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ORIGINAL PAPER

Imminent cognitive decline in normal elderly individuals is associated with hippocampal hyperconnectivity in the variant neural correlates of episodic memory

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Abstract

The secondary prevention trials of Alzheimer's disease (AD) require an enrichment strategy to recruit individuals with imminent cognitive decline at the preclinical stage. Previously, we demonstrated a variant neural correlates of episodic memory (EM) function in apolipoprotein E (APOE) ε4 carriers. Herein, we investigated whether this variation was associated with longitudinal EM performance. This 3-year longitudinal study included 88 normal elderly subjects with EM assessment and resting-state functional MRI data at baseline; 48 subjects (27 ε3 homozygotes and 21 ε4 carriers) underwent follow-up EM assessment. In the identified EM neural correlates, multivariable regression models examined the association between hippocampal functional connectivity (HFC) and longitudinal EM change. Independent validation was performed using the Alzheimer's Disease Neuroimaging Initiative (ADNI) dataset. At baseline, the EM neural correlates were characterized in the Papez circuit regions in the ε3 homozygotes, but in the sensorimotor cortex and cuneus in the ε4 carriers. Longitudinally, the ε4 carriers exhibited a negative association of the baseline HFC strength in the EM neural correlates with annual rate of EM change ($R^2 = 0.25$, $p = 0.05$). This association also showed a trend in the ADNI dataset ($R^2 = 0.42$, $p = 0.06$). These results indicate that hippocampal hyperconnectivity in the variant EM neural correlates is associated with imminent EM decline in ε4 carriers, which may serve as a promising enrichment strategy for secondary prevention trials of AD.

Keywords Episodic memory · Apolipoprotein E · Degeneracy · Alzheimer's disease · Hippocampus · Functional connectivity

Introduction

Alzheimer's disease (AD) begins with a decades-long preclinical stage in which AD pathological process insidiously arise before detectable cognitive impairment [1, 2]. This preclinical stage is the focus of current clinical trials because the pathological process could be potentially reversed by disease-modifying treatment [3]. To optimize clinical efficacy of the trials, an enrichment strategy is required to enroll a sample of cognitively normal (CN) individuals with greater cognitive decline over the duration of the trial. Unfortunately, such a strategy remains to be determined in current clinical trials of AD. For example, although the beta-amyloid (Aβ) plaque biomarkers serve as the defining signature of AD, a large proportion of Aβ-positive individuals maintain normal cognitive function even over a decade [4, 5]. These observations suggest that the enrichment using Aβ biomarker alone is insufficient to observe a treatment effect

Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in analysis or writing of this report. A complete listing of ADNI investigators can be found at: http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf.

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in AD prevention trials. A supplementary enrichment strategy that is strongly predictive of imminent cognitive decline in CN population is needed to increase clinical efficacy of AD prevention trials.

Relative to Aβ plaque, brain network dysfunction appears to be an upstream event in AD pathophysiology and links more closely to cognitive decline [6]. First, network hypersynchrony may emerge as early as at the preclinical stage [7–9], even before the detectable Aβ pathology [10]. Second, brain network dysfunction and Aβ pathology may promote each other. Greater neural activity increases Aβ burden [11, 12], and alternatively, pathological Aβ accumulation is associated with neuronal hyperactivity and network excitability [13, 14]. Third, antiepileptic treatments against network dysfunction could reverse synaptic and memory deficits in AD rodent models [15], and improve cognition in amnestic mild cognitive impairment (aMCI) subjects [16, 17]. These evidences indicate that brain network dysfunction closely connected with both Aβ pathology and cognitive impairment in AD pathological cascade, and thus may serve as a potential strategy to enrich the CN population in AD prevention trials.

Integration of genetic information into the enrichment strategy may further facilitate the trial efficacy, as genetic variation accounts for 70% risk of AD [18]. To date, the apolipoprotein E (APOE) ε4 allele is the strongest genetic risk factor for late-onset AD, and exacerbates Aβ accumulation [19], brain network dysfunction [20], and episodic memory (EM) decline [21] in CN cohorts. In an earlier cross-sectional study, we identified that the ε4 carriers exhibit a variant neural correlates of EM function in the sensorimotor cortex and cuneus but not in the Papez circuit regions as the ε3 homozygotes, and hypothesized that hyperactivity in the variant EM neural correlates advances longitudinal EM decline in ε4 carriers [22]. Therefore, this study followed up these subjects to examine the association between baseline hippocampal functional connectivity (HFC) in the EM neural correlates and longitudinal EM change by the APOE allele. This association was further validated by the Alzheimer's Disease Neuroimaging Initiative (ADNI) dataset. Altogether, this study demonstrates that higher HFC strength in the variant EM neural correlates is associated with greater EM decline later in life. It may serve as a supplementary enrichment strategy to be used jointly with the established AD pathologies for secondary prevention trials of AD.

Methods

Subjects

Subjects were from the Nanjing Aging and Dementia Study (NADS). The NADS is characterized by a non-demented longitudinal cohort enriched for the APOE allele status, with the aim to enhance the understanding of the early development of AD. Please refer to Supplemental Method S1 for subject recruitment and inclusion criteria. As a result, this study included 88 subjects, including 43 APOE ε3 homozygotes and 45 APOE ε4 carriers (with APOE ε3ε4 or ε4ε4 genotype), for baseline analysis. Among of them, 27 APOE ε3 homozygotes and 21 APOE ε4 carriers participated in the follow-up neurocognitive tests at an average of 3.09 years after the baseline visit. They were cognitively normal at the follow-up visits. Please refer to Figure S1 for the subject inclusion process at baseline and follow-up. The Affiliated ZhongDa Hospital of Southeast University Research Ethics Committee approved this study. Written informed consents were obtained from each individual.

Neurocognitive tests

Each subject's cognitive function was examined at both baseline and follow-up visits. The EM tests consisted of the Auditory Verbal Learning Test, Logical Memory Test, and Rey-Osterrieth Complex Figure Test [23]. Each test's 20-min delayed recall score measured the subject's EM function. To reduce measurement error and potential type I error related to multiple comparisons, we calculated the EM Composite Scores by first converting raw Scores to z-Scores using the mean and standard deviation of the sample for each test and then averaging the z-Scores of the three EM tests.

MRI image acquisition, preprocessing, and construction of HFC networks

We acquired rs-fMRI and high-resolution T1-weighted anatomical images at baseline, using a Siemens Verio 3.0 Tesla scanner (Siemens, Erlangen, Germany) with a 12-channel head coil. The rs-fMRI data were conventionally preprocessed using the Analysis of Functional NeuroImages (AFNI) software (https://afni.nimh.nih.gov/afni) and MATLAB programs (The MathWorks, Inc., Natick, MA, USA) [24]. Please refer to Supplemental Method S2 for MRI scan parameter and preprocessing pipeline.

The construction of HFC networks has been described in our earlier paper [22]. Briefly, the bilateral hippocampus regions of interest (ROIs) were extracted from the automated anatomical labeling (AAL) template [25]. Voxelwise correlation coefficients (CC) of the ROIs with the whole brain were calculated and then underwent a Fisher transformation to improve normality [$m = 0.5\ln(1+CC)/(1-CC)$]. The obtained HFC values were subjected to a voxelwise gray matter volume correction to control anatomical variation influence on the HFC. Finally, the gray matter corrected HFC values were smoothed with a 6-mm Gaussian kernel.

Statistical analysis

Demographic and clinical data

Independent two-sample *t* test and Chi-square test compared quantitative and qualitative demographic data, respectively, between the APOE ε3 homozygous and APOE ε4 carrier groups. The EM Scores were analyzed using the mixed-design analysis of variance (ANOVA), including time as the within-subjects factor and group as the between-subjects factor, to test if the EM Scores differed across times or groups. The statistical significance was set at $p < 0.05$.

Identification of EM neural correlates at baseline

We identified the EM neural correlates separately for the APOE ε3 homozygous group and ε4 carrier group, as previously described, by the voxelwise multivariable linear regression model below (3dRegAna, AFNI):

$$m_i = \beta 0 + \beta 1 EM + \beta 2 age + \beta 3 edu + \beta 4 sex + \beta 5 FH + \varepsilon \quad (1)$$

where m_i is the HFC value of the *i* th voxel. β0 is intercept of the fitting line. *EM* is the EM composite z-Score. Age, education years (*edu*), sex, and family history (*FH*) are demographic covariates in the model. ε denotes random errors. We identified clusters showing significant β1 as the EM neural correlates. These EM neural correlates were employed as ROIs in the longitudinal analysis. To correct for multiple comparisons on the statistical maps, we used the 3dFWHMx to estimate the smoothing parameter and 3dClustSim to calculate the cluster size threshold (AFNI version 16.2.06). The significance level was set at $\alpha \leq 0.05$, determined by voxelwise $p = 0.05$ and cluster size ≥ 5504 mm^3.

Longitudinal analysis

Longitudinal analysis also was performed separately for APOE ε3 homozygous and ε4 carrier groups. We examined the association between the baseline HFC and the annual rate of change in EM Composite Score, as shown below:

$$\Delta EM_{an.} = \beta 0 + \beta 1 HFC_{BL} + \beta 2 EM_{BL} + \beta 3 age + \beta 4 edu + \beta 5 sex + \beta 6 FH + \varepsilon \quad (2)$$

where $\Delta EM_{an.}$, the annual rate of change in EM Composite Score, is defined as the EM Composite Score at follow-up minus that at baseline divided by the follow-up interval between the two visits. HFC_{BL} is the mean HFC in the EM neural correlates identified at the baseline analysis. EM_{BL} is the EM Composite Score at baseline. In addition, a univariate regression analysis between the baseline HFC strength and the annual rate of change in EM Composite Score was performed to examine if the two variables was still correlated without other covariates.

Independent validation by the ADNI dataset

We found a total of 84 cognitively normal elderly subjects who underwent rs-fMRI scan in the ADNI dataset. Among of them, 14 eligible APOE ε4 carriers were included with the following criteria: (1) their ages were younger than 80 years; (2) they were right-handers; (3) their APOE genotypes were APOE ε3ε4 or ε4ε4; (4) they completed all neuropsychological episodic memory tests within one visit; (5) they underwent 1-year follow-up visits; (6) no significant image artifacts in rs-fMRI data. This sample size is comparable to other ADNI studies using the cognitively normal ε4 carriers [26, 27]. With respect to the ADNI dataset, data used in the preparation of this article were obtained from the ADNI database (adni.loni.usc.edu). The ADNI was launched in 2003 as a public–private partnership, led by Principal Investigator Michael W. Weiner, MD. The primary goal of ADNI has been to test whether serial MRI, positron emission tomography (PET), other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of mild cognitive impairment (MCI) and early AD. For up-to-date information, see www.adni-info.org.

According to the ADNI protocol, the EM tests consists of the Rey Auditory Verbal Learning Test and Logical Memory Test; both tests are performed at baseline and annual follow-up visits. To control the potential practice effect due to repeated neurocognitive measures [28], the test scores at the first-year follow-up visit were applied in this study. The MRI data processing was identical to that in the NADS as described above.

Paired-samples *t* tests compared EM test scores between baseline and follow-up visits. We applied Eq. (1) to identify the EM neural correlates in the ADNI ε4 carriers. The mean HFC value of the regions showing significant EM neural correlates was extracted; then, it was input in Eq. (2) as HFC_{BL} to test its relationship with longitudinal EM performance.

Results

Subjects' characteristics

As Table 1 shows, the APOE ε3 homozygous and ε4 carrier groups in NADS dataset at baseline were matched in age, sex, education years, family history distribution, and EM performances. Table 2 illustrates characteristics of the subjects who participated in the follow-up study. The mean follow-up interval was 3.09 years for all NADS

Table 1 Demographic and neurocognitive information of the NADS subjects at baseline

	APOE ε3ε3 ($n=43$)	APOE ε4+ ($n=45$)	p value
Age, mean (SD), years	68.60 (6.60)	66.76 (6.23)	0.18
Male, No. (%)	23 (53.49)	21 (46.67)	0.75
Education, mean (SD), years	12.38 (3.18)	11.72 (2.89)	0.31
Positive family history, No. (%)	10 (23.26)	12 (26.67)	0.89
MMSE, mean (SD)	28.65 (1.02)	28.33 (1.30)	0.20
MDRS-2, mean (SD)	138.26 (2.70)	138.04 (3.06)	0.73
AVLT–20-min DR, mean (SD)	7.60 (1.94)	7.67 (2.03)	0.88
LMT–20-min DR, mean (SD)	8.57 (2.65)	8.67 (2.51)	0.86
CFT–20-min DR, mean (SD)	18.20 (6.15)	18.63 (4.94)	0.71
Composite memory z-Score, mean (SD)	0.47 (0.58)	0.51 (0.45)	0.73

The p values were obtained by independent two-sample t tests for quantitative data, or by Chi-square test for qualitative data

NADS Nanjing Aging and Dementia Study, *SD* standard deviation, *No.* number, *MMSE* Mini-Mental State Examination, *MDRS-2* Mattis Dementia Rating Scale-2, *AVLT–20-min DR* auditory verbal learning test–20-min delayed recall, *LMT–20-min DR* logical memory test–20-min delayed recall, *CFT–20-min DR* Rey-Osterrieth Complex Figure test–20-min delayed recall

subjects. The ε4 carrier group was significantly younger ($t_{46}=-2.18$, $p=0.03$) and had a longer follow-up interval ($t_{46}=5.22$, $p<0.01$) relative to the ε3 homozygous group. The mixed-design ANOVA demonstrated significant main effects of time in Auditory Verbal Learning Test ($F_1=23.10$, $p<0.001$) and Logical Memory Test ($F_1=7.20$, $p=0.01$) scores, respectively, which the scores at follow-up were significantly lower than those at baseline. No significant interaction between time and group was found in the three EM tests.

The EM neural correlates in APOE ε3 homozygotes and ε4 carriers

The bilateral HFC patterns are provided in Fig. 1. The EM neural correlates of each group are illustrated in Fig. 2 and Table S1. The EM neural correlates difference between the NADS APOE ε3 homozygous and ε4 carrier groups was statistically demonstrated in our earlier paper [22]. Briefly, relative to the ε3 homozygous group whose positive EM neural correlates were characterized in the Papez circuit regions including the bilateral thalamus and medial temporal lobe (MTL), the ε4 carrier group exhibited positive EM neural correlates beyond the Papez circuit regions including the bilateral cuneus and premotor cortex / sensorimotor cortex / superior parietal lobule. Similar to the difference above, the ADNI APOE ε4 carrier group showed negative, rather than positive, EM neural correlates in the MTL. Their positive EM neural correlates were primarily in the right inferior parietal lobule and bilateral precuneus / posterior cingulate cortex (Fig. S2).

Association of the HFC strength at baseline with longitudinal EM change

In the ε4 carriers, the mean HFC strength in the EM neural correlates at baseline negatively correlated with the annual rate of change in the EM Composite Score after controlling for baseline EM Score and demographic covariates including age, education years, sex, and family history ($R^2=0.25$, $p=0.05$, Fig. 3A and Table 3). Please note that the R^2 value above was calculated by correlating residual HFC with residual EM Composite Score after regressing out effects of other variables in the model, rather than the R^2 value of the full model. In addition, the univariate regression analysis without demographic covariates also demonstrated the negative correlation between the baseline HFC and the annual rate of change in the EM Composite Score ($R^2=0.37$, $p<0.01$, Fig. S3A). These findings indicate that higher HFC strength at baseline is associated with a greater rate of decline in the EM performance during the follow-up period. By contrast, such a correlation was not observed in the APOE ε3 homozygotes. In addition, we found no significant correlation between the baseline HFC and longitudinal EM performance in the brain region outside of the EM neural correlates in the ε4 carriers.

Independent validation by the ADNI dataset

The associations described above were independently tested by the ADNI dataset, although the ADNI subjects relative

Table 2 Demographic and neurocognitive data of subjects participated in the longitudinal study

	NADS dataset				
	APOE ε3ε3 ($n=27$)		APOE ε4+ ($n=21$)		p value[a]
Age, mean (SD), years	70.11 (5.23)		66.62 (5.84)		0.03*
Male, No. (%)	12 (44.4)		11 (47.6)		0.59
Education, mean (SD), years	12.78 (3.29)		11.98 (2.74)		0.37
Positive family history, No. (%)	3 (11.1)		6 (28.6)		0.24
Follow-up interval, mean (SD), years	2.81 (0.32)		3.44 (0.52)		0.00*
	APOE ε3ε3 Baseline	APOE ε3ε3 Follow-up	APOE ε4+ Baseline	APOE ε4+ Follow-up	p value[b]
AVLT–20-min DR, mean (SD) (range 0–12)	7.37 (1.84)	5.89 (1.76)	7.43 (1.78)	6.24 (1.81)	0.00*
LMT–20-min DR, mean (SD) (range 0–20)	8.37 (2.62)	7.54 (2.26)	8.48 (2.67)	7.29 (2.27)	0.01*
CFT–20-min DR, mean (SD) (range 0–36)	18.50 (5.78)	20.65 (7.84)	18.98 (4.44)	19.36 (6.02)	0.15
	ADNI dataset				
	APOE ε4+ ($n=14$)				p value[c]
Age, mean (SD), years	71.95 (4.67)				0.01*
Male, No. (%)	5 (35.7)				0.33
Education, mean (SD), years	17.14 (2.2)				0.00*
Positive family history, No. (%)	11 (78.6)				0.00*
Follow-up interval, mean (SD), years	1.23 (0.45)				n/a
	Baseline		Follow-up		p value[d]
AVLT–20-min DR, mean (SD) (range 0–15)	7.21 (3.26)		6.93 (4.27)		0.70
LMT–20-min DR, mean (SD) (range 0–25)	13.50 (3.13)		13.36 (5.15)		0.88

Please note that the AVLT and LMT used in the NADS were different from those used in the ADNI. In addition, the follow-up interval was approximate 3 years in NADS, which was about 1 year in ADNI

NADS Nanjing Aging and Dementia Study, *ADNI* Alzheimer's Disease Neuroimaging Initiative, *SD* standard deviation, *No*. number, *n/a* non-applicable, *ANOVA* analysis of variance, *AVLT–20-min DR* auditory verbal learning test–20-min delayed recall, *LMT–20-min DR* logical memory test–20-min delayed recall, *CFT–20-min DR* Rey–Osterrieth Complex Figure test–20-min delayed recall

*Indicate significant differences among groups

[a]Independent two-sample t tests and Chi-square tests compared quantitative and qualitative demographic data, respectively, between the NADS APOE ε3ε3 and NADS APOE ε4+ groups

[b]The mixed-design ANOVA obtained main effects of time among the four groups

[c]Independent two-sample t tests compared demographic data between ADNI APOE ε4+ group and NADS APOE ε4+ group

[d]Paired-samples t tests compared episodic memory performances between follow-up and baseline visits within ADNI APOE ε4+ subjects

to the NADS subjects were significantly older ($t_{33}=2.86$, $p=0.01$), had higher education years ($t_{33}=5.88$, $p<0.01$), and had a higher proportion of a family history ($\chi^2=8.41$, $p<0.01$, Table 2). In the positive EM neural correlates of the ADNI ε4 carriers, the baseline HFC strength showed a negative trend with the annual rate of change in the EM Composite Score after controlling for baseline EM Score and demographic covariates ($R^2=0.42$, $p=0.06$, Fig. 3B and Table S2). This negative correlation was also demonstrated in the univariate regression analysis ($R^2=0.49$, $p<0.01$, Fig. S3B).

Discussion

Following our earlier cross-sectional study identifying a variant EM neural correlates in the sensorimotor and cuneus in CN individuals carrying the APOE ε4 allele, this follow-up study further demonstrates that hippocampal functional hyperconnectivity in the variant EM neural correlates is associated with longitudinal EM decline in ε4 carriers. This finding was independently validated by the ADNI dataset. These results indicate the EM neural correlates as a brain network mechanism linking APOE polymorphism and longitudinal EM change. Clinically, these results pave a new way

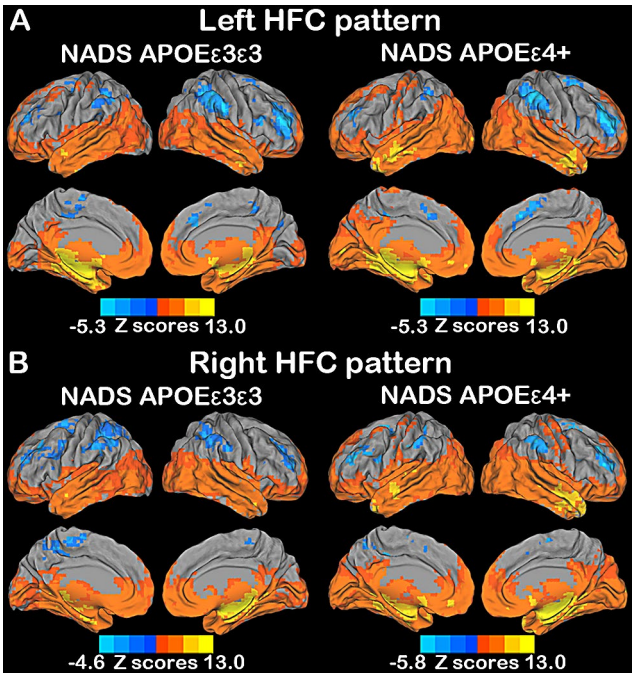

Fig. 1 Bilateral hippocampal functional connectivity (HFC) patterns in each group. A warm color indicates positive functional connectivity and a cool color indicates negative functional connectivity ($p < 0.05$, AlphaSim correction). The color bar presents *Z* Scores. *NADS* Nanjing Aging and Dementia Study, *HFC* hippocampal functional connectivity, *APOE* apolipoprotein E

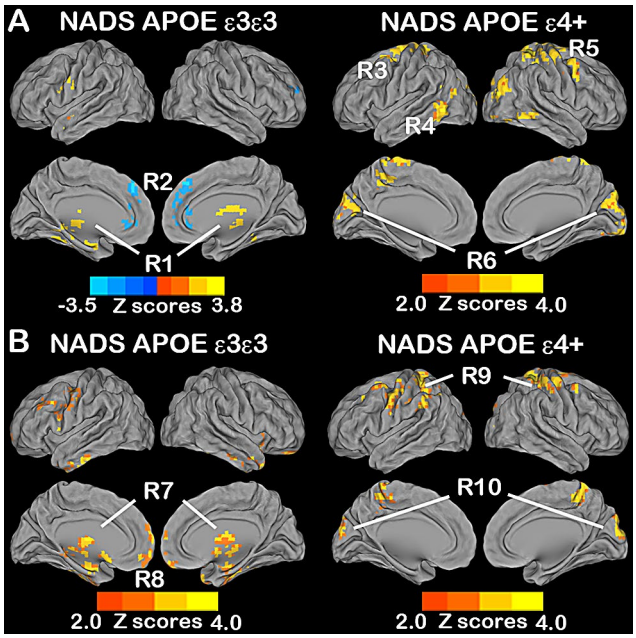

Fig. 2 Neural correlates of EM function in the left (**A**) and right (**B**) HFC networks from the NADS APOE ε3ε3 group and NADS APOE ε4 carrier group. A warm color indicates positive HFC correlation with EM function, while a cool color denotes negative HFC correlation with EM function. The color bar presents *Z* Scores. *R1* bilateral thalamus/left medial temporal lobe, *R2* bilateral dorsal medial prefrontal cortex/rostral anterior cingulate cortex, *R3* left premotor cortex/sensorimotor cortex/superior parietal lobule, *R4* left posterior middle temporal gyrus, *R5* right premotor cortex/sensorimotor cortex/superior parietal lobule, *R6* bilateral cuneus/right middle temporal gyrus, *R7* bilateral thalamus/medial temporal lobe/inferior temporal gyrus/left lateral prefrontal cortex, *R8* bilateral ventral/anterior medial prefrontal cortex, *R9* bilateral premotor cortex/sensorimotor cortex/superior parietal lobule, *R10* bilateral cuneus, *NADS* Nanjing Aging and Dementia Study, *ADNI* Alzheimer's Disease Neuroimaging Initiative, *HFC* hippocampal functional connectivity, *EM* episodic memory, *APOE* apolipoprotein E

to identify CN individuals with imminent EM decline over a brief follow-up period.

Identification of CN individuals with imminent cognitive decline remains a major challenge in conducting secondary prevention trails for AD. The APOE ε4 allele alone has limited utility to predict cognitive decline on an individual level [29], but may influence cognitive function through a variety of AD-related pathologies including Aβ, tau, and neurodegeneration [30, 31]. Our findings suggest that network hypersynchrony, measured by hippocampal functional hyperconnectivity herein, promotes longitudinal EM decline in ε4 carriers. Recently, network hypersynchrony was identified as an upstream pathophysiology that drives brain hyperactivity and cognitive impairment in the AD continuum [32]. Particularly, hippocampal hyperactivity, arising from the preclinical stage of AD [33], not only contributes to faster cognitive decline in non-demented individuals [34] but also correlates with longitudinal Aβ accumulation and greater cortical thinning in CN cohorts [35]. Our findings resonate with the findings from these studies and suggest the combination of hippocampal network hypersynchrony with the APOE ε4 allele as a pathogenic mechanism in advancing AD progression at the preclinical stage; this combination could be employed as a potential tool to indicate the risk of cognitive decline in CN individuals.

The association of network hypersynchrony with EM decline is detected in the variant EM neural correlates in ε4 carriers. Both our and other cross-sectional studies have documented that, relative to ε3 homozygotes who preferably use the acknowledged EM neural substrates involving the Papez circuit and prefrontal cortex, ε4 carriers may recruit structurally different brain regions to perform EM function [36–40]. This many-to-one structure–function relationship is defined as the degeneracy of brain network organization [41, 42]. According to the degeneracy theory, although the different EM neural substrates are comparable in terms of maintaining EM output, each may respond differently to EM-related tasks and thus offer a unique target for natural selection and biological evolution [41]. The longitudinal design of this study facilitates differentiating the cognitive influence over time between the EM neural substrates. In the ε4 carriers, higher HFC in their variant EM neural correlates

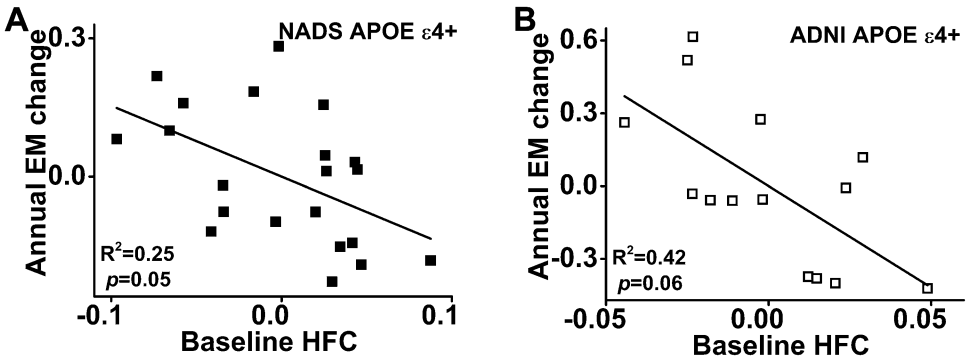

Fig. 3 Higher baseline HFC strengths in the EM neural correlates are associated with lower EM Scores during follow-up in the APOE ε4 carriers from both NADS (**A**) and ADNI (**B**) datasets. **A**, in the NADS APOE ε4 carriers, the mean HFC in the EM neural correlates at baseline was negatively correlated with the annual change in EM Composite Score. **B**, this negative correlation also showed a trend in the ADNI dataset. *NADS* Nanjing Aging and Dementia Study, *ADNI* Alzheimer's Disease Neuroimaging Initiative, *HFC* hippocampal functional connectivity, *EM* episodic memory, *APOE* apolipoprotein E

Table 3 Multivariable regression analyses for variables predicting annual rate of change in EM Composite Score in the NADS ε4 carriers

Variables	Regression coefficients (95% CI)	*p* value
Constant	0.33 (− 0.56 to 1.22)	0.44
HFC_{BL}	− 1.54 (− 3.05 to − 0.03)	0.05*
EM_{BL}	− 0.09 (− 0.26 to 0.08)	0.26
Sex	0.12 (− 0.04 to 0.29)	0.12
Age	− 0.01 (− 0.02 to 0.01)	0.29
Education years	0.01 (− 0.02 to 0.04)	0.41
Family history	− 0.01 (− 0.21 to 0.19)	0.90

HFC_{BL} hippocampal functional connectivity strength at baseline, EM_{BL} composite memory *z*-Score at baseline

*Indicates statistical significance

was associated with greater EM decline during the follow-up period. By contrast, we did not observe any significant association between the HFC and longitudinal EM change in the ε3 homozygotes. These results indicate that the greater the ε4 carriers employ the variant EM neural correlates, the more rapid the EM decline later. From a neural system perspective, the variant EM neural correlates primarily involve the bilateral sensorimotor cortex. Recent evidence suggests Aβ deposition in the sensorimotor cortex as a key contributor to gait dysfunction that serves as a predictive symptom of the incidents of MCI and dementia at the preclinical stage of AD [43–45]. Our study suggests that the sensorimotor cortex contribution to AD development may also involve the EM domain, in which greater employment of the sensorimotor cortex for EM formation is detrimental to longitudinal EM performances in ε4 carriers.

Several strengths distinguish our study from other clinical studies in the field. First, we independently validated the predictive correlation between hippocampal hyperconnectivity and longitudinal EM decline in ε4 carriers. Low reproducibility becomes a major concern in the biomedical research community, as it undermines research significance in science and impedes development of translational medicine [46]. Independent validation could evaluate reproducibility and screen coincidental findings that reach statistical significance by chance. Although the correlation showed a trend in the ADNI dataset, we believe that the correlation would reach the significance level if the sample size becomes larger. The achieved independent validation could enhance our confidence to apply the correlation in future AD prevention trials. Second, the HFC measure is obtained from a noninvasive and cost-effective MRI scan, and herein predicted EM decline over a short follow-up duration. These features suggest the HFC measure as a sensitive and easily applicable tool to screen CN individuals who should be referred for AD pathological biomarkers tests (i.e., Aβ and tau), which are invasive and more expensive. Third, HFC is a continuous variable that might be better than categorical variables to assess the risk of EM decline. Subjects with very high HFC would exhibit greater EM decline relative to those with medium–high HFC.

Our findings should be considered with the following caveats. First, the obtained EM neural correlates patterns between the NADS and ADNI datasets, although were convergent in the absence of Papez circuit regions in the positive EM neural correlates, were not exactly identical. It suggests that the EM neural correlates were not determined by the APOE alleles alone, but may also be modulated by multiple factors such as age, education years, and cerebral amyloid load. Further studies should disentangle the impact of these factors on the EM neural correlates. Second, 55% of the subjects participated in the 3-year follow-up visit. The follow-up rate was slightly lower than that of other datasets [e.g., 64% of the 3-year follow-up rate in the National

Alzheimer's Coordinating Center Uniform Data Set (NACC UDS)] [47]. In addition, in the follow-up data, the NADS ε4 carrier group was significantly younger than the NADS ε3 homozygous group and the ADNI ε4 carrier group. The relatively low follow-up rate and the younger age in the NADS ε4 carrier group may bias the results found in this study. Third, all of the NADS ε4 carriers that participated in the follow-up study maintained normal cognitive function at follow-up. It remains unknown whether the high HFC contributes to conversion to mild cognitive impairment or dementia in ε4 carriers. Studies with longer follow-up durations are needed to demonstrate this contribution. Fourth, the subjects were asked to close their eyes during the resting-state fMRI scan in this study. The reliability of the network connectivity when eyes closed would be relatively lower compared with that when eyes fixated on a cross [48]. The potential difference in reliability between different resting-state conditions should be noted when comparing results among different datasets. Accordingly, after addressing these challenges, we expect to establish a degeneracy framework to measure an individual's risk of cognitive impairment at the preclinical stage of AD.

In summary, our findings indicate that hippocampal hyperconnectivity in the variant EM neural correlates is associated with imminent EM decline in cognitively normal ε4 carriers, which may serve as a promising enrichment strategy for secondary prevention trials of AD. In addition, these findings may provide a supplementary tool to be used jointly with the established AD biomarkers to identify cognitively normal ε4 carriers who are likely on the path to AD with a high sensitivity.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00406-021-01310-7.

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Availability of data and material The data that support the findings of this study are available from the corresponding author upon reasonable request.

Code availability Not applicable.

Declarations

Conflict of interest The authors declare no conflict of interests.

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