complex, alveus, and fimbria. The boundaries were drawn using MRIcron software (http://www.mccauslandcenter.sc.edu/mricro/mricron/) by one trained rater (XW) who was blind to the clinical information and *APOE* genotype. All individual anatomical tracings were carefully reviewed by another senior rater (HW) who was also blind to all clinical information. Corrections were made if necessary. The number of voxels was then quantified within the mask and multiplied by the voxel volume (1 mm^3 here) using in-house Matlab codes. Importantly, the hippocampal volume was measured in the individual native space. For the hippocampus-related functional analyses, individual hippocampal ROIs were normalized into the Montreal Neurological Institute (MNI) space in terms of corresponding transformation matrices derived from the normalization of individual T1 images to the ICBM152 T1 template provided in the SPM8 toolbox (http://www.fil.ion.ucl.ac.uk/spm/software/spm8/).

To examine the reliability of the manual hippocampal masks, we randomly selected 10 of the 42 participants and manually traced the borders of the bilateral hippocampi a second time. The intra-class correlation coefficient (ICC) and dice coefficient (DC) were used to test the intra-rater reliability of the hippocampal volume and spatial overlap of the hippocampal topography, respectively. The ICC was 0.892 for the left and 0.896 for the right hippocampal ROIs. The DC was 0.858 ± 0.025 for the left and 0.855 ± 0.029 for the right hippocampal ROIs. These quantitative analyses indicated that the manual hippocampal masks were reliable and were thus adequate for the current study. The hippocampal probability map over all participants is presented in Fig. 1.

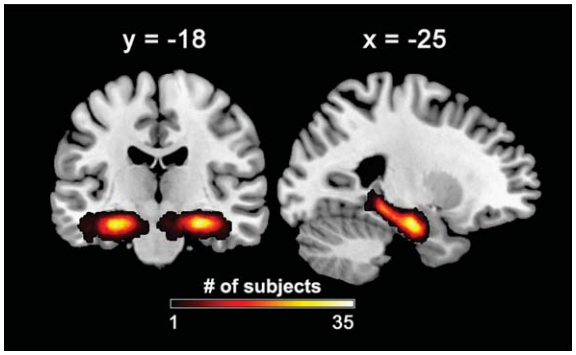

Fig. 1. Probability map of the hippocampal mask over all participants. Individual hippocampal ROIs that were drawn manually were first transformed from the native space into the MNI space by applying the corresponding transformation matrices derived from the spatial normalization of individual T1 images to the ICBM152 T1 template. The transformed hippocampal ROIs were then summed over all of the participants to demonstrate the consistency of the hippocampal location.

Functional image analysis

Image pre-processing

Pre-processing of the R-fMRI data was performed using the SPM8 package (http://www.fil.ion.ucl.ac.uk/spm/software/spm8/). After removal of the first five volumes to allow for T1 equilibration effects, the functional images were corrected for intra-volume time offsets between slices and inter-volume geometrical displacements due to head movement. None of the participants were excluded on the basis of the criterion of displacement >3 mm or angular rotation >3 degree in any direction. All corrected functional data were then normalized to the MNI space (12-parameter affine transformation and non-linear deformations) and resampled to a 3-mm isotropic resolution. The resulting images were further spatially smoothed (Gaussian kernel of 6-mm full width at half maximum) and temporally band-pass filtered (0.01–0.1 Hz). The linear trends were also removed. Finally, several nuisance signals (six head-motion profiles, mean white matter and cerebrospinal fluid time series as well as their first derivatives) were regressed out from each voxel's time course.

Intra-regional functional measures within the hippocampus

To measure the local functional characteristics of the hippocampus, we adopted the following two measures: ALFF [35] and COSLOF [22]. (i) ALFF: For each voxel within a given hippocampal ROI, the time series was first converted to the frequency domain using a Fast Fourier Transform. The square root of the power spectrum was then computed and summed across a pre-defined frequency interval (0.01–0.1 Hz in the current study). This summed square root was termed ALFF at the given voxel [35]. The ALFF for the given hippocampal ROI was simply calculated as

the mean ALFF value across all voxels. The ALFF measured the strength or intensity of low-frequency oscillations embedded in spontaneous neural activity. (ii) COSLOF: For any pair of voxels within a given hippocampal ROI, we first computed the cross correlation coefficient (zero lag) between the voxels [22]. This resulted in a correlation matrix with a dimensionality of $N \times N$ (N = the number of voxels in the given hippocampal ROI). The COSLOF index was then calculated as the mean of all elements in the upper triangular portion of the correlation matrix. The COSLOF reflected the overall functional integration within a given hippocampal ROI.

Inter-regional functional connectivity with the hippocampus

In addition to the local functional characteristics, we further explored the RSFC of the hippocampus with other distant brain regions [24, 27]. For a given hippocampal ROI, a seed reference was first obtained by averaging all of the voxels' time series within the ROI. The seed reference time course was then correlated with the time series extracted over the entire brain in a voxel-wise manner. Finally, a Fisher's r-to-z transformation was applied to the resulting whole-brain correlation map to improve the normality of these correlation coefficients. Thus, for each participant, we obtained two RSFC maps for the left and right hippocampi.

Statistical analysis

To determine the presence of statistically significant differences between the *APOE* ε4-carriers and non-carriers in cognitive performance, structural volume, and functional ALFF and COSLOF of the hippocampus, multiple general linear models were performed with age, gender, education, and disease severity measured using the CDR-SB as covariates. The significance level was established at $p < 0.05$. For the hippocampal RSFC analysis, we first performed one-sample *t*-tests in a voxel-wise manner to examine within-group RSFC patterns. The statistical threshold was set at a corrected $p < 0.05$ by combining a height threshold of $p < 0.0001$ and an extent threshold of $p < 0.05$ [56]. Voxel-by-voxel general linear models were then performed to test between-group differences with age, gender, education, and CDR-SB as covariates. The statistical threshold was established at a corrected $p < 0.05$ by combining the height threshold of $p < 0.01$ and extent threshold of $p < 0.05$ [56]. To further elucidate the relationship between brain characteristics and cognitive performance, multiple linear regressions were performed on all of the subjects with regression of the effects of age, gender, education, and CDR-SB. Notably, the group status was also entered into the regression model as a covariate to avoid spurious correlations driven by the *APOE* genotyping differences.

RESULTS

Demographic data and cognitive performance

The demographics and cognitive performances of all subjects are illustrated in Table 1. There were no significant ($p > 0.05$) differences in age, gender, years of education, duration of illness, and severity of dementia (as measured using CDR-SB) between *APOE* ε4-carriers and non-carriers. After controlling for gender, age, education, and CDR-SB, the *APOE* ε4-carriers demonstrated poorer performance on the 5-min [$t(33) = -3.622$, $p = 0.001$] and 30-min [$t(33) = -2.828$, $p = 0.012$] recognition test and a trend toward poorer performance on the verbal fluency test during the first 15 s [$t(33) = 2.026$, $p = 0.051$] compared to the non-carrier group (Table 1). In contrast, carriers performed better [$t(33) = 3.319$, $p = 0.002$] on the verbal fluency during 46–60 s (which is associated with executive function) compared to non-carriers (Table 1). There were no between-group differences on the other cognitive measures.

Hippocampal volume

Compared with non-carriers, *APOE* ε4-carriers exhibited significantly smaller volumes in the left hippocampus [carriers versus non-carriers: 2085 ± 351 mm^3 versus 2384 ± 402 mm^3; $t(36) = 2.188$, $p = 0.035$]. There was no significant difference in the right hippocampus between the two groups [2035 ± 373 mm^3 versus 2293 ± 350 mm^3; $t(36) = 1.927$, $p = 0.062$]. When corrected for additional individual brain size (calculated by total voxel number within individual brain masks multiplied by the voxel volume), the results demonstrated little change ($p = 0.048$ for the left hippocampus and $p = 0.061$ for the right hippocampus).

Hippocampal ALFF and COSLOF

Compared with non-carriers, *APOE* ε4-carriers exhibited significantly lower ALFF for the left hippocampus [$t(36) = 2.414$, $p = 0.021$] and

non-significant differences for the right hippocampus [t(36) = 1.409, $p = 0.168$]. After further regressing out the hippocampal volume, we observed similar results ($p = 0.034$ for the left hippocampus and $p = 0.203$ for the right hippocampus). There were no significant between-group differences in COSLOF within the hippocampus independent of correction for the additional hippocampal volume (all $p > 0.30$). These results of functional analyses were little changed after correcting for the effects of total brain volume.

Hippocampal functional connectivity

Within the *APOE* ε4 non-carriers group, the left hippocampus showed extensive RSFCs with the frontal, parietal, occipital, and temporal regions (Fig. 2A). In contrast, within the *APOE* ε4-carriers group, the left hippocampus showed a much more restricted RSFC pattern that was primarily located in the temporal lobe and limbic system (Fig. 2A). Notably, the within-group RSFC pattern of the right hippocampus was very similar to that of the left hippocampus (Fig. 2A, B). Subsequent between-group comparisons revealed decreased RSFCs in the *APOE* ε4-carriers compared with the non-carriers. Specifically, the decreased RSFCs in the left hippocampus were mainly involved in several default-mode regions including the right medial prefrontal cortex (MPFC), middle frontal gyrus (MFG), left supramarginal gyrus, and left inferior temporal gyrus (ITG) (Table 2 and Fig. 2A). However, for the right hippocampus, a spatially more extensive and inter-hemispheric symmetrical pattern of decreased RSFCs was identified that was also mainly located in the default-mode network, such as the bilateral MPFC/MFG, posterior cingulate gyrus (PCC), ITG, and middle temporal gyrus (MTG) (Table 3 and Fig. 2B). In addition, the *APOE* ε4-carriers also showed decreased RSFCs in the right hippocampus with the bilateral middle occipital gyrus and putamen. However, no regions showed increased RSFC with the bilateral hippocampi in the *APOE* ε4-carriers.

Correlation analysis

No significant correlations were found between the hippocampus-related neuroimaging measures

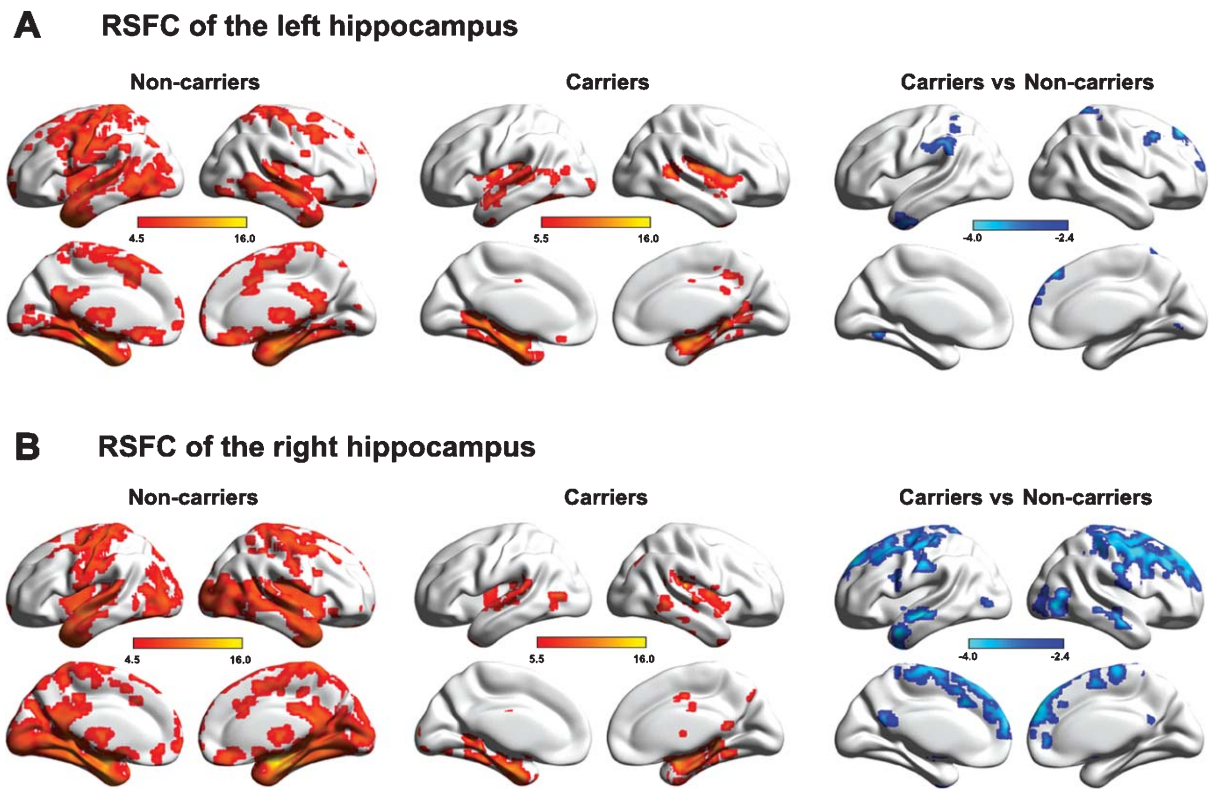

Fig. 2. Within-group RSFC patterns and between-group RSFC differences in the bilateral hippocampi. A) Left hippocampus. B) Right hippocampus. The color bars represent the T scores. The results were mapped onto the brain surface using the BrainNet viewer (http://www.nitrc.org/projects/bnv) [101].

Table 2
Regions showing decreased RSFC with the left hippocampus in *APOE ε4*-carriers compared to non-carriers

Brain regions	BA	Vol (mm^3)	MNI coordinate (mm)			Maximum T
			X	Y	Z	
Right MPFC	9/44	1998	15	39	48	−4.03
Left SMG/IPL	2/40	1107	−60	−42	42	−3.60
Right MFG	8/9	972	36	33	48	−3.44
Right LING	17/18	810	3	−72	−3	−3.32
Left LING/PLC	18/19	1053	−24	−60	−90	−3.31
Right MPFC	10	1134	15	54	18	−3.29
Left IPL	40	810	−45	−42	51	−3.10
Right PoCG	2/5/7	1026	18	−51	72	−3.09
Left ITG	20	999	−48	0	−36	−2.99

BA, Brodmann's area; Vol, cluster volume; X, Y, Z, coordinates of peak locations; Maximum T, t statistical values of peak locations. MPFC, medial prefrontal gyrus; ITG, inferior temporal gyrus; LING, lingual gyrus; PLC, posterior lobe of cerebellum; SMG, supramarginal gyrus; IPL, inferior parietal lobule; MFG, middle frontal gyrus; PoCG, postcentral gyrus.

Table 3
Regions showing decreased RSFC with the right hippocampus in *APOE ε4*-carriers compared to non-carriers

Brain regions	BA	Vol (mm^3)	MNI coordinate (mm)			Maximum T
			X	Y	Z	
Bilateral MPFC/MFG/PreCG/PoCG	3/4/6/8/9/10/32/44/46	84132	18	45	24	−5.20
Left MTG/ITG	21/21/22	4590	−60	−21	−9	−4.52
Right MTG/MOG	19/37	3186	51	−69	0	−4.45
Left PUT	25/34/48	1674	−15	6	−6	−4.36
Right MTG/ITG	21/22	2592	60	−3	−15	−4.12
Right LN/PUT	25/48	1026	12	3	−9	−4.11
Left SMG/PreCG/PoCG	3/43/48	1215	−60	−18	42	−3.84
Left MOG	19/37	1161	−54	−72	0	−3.75
Left IFG	44	1026	−51	9	27	−3.63
Left PCC	23/30	2376	−3	−57	21	−3.49
Right MOG	18/19	918	39	−84	12	−3.14

BA, Brodmann's area; Vol, cluster volume; X, Y, Z, coordinates of peak locations; Maximum T, t statistical values of peak locations. MPFC, medial prefrontal gyrus; MFG, middle frontal gyrus; PreCG, precentral gyrus; PoCG, postcentral gyrus; MTG, middle temporal gyrus; ITG, inferior temporal gyrus; MOG, middle occipital gyrus; PUT, putamen; LN, lentiform nucleus; SMG, supramarginal gyrus; IFG, inferior frontal gyrus; PCC, posterior cingulate gyrus.

(structural volume and functional ALFF, COSLOF, and RSFC) and neuropsychological data (all $ps > 0.05$).

Confounding effects of head motion

Several recent R-fMRI studies have suggested that head motion during scanning might affect RSFC analysis results [57–59]. For the current dataset, we compared the maximum, root mean square and frame-wise displacement of head motion and found no significant differences between groups (all $p > 0.107$). After treating these confounding factors as extra covariates for functional neuroimaging analyses (ALFF, COSLOF, and RSFC), these results remain little changed. Lastly, we employed another scrubbing approach to censor "bad" volumes based on a criterion of framewise displacement > 0.2 mm [57] and found that the results of RSFC analyses largely preserved (Fig. 3). These validation analyses indicate that our main findings were not affected by head motion.

DISCUSSION

The present study investigated the effects of the *APOE* genotype on cognitive performance, hippocampal volume, and functional architecture in the very mild AD patients. Consistent with our hypothesis, *APOE ε4* carriers in patient with AD showed impaired recognition performance and smaller left hippocampal volume as compared to non-carriers. Further, we demonstrated that *APOE* genotype modulates hippocampal function, including ALFF in the left hippocampus and inter-regional functional connectivity primarily in the default-mode regions.

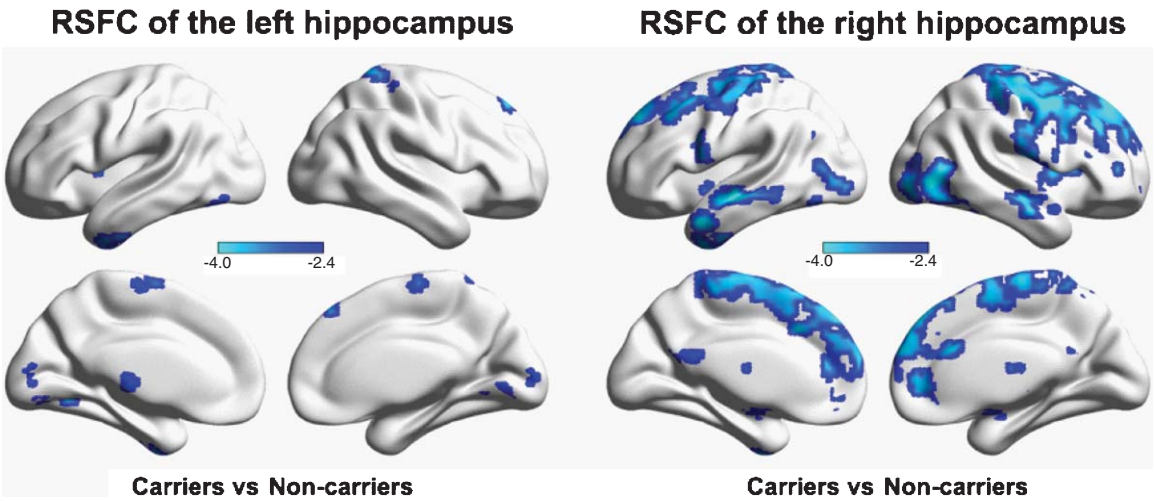

Fig. 3. The effects of head motion on between-group RSFC differences in the bilateral hippocampi. A "scrubbing" procedure in the imaging preprocessing was first used to censor "bad" volumes based on a criterion of framewise displacement [57], and between-group RSFC differences in the bilateral hippocampi were re-analyzed.

In the present study, we found that compared with non-carriers, *APOE* ε4 carriers had greater memory deficits in AD patients, which was largely consistent with previous studies [4–11]. Disruptions in the episodic memory system have been previously demonstrated to be the earliest signs and symptoms of AD [60]. Episodic memory consists of two major subsystems: recall and recognition [61]. Compared with free recall, recognition tasks may rely less on strategic memory and produce lower search demands [62]. Specifically, Walhovd et al. [62] showed that *APOE* was only significantly related to recognition in patients with AD, which provided important support for our findings. Previous studies suggest that delayed recall is associated with hippocampal atrophy [63, 64]. However, we found that the differences in episodic memory were significant only for recognition, not for delayed recall. Previous studies show that recognition memory is also related to the region of hippocampus. Recognition memory is widely viewed as being composed of at least two processes: recollection and familiarity by the dual-process theories, and they can be mapped onto the hippocampus and perirhinal cortex, respectively [65–67]. An alternative theory is that the hippocampus supports both processes of recognition memory [68–70]. Given that the seed-based connectivity analysis used here was performed only on the hippocampus, further works will be necessary to examine the connectivity patterns of hippocampus and perirhinal cortex. These would be helpful for clarifying the brain connectivity basis underlying the different effects of recognition and recall.

In this study, we also observed a trend toward greater impairment of executive function, namely verbal fluency (the total output of animal names) in *APOE* ε4 non-carriers. This finding was consistent with several previous studies demonstrating that non-carriers performed more poorly than carriers on non-memory performance [4, 6, 10, 11]. Notably, in this study, *APOE* ε4-carriers performed poorly in the early item generation time periods (0–15 s) but not in the late generation periods (46–60 s). Successful performance on semantic category fluency is thought to depend on executive functions and the ability to initiate systematic search and retrieval data from the lexicon or the semantic memory system [50, 52, 53]. Different subject groups produced different correct answers in the initial phases (e.g., children, young and old adults; patients with schizophrenia, aphasia, depression, and dementia) [71], which was considered to be related to automatic retrieval from the available word store, and the number decreased in the subsequent time intervals due to the fact that the word store was exhausted and search became more effortful [50, 52]. The performance of the subsequent time intervals in the verbal fluency was actually more dependent on executive functioning [52]. The results of verbal fluency as a function time in this study were consistent with previous studies [50, 52–54]. Memory impairment might underlie the degraded semantic store [50, 72] and might explain the poorer semantic memory performance in initiating the fluency task in *APOE* ε4-carriers. The mechanism of more pronounced performance on the fluency task in the late period in *APOE* ε4-carriers may

correlate with the notion that the non-carriers showed greater difficulties on tasks of the executive function [51].

The theory of cognitive reserve [73] suggests that individuals with higher levels of ability have greater neuronal changes or more efficient neuronal network. Education level has been used as a substitute for cognitive reserve [74–79], however, there was no significant difference in education between the *APOE* ε4-carriers and non-carriers in the present study. Furthermore, we compared the cognitive performance between the two groups after further adjusting for gender, age, and education, and found that the results were little changed. Notably, several previous studies supported that the premorbid IQ may be a better proxy of cognitive reserve [73, 76] but was not examined in the pilot work. Further works will be important to include the premorbid IQ measurement.

Our finding of *APOE* ε4-related hippocampal atrophy corroborates the results of previous structural imaging studies demonstrating smaller hippocampal volume in *APOE* ε4 carriers with AD [15–18]. However, several other studies did not detect any pronounced ε4-related hippocampal volumetric difference in AD [19, 20]; thus the modulation of the *APOE* ε4 allele on anatomical phenotypic expression is inconclusive in AD. Several potential confounding factors, specifically demographic characteristics and disease-relevant variables such as the onset age of symptom, disease duration, and severity, may partially contribute to the discrepancy of earlier studies. Another speculative interpretation of this discrepancy might be attributed to different sampling and analysis approaches. In studies performed by Jack et al. [19] and Drzezga et al. [20] patients with the *APOE* ε2 allele were included. Given the potential protective effect of the *APOE* ε2 allele on AD risk [43], participants with the *APOE* ε2 allele may lessen the ability to detect hippocampal volumetric differences between *APOE* ε4-carriers and non-carriers. In this study, we observed a decreased hippocampal volume in *APOE* ε4-carriers compared to non-carriers after removal of the effects of several confounding factors including age, gender, education, and severity of dementia. Moreover, subjects with only the *APOE* genotype of ε3/ε4, ε4/ε4, and ε3/ε3 were included. Thus, our findings indicated that the *APOE* ε4 allele decreased the hippocampal volume in AD.

With respect to hippocampal functioning, we observed a decreased local spontaneous activity (i.e., ALFF) in the left hippocampus in *APOE* ε4-carriers. Several previous R-fMRI studies have shown that the hippocampus displayed abnormal regional spontaneous activities in patients with AD [21, 23]. Moreover, under cognitive engagement of a memory encoding task, Adamson and colleagues [80] reported that *APOE* ε4-carriers who were healthy elderly subjects exhibited reduced activation in the hippocampus compared to non-carriers. In this study, we did not observe significant difference in ALFF in the right hippocampus between two groups. These results suggest that the local spontaneous activity in the left hippocampus might be more vulnerable to *APOE* ε4 than that in the right hippocampus. This speculation is compatible with previous morphological studies in AD [81–83]. Together, we provided further evidence for the effect of the *APOE* ε4 allele on the level of spontaneous or intrinsic brain activity in the hippocampus. Although a previous R-fMRI study showed AD-related reduction in the COSLOF index in the hippocampus [22], we did not find significant differences in this index between *APOE* ε4-carriers and non-carriers. This result indicates that the intra-regional functional synchronization within the hippocampus was not significantly influenced by the *APOE* ε4 allele.

In addition to local functional properties, we also observed *APOE* related alterations in hippocampal RSFC. Compared to non-carriers, *APOE* ε4-carriers showed decreased hippocampal RSFC predominately in several components of the default-mode network (DMN) (PCC, MPFC, MFG, MTG, and ITG). The DMN consists of a specific set of regions that routinely display high levels of activity during rest but decreased activity during the performance of attention-demanding cognitive tasks [84, 85]. Converging evidence suggests that the DMN regions are important functional hubs of the brain and show preferential vulnerability to AD pathology [86–88]. Several previous R-fMRI studies have also consistently reported intrinsic RSFC changes in the DMN in AD patients [24, 26]. Furthermore, accumulating evidence from healthy adults has demonstrated the effect of the *APOE* ε4 allele on DMN integrity [36–39]. A previous study proposed the model of disruption of DMN connectivity without amyloid-β deposition [89] that abnormally increased network connectivity in young *APOE* ε4 carriers [37] might be followed by subsequent decreases of network interconnectivity in elderly *APOE* ε4 carriers [38]. Several studies reported initially increased functional connectivity as a consequence of Aβ-induced hyperexcitability of neurons, representing a transient phase of impending breakdown of neuronal networks and turning into activation deficit with further increase in pathology [90–92].

One interesting discrepancy was observed between the increased connectivity in regions associated with successful memory performance (the precuneus and the gyrus rectus in the medial orbitofrontal cortex) [39] versus decreased connectivity in these regions (amygdala, hippocampus, PCC, and precuneus) [36] in elderly APOE ε4 carriers. The results were supported by another study reporting that increased Aβ deposition was associated with decreased DMN connectivity in the same regions implicated in episodic memory processing (posteromedial cortex, ventral medial prefrontal cortex, and angular gyrus) in elderly adults [91]. Here, our results provided the first evidence for the modulation of the *APOE* genotype on DMN connectivity in AD. The primary biological effect of *APOE* ε4 appears to increase Aβ accumulation [93, 94], which potentially affect soluble Aβ metabolism [95]. This increase is spatially specific predominantly in the medial prefrontal and parietal cortices [20, 96], overlapping largely with the main components of the DMN. The elevated amyloid burden was found to be related to a disrupted connectivity in these corresponding regions [97, 98], potentially via a possible mechanism of synaptic loss [99]. Thus, the disrupted DMN connectivity in *APOE* ε4 carriers might be an outcome of increased amyloid-β burden. Given the implications of the DMN in episodic memory processing [26], we proposed that the disrupted hippocampus-DMN RSFC might underlie the deficits of episodic memory performance in *APOE* ε4 carriers compared to non-carriers as observed in the current study. In addition, we observed a visual asymmetrical effect of the *APOE* ε4 allele on hippocampal RSFC in which the right hippocampus was more severely disrupted. Thus, investigation of the modulation of *APOE* ε4 allele on brain structure and functioning from the perspective of asymmetry will be an interesting topic in future studies.

Several issues need to be further addressed. First, the sample size between carriers and non-carriers was not balanced. The imbalance of sample size has been observed in numerous genetic neuroimaging studies [19, 38, 39, 100]. Given the relative small simple size in this study, we did not explore the effects using bootstrapping approaches. This small sample also made us not examine the dose effect of ε4 genotype. We are continuing to collect more AD patients with different *APOE* genes, and a larger sample is expectable to address these issues in future. Second, we exclusively studied structural and functional features of the bilateral hippocampi by manually tracing their boundaries. However, such an approach cannot be used for hippocampal subregions due to that the structural images collected in this study did not have enough spatial resolution. Further studies are warranted to assess the *APOE* ε4-related effects on structural and functional characteristics of hippocampal subregions in AD using high-resolution MRI techniques. Lastly, major concerns have been raised about the effects of head motion on R-fMRI analysis [57–59]. In this study, we used both regression and scrubbing methods to validate our results, and the main findings were preserved. Nonetheless, the effects of residual motion might exist, which needs to be further validated by advanced head motion approaches developed in the future.

Taken together, these pilot data suggest that compared with non-carriers, *APOE* ε4-carriers exhibited episodic memory impairment, left hippocampal atrophy, and functional connectivity disruption in patients with AD. Thus, these results provided preliminary evidence for the possible effects of *APOE* on the neurocognitive phenotype and the functional integrity of the hippocampus in AD. These findings need to be confirmed in future studies using large sample data.

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REFERENCES

[1] Corder EH, Saunders AM, Strittmatter WJ, Schmechel DE, Gaskell PC, Small GW, Roses AD, Haines JL, Pericak-Vance MA (1993) Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science* **261**, 921-923.

[2] Strittmatter WJ, Saunders AM, Schmechel D, Pericak-Vance M, Enghild J, Salvesen GS, Roses AD (1993) Apolipoprotein E: High-avidity binding to beta-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. *Proc Natl Acad Sci U S A* **90**, 1977-1981.

[3] Roses AD (1996) Apolipoprotein E alleles as risk factors in Alzheimer's disease. *Annu Rev Med* **47**, 387-400.

[4] Lehtovirta M, Soininen H, Helisalmi S, Mannermaa A, Helkala EL, Hartikainen P, Hanninen T, Ryynanen M, Riekkinen PJ (1996) Clinical and neuropsychological characteristics in familial and sporadic Alzheimer's disease: Relation to apolipoprotein E polymorphism. *Neurology* **46**, 413-419.

[5] Smith GE, Bohac DL, Waring SC, Kokmen E, Tangalos EG, Ivnik RJ, Petersen RC (1998) Apolipoprotein E genotype influences cognitive 'phenotype' in patients with Alzheimer's disease but not in healthy control subjects. *Neurology* **50**, 355-362.

[6] Johnson JK, Head E, Kim R, Starr A, Cotman CW (1999) Clinical and pathological evidence for a frontal variant of Alzheimer disease. *Arch Neurol* **56**, 1233-1239.

[7] Wilson RS, Bienias JL, Berry-Kravis E, Evans DA, Bennett DA (2002) The apolipoprotein E epsilon 2 allele and decline in episodic memory. *J Neurol Neurosurg Psychiatry* **73**, 672-677.

[8] Marra C, Bizzarro A, Daniele A, De Luca L, Ferraccioli M, Valenza A, Brahe C, Tiziano FD, Gainotti G, Masullo C (2004) Apolipoprotein E epsilon4 allele differently affects the patterns of neuropsychological presentation in early- and late-onset Alzheimer's disease patients. *Dement Geriatr Cogn Disord* **18**, 125-131.

[9] van der Flier WM, Schoonenboom SN, Pijnenburg YA, Fox NC, Scheltens P (2006) The effect of APOE genotype on clinical phenotype in Alzheimer disease. *Neurology* **67**, 526-527.

[10] van der Vlies AE, Pijnenburg YA, Koene T, Klein M, Kok A, Scheltens P, van der Flier WM (2007) Cognitive impairment in Alzheimer's disease is modified by APOE genotype. *Dement Geriatr Cogn Disord* **24**, 98-103.

[11] Wolk DA, Dickerson BC (2010) 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* **107**, 10256-10261.

[12] Pievani M, Rasser PE, Galluzzi S, Benussi L, Ghidoni R, Sabattoli F, Bonetti M, Binetti G, Thompson PM, Frisoni GB (2009) Mapping the effect of APOE epsilon4 on gray matter loss in Alzheimer's disease *in vivo*. *Neuroimage* **45**, 1090-1098.

[13] Hyman BT, Van Hoesen GW, Damasio AR, Barnes CL (1984) Alzheimer's disease: Cell-specific pathology isolates the hippocampal formation. *Science* **225**, 1168-1170.

[14] Ghebremedhin E, Schultz C, Braak E, Braak H (1998) High frequency of apolipoprotein E epsilon4 allele in young individuals with very mild Alzheimer's disease-related neurofibrillary changes. *Exp Neurol* **153**, 152-155.

[15] Lehtovirta M, Laakso MP, Soininen H, Helisalmi S, Mannermaa A, Helkala EL, Partanen K, Ryynanen M, Vainio P, Hartikainen P, et al. (1995) Volumes of hippocampus, amygdala and frontal lobe in Alzheimer patients with different apolipoprotein E genotypes. *Neuroscience* **67**, 65-72.

[16] Geroldi C, Laakso MP, DeCarli C, Beltramello A, Bianchetti A, Soininen H, Trabucchi M, Frisoni GB (2000) Apolipoprotein E genotype and hippocampal asymmetry in Alzheimer's disease: A volumetric MRI study. *J Neurol Neurosurg Psychiatry* **68**, 93-96.

[17] Hashimoto M, Yasuda M, Tanimukai S, Matsui M, Hirono N, Kazui H, Mori E (2001) Apolipoprotein E epsilon 4 and the pattern of regional brain atrophy in Alzheimer's disease. *Neurology* **57**, 1461-1466.

[18] Agosta F, Vossel KA, Miller BL, Migliaccio R, Bonasera SJ, Filippi M, Boxer AL, Karydas A, Possin KL, Gorno-Tempini ML (2009) Apolipoprotein E epsilon4 is associated with disease-specific effects on brain atrophy in Alzheimer's disease and frontotemporal dementia. *Proc Natl Acad Sci U S A* **106**, 2018-2022.

[19] Jack CR Jr, Petersen RC, Xu YC, O'Brien PC, Waring SC, Tangalos EG, Smith GE, Ivnik RJ, Thibodeau SN, Kokmen E (1998) Hippocampal atrophy and apolipoprotein E genotype are independently associated with Alzheimer's disease. *Ann Neurol* **43**, 303-310.

[20] Drzezga A, Grimmer T, Henriksen G, Muhlau M, Perneczky R, Miederer I, Praus C, Sorg C, Wohlschlager A, Riemenschneider M, Wester HJ, Foerstl H, Schwaiger M, Kurz A (2009) Effect of APOE genotype on amyloid plaque load and gray matter volume in Alzheimer disease. *Neurology* **72**, 1487-1494.

[21] Wang Z, Yan C, Zhao C, Qi Z, Zhou W, Lu J, He Y, Li K (2011) Spatial patterns of intrinsic brain activity in mild cognitive impairment and Alzheimer's disease: A resting-state functional MRI study. *Hum Brain Mapp* **32**, 1720-1740.

[22] Li SJ, Li Z, Wu G, Zhang MJ, Franczak M, Antuono PG (2002) Alzheimer disease: Evaluation of a functional MR imaging index as a marker. *Radiology* **225**, 253-259.

[23] He Y, Wang L, Zang Y, Tian L, Zhang X, Li K, Jiang T (2007) Regional coherence changes in the early stages of Alzheimer's disease: A combined structural and resting-state functional MRI study. *Neuroimage* **35**, 488-500.

[24] Wang L, Zang Y, He Y, Liang M, Zhang X, Tian L, Wu T, Jiang T, Li K (2006) Changes in hippocampal connectivity in the early stages of Alzheimer's disease: Evidence from resting state fMRI. *Neuroimage* **31**, 496-504.

[25] Allen G, Barnard H, McColl R, Hester AL, Fields JA, Weiner MF, Ringe WK, Lipton AM, Brooker M, McDonald E, Rubin CD, Cullum CM (2007) Reduced hippocampal functional connectivity in Alzheimer disease. *Arch Neurol* **64**, 1482-1487.

[26] Greicius MD, Srivastava G, Reiss AL, Menon V (2004) Default-mode network activity distinguishes Alzheimer's disease from healthy aging: Evidence from functional MRI. *Proc Natl Acad Sci U S A* **101**, 4637-4642.

[27] Biswal B, Yetkin FZ, Haughton VM, Hyde JS (1995) Functional connectivity in the motor cortex of resting human brain using echo-planar MRI. *Magn Reson Med* **34**, 537-541.

[28] Fox MD, Raichle ME (2007) Spontaneous fluctuations in brain activity observed with functional magnetic resonance imaging. *Nat Rev Neurosci* **8**, 700-711.

[29] Logothetis NK, Pauls J, Augath M, Trinath T, Oeltermann A (2001) Neurophysiological investigation of the basis of the fMRI signal. *Nature* **412**, 150-157.

[30] Qi Z, Wu X, Wang Z, Zhang N, Dong H, Yao L, Li K (2010) Impairment and compensation coexist in amnestic MCI default mode network. *Neuroimage* **50**, 48-55.

[31] Machulda MM, Ward HA, Borowski B, Gunter JL, Cha RH, O'Brien PC, Petersen RC, Boeve BF, Knopman D, Tang-Wai DF, Ivnik RJ, Smith GE, Tangalos EG, Jack CR Jr (2003) Comparison of memory fMRI response among normal, MCI, and Alzheimer's patients. *Neurology* **61**, 500-506.

[32] Xi Q, Zhao X, Wang P, Guo Q, Jiang H, Cao X, He Y, Yan C (2012) Spontaneous brain activity in mild cognitive impairment revealed by amplitude of low-frequency fluctu-

ation analysis: A resting-state fMRI study. *Radiol Med* **117**, 865-871.

[33] Liu X, Wang S, Zhang X, Wang Z, Tian X, He Y (2014) Abnormal amplitude of low-frequency fluctuations of intrinsic brain activity in Alzheimer's disease. *J Alzheimers Dis* **40**, 387-397.

[34] Han Y, Wang J, Zhao Z, Min B, Lu J, Li K, He Y, Jia J (2011) Frequency-dependent changes in the amplitude of low-frequency fluctuations in amnestic mild cognitive impairment: A resting-state fMRI study. *Neuroimage* **55**, 287-295.

[35] Zang YF, He Y, Zhu CZ, Cao QJ, Sui MQ, Liang M, Tian LX, Jiang TZ, Wang YF (2007) Altered baseline brain activity in children with ADHD revealed by resting-state functional MRI. *Brain Dev* **29**, 83-91.

[36] Fleisher AS, Sherzai A, Taylor C, Langbaum JB, Chen K, Buxton RB (2009) Resting-state BOLD networks versus task-associated functional MRI for distinguishing Alzheimer's disease risk groups. *Neuroimage* **47**, 1678-1690.

[37] Filippini N, MacIntosh BJ, Hough MG, Goodwin GM, Frisoni GB, Smith SM, Matthews PM, Beckmann CF, Mackay CE (2009) Distinct patterns of brain activity in young carriers of the APOE-epsilon4 allele. *Proc Natl Acad Sci U S A* **106**, 7209-7214.

[38] Sheline YI, Morris JC, Snyder AZ, Price JL, Yan Z, D'Angelo G, Liu C, Dixit S, Benzinger T, Fagan A, Goate A, Mintun MA (2010) APOE4 allele disrupts resting state fMRI connectivity in the absence of amyloid plaques or decreased CSF Abeta42. *J Neurosci* **30**, 17035-17040.

[39] Westlye ET, Lundervold A, Rootwelt H, Lundervold AJ, Westlye LT (2011) Increased hippocampal default mode synchronization during rest in middle-aged and elderly APOE epsilon4 carriers: Relationships with memory performance. *J Neurosci* **31**, 7775-7783.

[40] Morris JC (1993) The Clinical Dementia Rating (CDR): Current version and scoring rules. *Neurology* **43**, 2412-2414.

[41] World Health Organization (1999) *International Classification of Diseases, 10th Revision (ICD-10)*. World Health Organization, Geneva.

[42] McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM (1984) 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* **34**, 939-944.

[43] Talbot C, Lendon C, Craddock N, Shears S, Morris JC, Goate A (1994) Protection against Alzheimer's disease with apoE epsilon 2. *Lancet* **343**, 1432-1433.

[44] Wenham PR, Price WH, Blandell G (1991) Apolipoprotein E genotyping by one-stage PCR. *Lancet* **337**, 1158-1159.

[45] Teng EL, Hasegawa K, Homma A, Imai Y, Larson E, Graves A, Sugimoto K, Yamaguchi T, Sasaki H, Chiu D, et al. (1994) The Cognitive Abilities Screening Instrument (CASI): A practical test for cross-cultural epidemiological studies of dementia. *Int Psychogeriatr* **6**, 45-58; discussion 62.

[46] Lin KN, Wang PN, Liu HC, Teng EL (2012) Cognitive Abilities Screening Instrument, Chinese Version 2.0 (CASI C-2.0): Administration and clinical application. *Acta Neurol Taiwan* **21**, 180-189.

[47] Dick MB, Teng EL, Kempler D, Davis DS, Taussig IM (2002) The cross-cultural neuropsychological test battery (CCNB): Effects of age, education, ethnicity, and cognitive status on performance. In *Minority and Cross-cultural Aspects of Neuropsychological Assessment*, Ferraro FR, ed. Swets & Zeitlinger B.V., Lisse, pp. 17-41.

[48] Kempler D, Teng EL, Taussig M, Dick MB (2010) The common objects memory test (COMT): A simple test with cross-cultural applicability. *J Int Neuropsychol Soc* **16**, 537-545.

[49] Fillenbaum GG, van Belle G, Morris JC, Mohs RC, Mirra SS, Davis PC, Tariot PN, Silverman JM, Clark CM, Welsh-Bohmer KA, Heyman A (2008) Consortium to Establish a Registry for Alzheimer's Disease (CERAD): The first twenty years. *Alzheimers Dement* **4**, 96-109.

[50] Crowe SF (1998) Decrease in performance on the verbal fluency test as a function of time: Evaluation in a young healthy sample. *J Clin Exp Neuropsychol* **20**, 391-401.

[51] Fernaeus SE, Almkvist O (1998) Word production: Dissociation of two retrieval modes of semantic memory across time. *J Clin Exp Neuropsychol* **20**, 137-143.

[52] Hurks PP, Vles JS, Hendriksen JG, Kalff AC, Feron FJ, Kroes M, van Zeben TM, Steyaert J, Jolles J (2006) Semantic category fluency versus initial letter fluency over 60 seconds as a measure of automatic and controlled processing in healthy school-aged children. *J Clin Exp Neuropsychol* **28**, 684-695.

[53] Lee SH, Kim H, Kim J, Yoon JH, Kim SR (2014) Initial phase performance in a 30-s verbal fluency task as being reflective of aging effect. *Geriatr Gerontol Int*. doi: 10.1111/ggi.12284

[54] Hall JR, Harvey M, Vo HT, O'Bryant SE (2011) Performance on a measure of category fluency in cognitively impaired elderly. *Neuropsychol Dev Cogn B Aging Neuropsychol Cogn* **18**, 353-361.

[55] Wang H, Shu L, Xie J, Zhang H, Zhang D (2004) Diagnostic utility of neuropsychological performance and quantitative MRI-based measurement in Alzheimer disease. *Alzheimer Dis Assoc Disord* **18**, 163-170.

[56] Ledberg A, Akerman S, Roland PE (1998) Estimation of the probabilities of 3D clusters in functional brain images. *Neuroimage* **8**, 113-128.

[57] Power JD, Barnes KA, Snyder AZ, Schlaggar BL, Petersen SE (2012) Spurious but systematic correlations in functional connectivity MRI networks arise from subject motion. *Neuroimage* **59**, 2142-2154.

[58] Satterthwaite TD, Wolf DH, Loughead J, Ruparel K, Elliott MA, Hakonarson H, Gur RC, Gur RE (2012) Impact of in-scanner head motion on multiple measures of functional connectivity: Relevance for studies of neurodevelopment in youth. *Neuroimage* **60**, 623-632.

[59] Van Dijk KR, Sabuncu MR, Buckner RL (2012) The influence of head motion on intrinsic functional connectivity MRI. *Neuroimage* **59**, 431-438.

[60] Gold CA, Budson AE (2008) Memory loss in Alzheimer's disease: Implications for development of therapeutics. *Expert Rev Neurother* **8**, 1879-1891.

[61] Nilsson LG, Adolfsson R, Backman L, Cruts M, Nyberg L, Small BJ, Van Broeckoven C (2006) The influence of APOE status on episodic and semantic memory: Data from a population-based study. *Neuropsychology* **20**, 645-657.

[62] Walhovd KB, Fjell AM, Dale AM, McEvoy LK, Brewer J, Karow DS, Salmon DP, Fennema-Notestine C (2010) Multimodal imaging predicts memory performance in normal aging and cognitive decline. *Neurobiol Aging* **31**, 1107-1121.

[63] Chen KH, Chuah LY, Sim SK, Chee MW (2010) Hippocampal region-specific contributions to memory performance in normal elderly. *Brain Cogn* **72**, 400-407.

[64] Mortimer JA, Gosche KM, Riley KP, Markesbery WR, Snowdon DA (2004) Delayed recall, hippocampal volume

and Alzheimer neuropathology: Findings from the Nun Study. *Neurology* **62**, 428-432.

[65] Brown MW, Aggleton JP (2001) Recognition memory: What are the roles of the perirhinal cortex and hippocampus? *Nat Rev Neurosci* **2**, 51-61.

[66] Winters BD, Saksida LM, Bussey TJ (2008) Object recognition memory: Neurobiological mechanisms of encoding, consolidation and retrieval. *Neurosci Biobehav Rev* **32**, 1055-1070.

[67] Staresina BP, Fell J, Dunn JC, Axmacher N, Henson RN (2013) Using state-trace analysis to dissociate the functions of the human hippocampus and perirhinal cortex in recognition memory. *Proc Natl Acad Sci U S A* **110**, 3119-3124.

[68] Squire LR, Wixted JT, Clark RE (2007) Recognition memory and the medial temporal lobe: A new perspective. *Nat Rev Neurosci* **8**, 872-883.

[69] Wixted JT, Squire LR (2011) The medial temporal lobe and the attributes of memory. *Trends Cogn Sci* **15**, 210-217.

[70] Dede AJ, Wixted JT, Hopkins RO, Squire LR (2013) Hippocampal damage impairs recognition memory broadly, affecting both parameters in two prominent models of memory. *Proc Natl Acad Sci U S A* **110**, 6577-6582.

[71] Raboutet C, Sauzeon H, Corsini MM, Rodrigues J, Langevin S, N'kaoua B (2010) Performance on a semantic verbal fluency task across time: Dissociation between clustering, switching, and categorical exploitation processes. *J Clin Exp Neuropsychol* **32**(3), 268–280.

[72] Fagundo AB, Lopez S, Romero M, Guarch J, Marcos T, Salamero M (2008) Clustering and switching in semantic fluency: Predictors of the development of Alzheimer's disease. *Int J Geriatr Psychiatry* **23**, 1007-1013.

[73] Stern Y (2002) What is cognitive reserve? Theory and research application of the reserve concept. *J Int Neuropsychol Soc* **8**, 448-460.

[74] Brayne C, Beardsall L (1990) Estimation of verbal intelligence in an elderly community: An epidemiological study using NART. *Br J Clin Psychol* **29**(Pt 2), 217-223.

[75] Amieva H, Jacqmin-Gadda H, Orgogozo JM, Le Carret N, Helmer C, Letenneur L, Barberger-Gateau P, Fabrigoule C, Dartigues JF (2005) The 9 year cognitive decline before dementia of the Alzheimer type: A prospective population-based study. *Brain* **128**, 1093-1101.

[76] Scarmeas N, Albert SM, Manly JJ, Stern Y (2006) Education and rates of cognitive decline in incident Alzheimer's disease. *J Neurol Neurosurg Psychiatry* **77**, 308-316.

[77] Bracco L, Piccini C, Baccini M, Bessi V, Biancucci F, Nacmias B, Bagnoli S, Sorbi S (2007) Pattern and progression of cognitive decline in Alzheimer's disease: Role of premorbid intelligence and ApoE genotype. *Dement Geriatr Cogn Disord* **24**, 483-491.

[78] Starr JM, Lonie J (2008) Estimated pre-morbid IQ effects on cognitive and functional outcomes in Alzheimer disease: A longitudinal study in a treated cohort. *BMC Psychiatry* **8**, 27.

[79] Alves L, Simoes MR, Martins C (2012) The estimation of premorbid intelligence levels among Portuguese speakers: The Irregular Word Reading Test (TeLPI). *Arch Clin Neuropsychol* **27**, 58-68.

[80] Adamson MM, Hutchinson JB, Shelton AL, Wagner AD, Taylor JL (2011) Reduced hippocampal activity during encoding in cognitively normal adults carrying the APOE varepsilon4 allele. *Neuropsychologia* **49**, 2448-2455.

[81] Muller MJ, Greverus D, Dellani PR, Weibrich C, Wille PR, Scheurich A, Stoeter P, Fellgiebel A (2005) Functional implications of hippocampal volume and diffusivity in mild cognitive impairment. *Neuroimage* **28**, 1033-1042.

[82] Thompson PM, Hayashi KM, De Zubicaray GI, Janke AL, Rose SE, Semple J, Hong MS, Herman DH, Gravano D, Doddrell DM, Toga AW (2004) Mapping hippocampal and ventricular change in Alzheimer disease. *Neuroimage* **22**, 1754-1766.

[83] Morra JH, Tu Z, Apostolova LG, Green AE, Avedissian C, Madsen SK, Parikshak N, Toga AW, Jack CR Jr, Schuff N, Weiner MW, Thompson PM (2009) Automated mapping of hippocampal atrophy in 1-year repeat MRI data from 490 subjects with Alzheimer's disease, mild cognitive impairment, and elderly controls. *Neuroimage* **45**, S3-15.

[84] Raichle ME, MacLeod AM, Snyder AZ, Powers WJ, Gusnard DA, Shulman GL (2001) A default mode of brain function. *Proc Natl Acad Sci U S A* **98**, 676-682.

[85] Buckner RL, Andrews-Hanna JR, Schacter DL (2008) The brain's default network: Anatomy, function, and relevance to disease. *Ann N Y Acad Sci* **1124**, 1-38.

[86] Buckner RL, Sepulcre J, Talukdar T, Krienen FM, Liu H, Hedden T, Andrews-Hanna JR, Sperling RA, Johnson KA (2009) Cortical hubs revealed by intrinsic functional connectivity: Mapping, assessment of stability, and relation to Alzheimer's disease. *J Neurosci* **29**, 1860-1873.

[87] Buckner RL, Snyder AZ, Shannon BJ, LaRossa G, Sachs R, Fotenos AF, Sheline YI, Klunk WE, Mathis CA, Morris JC, Mintun MA (2005) Molecular, structural, and functional characterization of Alzheimer's disease: Evidence for a relationship between default activity, amyloid, and memory. *J Neurosci* **25**, 7709-7717.

[88] Mintun MA, Larossa GN, Sheline YI, Dence CS, Lee SY, Mach RH, Klunk WE, Mathis CA, DeKosky ST, Morris JC (2006) [11C]PIB in a nondemented population: Potential antecedent marker of Alzheimer disease. *Neurology* **67**, 446-452.

[89] Prvulovic D, Bokde AL, Faltraco F, Hampel H (2011) Functional magnetic resonance imaging as a dynamic candidate biomarker for Alzheimer's disease. *Prog Neurobiol* **95**, 557-569.

[90] Jacobs HI, Radua J, Luckmann HC, Sack AT (2013) Meta-analysis of functional network alterations in Alzheimer's disease: Toward a network biomarker. *Neurosci Biobehav Rev* **37**, 753-765.

[91] Mormino EC, Smiljic A, Hayenga AO, Onami SH, Greicius MD, Rabinovici GD, Janabi M, Baker SL, Yen IV, Madison CM, Miller BL, Jagust WJ (2011) Relationships between beta-amyloid and functional connectivity in different components of the default mode network in aging. *Cereb Cortex* **21**, 2399-2407.

[92] Ewers M, Sperling RA, Klunk WE, Weiner MW, Hampel H (2011) Neuroimaging markers for the prediction and early diagnosis of Alzheimer's disease dementia. *Trends Neurosci* **34**, 430-442.

[93] Polvikoski T, Sulkava R, Haltia M, Kainulainen K, Vuorio A, Verkkoniemi A, Niinisto L, Halonen P, Kontula K (1995) Apolipoprotein E, dementia, and cortical deposition of beta-amyloid protein. *N Engl J Med* **333**, 1242-1247.

[94] Kim J, Basak JM, Holtzman DM (2009) The role of apolipoprotein E in Alzheimer's disease. *Neuron* **63**, 287-303.

[95] Verghese PB, Castellano JM, Garai K, Wang Y, Jiang H, Shah A, Bu G, Frieden C, Holtzman DM (2013) ApoE influences amyloid-beta (Abeta) clearance despite minimal apoE/Abeta association in physiological conditions. *Proc Natl Acad Sci U S A* **110**, E1807-E1816.

[96] Reiman EM, Chen K, Liu X, Bandy D, Yu M, Lee W, Ayutyanont N, Keppler J, Reeder SA, Langbaum JB, Alexander GE, Klunk WE, Mathis CA, Price JC, Aizenstein HJ, DeKosky ST, Caselli RJ (2009) Fibrillar amyloid-beta burden in cognitively normal people at 3 levels of genetic risk for Alzheimer's disease. *Proc Natl Acad Sci U S A* **106**, 6820-6825.

[97] Hedden T, Van Dijk KR, Becker JA, Mehta A, Sperling RA, Johnson KA, Buckner RL (2009) Disruption of functional connectivity in clinically normal older adults harboring amyloid burden. *J Neurosci* **29**, 12686-12694.

[98] Drzezga A, Becker JA, Van Dijk KR, Sreenivasan A, Talukdar T, Sullivan C, Schultz AP, Sepulcre J, Putcha D, Greve D, Johnson KA, Sperling RA (2011) Neuronal dysfunction and disconnection of cortical hubs in non-demented subjects with elevated amyloid burden. *Brain* **134**, 1635-1646.

[99] Talantova M, Sanz-Blasco S, Zhang X, Xia P, Akhtar MW, Okamoto S, Dziewczapolski G, Nakamura T, Cao G, Pratt AE, Kang YJ, Tu S, Molokanova E, McKercher SR, Hires SA, Sason H, Stouffer DG, Buczynski MW, Solomon JP, Michael S, Powers ET, Kelly JW, Roberts A, Tong G, Fang-Newmeyer T, Parker J, Holland EA, Zhang D, Nakanishi N, Chen HS, Wolosker H, Wang Y, Parsons LH, Ambasudhan R, Masliah E, Heinemann SF, Pina-Crespo JC, Lipton SA (2013) Abeta induces astrocytic glutamate release, extrasynaptic NMDA receptor activation, and synaptic loss. *Proc Natl Acad Sci U S A* **110**, E2518-2527.

[100] Gutierrez-Galve L, Lehmann M, Hobbs NZ, Clarkson MJ, Ridgway GR, Crutch S, Ourselin S, Schott JM, Fox NC, Barnes J (2009) Patterns of cortical thickness according to APOE genotype in Alzheimer's disease. *Dement Geriatr Cogn Disord* **28**, 476-485.

[101] Xia M, Wang J, He Y (2013) BrainNet Viewer: A network visualization tool for human brain connectomics. *PLoS One* **8**, e68910.