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RESEARCH ARTICLE

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Functional segregation loss over time is moderated by *APOE* genotype in healthy elderly

Kwun Kei Ng[1†] | Yingwei Qiu[1,2†] | June Chi-Yan Lo[1] | Evelyn Siew-Chuan Koay[3,4] | Woon-Puay Koh[5,6] | Michael Wei-Liang Chee[1] | Juan Zhou[1,7]

[1]Centre for Cognitive Neuroscience, Neuroscience and Behavioural Disorders Programme, Duke-National University of Singapore Medical School, Singapore 169857, Singapore

[2]Department of Radiology, Third Affiliated Hospital of Guangzhou Medical University, Guangzhou Shi, Guangdong Sheng 510000, China

[3]Department of Pathology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore 117549, Singapore

[4]Molecular Diagnosis Centre, Department of Laboratory Medicine, National University Hospital, Singapore 119074, Singapore

[5]Office of Clinical Sciences, Duke-National University of Singapore Medical School, Singapore 169857, Singapore

[6]Saw Swee Hock School of Public Health, National University of Singapore, Singapore 117549, Singapore

[7]Clinical Imaging Research Centre, the Agency for Science, Technology and Research and National University of Singapore, Singapore 117599, Singapore

Correspondence
Juan Zhou, PhD, Centre for Cognitive Neuroscience, Neuroscience and Behavioural Disorders Programme, Duke-National University of Singapore Medical School, 8 College Road, #06-15, Singapore 169857, Singapore.
Email: helen.zhou@duke-nus.edu.sg

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Abstract

We investigated the influence of the apolipoprotein E-ε4 allele (*APOE*-ε4) on longitudinal age-related changes in brain functional connectivity (FC) and cognition, in view of mixed cross-sectional findings. One hundred and twenty-two healthy older adults (aged 58–79; 25 *APOE*-ε4 carriers) underwent task-free fMRI scans at baseline. Seventy-eight (16 carriers) had at least one follow-up (every 2 years). Changes in intra- and internetwork FCs among the default mode (DMN), executive control (ECN), and salience (SN) networks, as well as cognition, were quantified using linear mixed models. Cross-sectionally, *APOE*-ε4 carriers had lower functional connectivity between the ECN and SN than noncarriers. Carriers also showed a stronger age-dependent decrease in visuospatial memory performance. Longitudinally, carriers had steeper increase in inter-ECN-DMN FC, indicating loss of functional segregation. The longitudinal change in processing speed performance was not moderated by APOE-ε4 genotype, but the brain–cognition association was. In younger elderly, faster loss of segregation was correlated with greater decline in processing speed regardless of genotype. In older elderly, such relation remained for noncarriers but reversed for carriers. *APOE*-ε4 may alter aging by accelerating the change in segregation between high-level cognitive systems. Its modulation on the longitudinal brain–cognition relationship was age-dependent.

KEYWORDS
APOE-ε4, functional MRI, functional segregation, healthy aging, intrinsic connectivity networks, longitudinal study

1 | INTRODUCTION

The apolipoprotein-ε4 (*APOE*-ε4) allele has been suggested to accelerate normal ageing or induce pathological ageing. It is a well-established genetic susceptibility factor for Alzheimer's disease (AD), advancing its onset from 84 to 68 years old (Corder et al., 1993) and affecting younger elderly more readily compared to the oldest old (Gardner, Valcour, & Yaffe, 2013). *APOE*-ε4 carriers are reported to have preclinical cognitive decline (Deary et al., 2002; Reiman et al., 1996), more severe cortical thinning (Espeseth et al., 2008), abnormalities in

†Contributed equally to the work

functional activation (Matura et al., 2014), earlier amyloid β deposition (Resnick et al., 2015), and higher risk of mortality (Liu, Liu, Kanekiyo, Xu, & Bu, 2013). Yet, how *APOE*-ε4 genotype affects functional brain organization underlying cognitive decline in healthy older adults remains largely unknown.

Task-free blood oxygen level-dependent (BOLD) functional magnetic resonance imaging (fMRI) enables researchers to examine the *APOE*-ε4 effect on the human brain functional architecture in vivo by studying changes in functional connectivity (FC), defined as synchronous fluctuations of BOLD signals among different brain regions (Biswal, Yetkin, Haughton, & Hyde, 1995; Harrison and Bookheimer, 2016). Functional intrinsic connectivity networks (ICN), formed by spatially distributed brain regions showing high FC and subserving different sensorimotor and cognitive functions, have been mapped in the healthy brain (Biswal et al., 1995, 2010). The "core cognitive networks" have been suggested to comprise the default mode network (DMN), the executive control network (ECN), and the salience network (SN) (Uddin, 2015). The DMN is involved in self-referential functions such as episodic memory (Buckner, Andrews-Hanna, & Schacter, 2008); the ECN is associated with externally oriented cognitive functions such as working memory and decision making; and the SN is implicated in directing attention to the most homeostatically salient events (Seeley et al., 2007). Healthy aging has been consistently associated with reduced brain network functional specialization (decreased intranetwork FC) (Ferreira et al., 2016; Geerligs, Renken, Saliasi, Maurits, & Lorist, 2014) and segregation (increased inter-network FC) (Chan, Park, Savalia, Petersen, & Wig, 2014; Geerligs et al., 2014), two critical principles underlying the modular network organization that is essential for efficient information processing (Park and Friston, 2013; Sporns, 2013). The *APOE*-ε4 allele may impact ageing by disrupting the normal course of reducing network specialization and segregation.

Despite evidence from cross-sectional studies suggesting that *APOE*-ε4 alters brain functional organization in healthy young (Filippini et al., 2009), middle-aged (Westlye, Lundervold, Rootwelt, Lundervold, & Westlye, 2011), and older adults (Machulda et al., 2011; Wu et al., 2016), systematic and consistent patterns have yet to be fully discovered and explained (Reinvang, Espeseth, & Westlye, 2013; Shu et al., 2014). This is true even for studies focusing on older adult participants. For instance, Westlye et al. (2011) observed increased hippocampal-DMN FC in *APOE*-ε4 middle-aged and older carriers compared to noncarriers, while Machulda et al. (2011) reported lower regional intra-DMN FC and higher intra-SN FC in elderly carriers compared to noncarriers. Sheline et al. (2010) found both precuneus-related decreased FC (temporal regions and the dorsal anterior cingulate) and increased FC (dorsal occipital cortex and anterior frontal regions) in Aβ-negative *APOE*-ε4 carriers compared to noncarriers. Finally, a small study by Wu et al. (2016) reported both increased and decreased FC within the DMN, ECN, and SN in *APOE*-ε4 carriers but comparable internetwork FC between carriers and noncarriers aged 50–65 years.

Moreover, cross-sectional findings do not necessarily generalize to individual ageing process as such design mixes interindividual differences and intraindividual changes over time (Kraemer, Yesavage, Taylor, & Kupfer, 2000). Longitudinal studies are necessary to dissociate intra- and interindividual differences to characterize the role of *APOE*-ε4 genotype in brain and cognitive ageing more clearly. To date, few longitudinal task-free FC studies have compared cognitively normal elderly individuals with different *APOE*-ε4 genotype. Ye et al. (2016) recently reported longitudinal differences in hippocampal FC between *APOE*-ε4 carriers and noncarriers who were cognitively normal or mild cognitively impaired (MCI), but the sample size for healthy carriers was relatively small (10 carriers in cognitively normal subjects, 16 in MCI), and only hippocampus-based FC was examined. The changes within and between large-scale core cognitive brain networks over time, a hallmark of healthy aging (Chan et al., 2014; Ng, Lo, Lim, Chee, & Zhou, 2016), remain to be elucidated.

To this end, we examined the association between *APOE*-ε4 genotype and task-free FC changes in cognitively normal older adults in a cross-sectional dataset and a longitudinal subset simultaneously. By expanding on the dataset from our previous study (Ng et al., 2016) and incorporating genotype information in the analysis, we investigated whether intra- and internetwork FC of the three core cognitive ICNs and the cognitive performance in five domains underwent diverse changes according to *APOE*-ε4 genotype over time. In line with the proposals of the effect of *APOE*-ε4 allele on the aging process, we hypothesized the intranetwork FC within the three ICNs to decrease over time, and more sharply in *APOE*-ε4 carriers than noncarriers, indicating greater loss of functional specialization over time. Second, we anticipated internetwork FC between task-positive networks (ECN and SN) and the DMN to increase over time, again more so in *APOE*-ε4 carriers than noncarriers, indicating faster loss of functional segregation. Last, we determined whether the association between the rate of change in FC and the longitudinal cognitive decline would be affected by *APOE*-ε4 genotype.

2 | MATERIALS AND METHODS

2.1 | Participants

One hundred and twenty-two cognitively normal, healthy older adults (61 female; 25 ε4-carriers (24 ε3ε4 and 1 ε2ε4) and 97 noncarriers (87 ε3ε3 and 10 ε2ε3; mean age = 70.11 years, *SD* = 5.62 years at the baseline, range = 58–79 years) were recruited at Duke-NUS Medical School (Chee et al., 2009; Pan, De Silva, Yuan, & Koh, 2014). Of the 122 participants, 78 had longitudinal data (16 ε4-carriers (15 ε3ε4 and 1 ε2ε4) and 62 noncarriers (52 ε3ε3, and 10 ε2ε3)). They were evaluated for a second and/or a third time every 18–24 months. Thirty-eight had data from all three time points, 37 had data for two consecutive time points, and 3 had data from the first and third time points. Eligible participants had to complete brain imaging with satisfactory data quality, a Mini-Mental State Examination (MMSE) (Folstein, Folstein, & McHugh, 1975) score of 26 or greater at all time points, and did not have any of the following clinical conditions: (1) a history of significant vascular events (i.e., myocardial infarction, stroke, or peripheral vascular disease); (2) a history of malignant neoplasia of any form; (3) a history of cardiac, lung, liver, or kidney failure; (4) active or inadequately treated thyroid disease; (5) active neurological or psychiatric conditions;

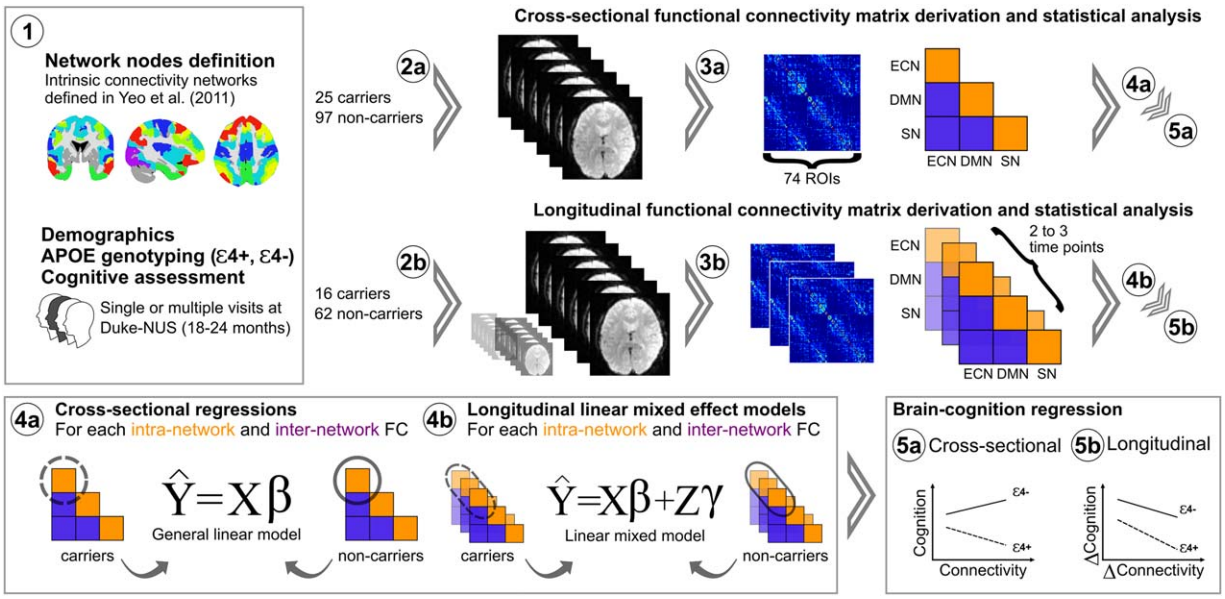

FIGURE 1 Study design schematic. Cross-sectional (steps 1, 2a, 3a, 4a, 5a) and longitudinal (steps 1, 2b, 3b, 4b, 5b) data analyses are presented. Demographics, *APOE* genotype, brain, and cognitive measures were obtained in Singapore (1). The functional connectivity (FC) matrix among 74 regions of interest (ROIs) covering the three intrinsic connectivity networks (ICN) of interest, namely, default mode network (DMN), executive control network (ECN), and salience network (SN), was derived for each participant and at each time point. Data were then collated separately for cross-sectional (2a) and longitudinal (2b) analyses. The intranetwork (orange cells) and internetwork FCs (violet cells) of the three ICNs were obtained by averaging the corresponding cells in the matrices (3a and 3b). We then assessed *APOE*-ε4 effects on the cross-sectional (4a) or longitudinal (4b) FC and cognitive scores using corresponding linear models. Finally, brain–cognition associations were examined by correlating the individuals' intra- and internetwork FC and cognitive test. In the cross-sectional analysis (5a), the cognitive score and FC was correlated using multiple regression; in the longitudinal analysis (5b), the rate of change in cognition and FC (represented as Δ in the axes) were derived from the linear mixed models and correlated using multiple regression [Color figure can be viewed at wileyonlinelibrary.com]

or (6) a history of head trauma with loss of consciousness. The study was approved by the Institutional Review Board of the National University of Singapore. Written informed consent was obtained prior to recruitment. Figure 1 gives the schematic summary of the data collection and analytical steps.

2.2 | APOE genotyping

APOE genotyping was achieved using established protocols. Genomic DNA was extracted from peripheral blood (PUREGENE®, Gentra System, Inc) drawn between 8:30 am and 9:30 am after an overnight fast at the National University Hospital Laboratory (Chee et al., 2009). *APOE* genotype was determined using high-resolution melting (HRM) with quality-controlled procedures (Hixson and Vernier, 1990). Briefly, the genomic DNA was amplified for a 277 bp region that includes *APOE* polymorphisms at both codons 112 and 158 using a 3′-blocked unlabeled oligonucleotide probe (LunaProbes) in a Light Scanner 32 real-time PCR instrument (Idaho Technology Inc., UT). *APOE* alleles were classified as having an ε2, ε3, or ε4 isoform. Participants were classified as carriers (one or more ε4 alleles present) or noncarriers (no ε4 allele present).

2.3 | Neuropsychological assessments

Participants were evaluated by trained researchers on five cognitive domains within three months of undergoing MRI: processing speed, attention, verbal memory, visuospatial memory, and executive functioning (Chee et al., 2009), as detailed in Supporting Information.

2.4 | MRI acquisition

Participants underwent an 8-min task-free fMRI scan with fixation in a 3 T Magnetom Tim Trio System (single-shot EPI, 36 continuous axial slices, TR/TE = 2,000/30 ms, flip angle = 90°, FOV = 192 × 192, matrix size = 64 × 64, isotropic voxel size = 3.0 × 3.0 × 3.0 mm^3, bandwidth = 2,112 Hz/pixel). High-resolution T1-weighted structural MRI was acquired in the same session using a magnetization-prepared rapid gradient echo (MPRAGE) sequence (192 continuous sagittal slices, TR/TE/TI = 2,300/2.98/900 ms, flip angle = 9°, FOV = 256 × 240 mm^2, matrix = 256 × 256, isotropic voxel size = 1.0 × 1.0 × 1.0 mm^3, bandwidth = 240 Hz/pixel).

2.5 | Image processing and functional connectivity derivation

All functional and structural images were preprocessed using a standard volume-based pipeline following our previous report (Ng et al., 2016; Zhou, Gennatas, Kramer, Miller, & Seeley, 2012) based on FSL 5.0.2.2 (https://www.fmrib.ox.ac.uk/fsl) and AFNI 2011_12_11 (https://afni.nimh.nih.gov/) on a Centos 7.2 Linux system. For the structural images, steps included (1) image noise reduction with

SUSAN, (2) skull stripping with the Brain Extraction Tool (BET), (3) linear (FLIRT) and nonlinear (FNIRT) registration to the Montreal Neurological Institute (MNI) 152 standard space, and (4) segmentation of the brain into gray matter, white matter, and cerebrospinal fluid (CSF) compartments. For the functional data, we (1) excluded the first five volumes, before performing, (2) slice-time correction, (3) motion correction, (4) despiking and grand-mean scaling, (5) spatial smoothing with a 6-mm FWHM Gaussian kernel, temporal band-pass filtering (0.009–0.1 Hz) and detrending (first and second order), (6) structural MRI coregistration using boundary-based registration (BBR) and nonlinear (FNIRT) registration to the MNI space, and (7) nuisance signals reduction by regressing out signals estimated from CSF, white matter, whole-brain global signal (Power, Schlaggar, & Petersen, 2015), and six motion parameters. Motion quality control was performed for each session of all participants (maximum absolute motion $\leq$ 3mm). Global signal regression was performed for consistency with past studies on ageing (Chan et al., 2014; Ferreira et al., 2016; Geerligs et al., 2014) and its potential effectiveness in nuisance signals suppression (Nalci, Rao, & Liu, 2017; Power, Plitt, Laumann, & Martin, 2017; Satterthwaite et al., 2017). In our previous study, we used the global negativity index (Chen et al., 2012) as a complementary tool to computes the proportion of voxels showing negative correlation with the global signal for each participant at each time point. Most percentages fell below 3%, suggesting that the global signal was more representative of nuisance signals and should be removed.

To quantify within- and between-network FCs, the DMN, the ECN, and the SN were spatially defined by a prior brain functional parcellation scheme in the standard MNI space (Arslan et al., 2017; Yeo et al., 2011). The labels and coordinates of the regions of interest (ROIs) used for each ICN are provided in Supporting Information, Table S1 and Figure S1. These ROIs were applied to functional data that were normalized to the same MNI space to extract the functional time series. At subject level, FC between two cortical ROIs was computed as the Pearson's correlation coefficient between the mean fMRI time series (across all voxels in an ROI) of the two ROIs. Fisher's z transformation was then applied to each correlation coefficient. A subject-level FC z-score matrix involving 74 ROIs from the three ICNs was then constructed for each study time point. Average z-scores of intranetwork FC (DMN, ECN, and SN) and internetwork FC (ECN-DMN, ECN-SN, and DMN-SN) were then calculated for each participant at each time point (e.g., averaging FCs between all pairs of ROIs within the DMN gave intra-DMN FC).

To adjust for the possible effect of grey matter volume (GMV) changes (Hostage, Choudhury, Murali Doraiswamy, & Petrella, 2013) on the FC aberration, we included average GMV of each ICN as covariates in our statistical models. GMV derivation was based on the standard VBM8 procedure (Supporting Information).

2.6 | Statistical analysis

2.6.1 | Demographic analysis

Demographic differences between the *APOE*-ε4 carriers and noncarriers at the baseline were tested using χ^2 tests for categorical variables (gender) and independent *t* tests for continuous variables (age, education, MMSE, and mean frame-wise relative head motion) (Table 1).

TABLE 1 Subject characteristics of the cross-sectional and longitudinal dataset

Participant characteristic	Noncarrier	Carrier	*p* value
Cross-sectional cohort			
Sample size	97	25	
Age (years)	70.30 (5.70)	69.14 (5.45)	.35
Male/female (number)	48/49	13/12	1
Education level (years)	10.66 (3.80)	11.08 (4.76)	.69
MMSE	28.2 (1.30)	27.7 (1.23)	.09
Mean absolute motion (mm)	0.29 (0.17)	0.26 (0.15)	.23
Mean relative motion (mm)	0.085 (0.049)	0.069 (0.035)	.06
Longitudinal subset			
Sample size	62	16	
Age (years)	68.20 (5.92)	67.03 (4.94)	.44
Male/female (number)	31/31	9/7	.78
Education level (years)	12.24 (3.03)	13.30 (3.26)	.28
MMSE (at baseline)	28.43 (1.19)	28.0 (1.10)	.18
GDS	0.91 (1.11)	1.25 (1.07)	.27

Note. Abbreviations: GDS = Geriatric Depression Scale; MMSE = Mini-Mental State Examination.
Numbers in brackets indicate standard deviations. Group difference in age, education, MMSE, GDS, and motion during scans were evaluated with independent-sample *t* tests, while that of gender proportion was evaluated with a χ^2 test.

2.6.2 | Brain and cognition analysis

Cross-sectional analyses

Each of the six cross-sectional intranetwork (DMN, SN, and ECN) and internetwork (DMN-ECN, DMN-SN, and SN-ECN) FCs and five cognitive performances was compared between *APOE*-ε4 carriers and noncarriers separately using a general linear model (GLM) including an interactive effect between age and *APOE*-ε4 genotype and adjusting for gender and years of education; FC analyses were further controlled for mean framewise displacement (FD) and network GMV (Equation 1):

$$Y_j = \beta_0 + \beta_1(\text{Gender}_j) + \beta_2(\text{Education}_j) + \beta_3(\text{Mean FD}_j) + \beta_4(\text{GMV}_j) + \beta_5(\text{Age}_j) + \beta_6(\text{APOE}_j) + \beta_7(\text{Age}_j \times \text{APOE}_j) + r_j \quad (1)$$

where the effect of each predictor on measure *Y* of individual j was denoted by the specific βs and × represented interaction. For cognition, we examined attention and processing speed across the whole cross-sectional cohort as these domains were examined with the same assessment materials for all participants in all visits. We then examined verbal memory, visuospatial memory, and executive function using all participants from the longitudinal protocol who contributed at least one time point.

To investigate whether *APOE*-ε4 genotype influences brain–cognition association cross-sectionally, if any FC or cognitive domain showed *APOE*-ε4 genotype effects, the association between each FC measure and cognitive domain was assessed using a GLM with the cognitive domain as the outcome variable, age, FC, *APOE*-ε4 genotype, and their interactions as the predictors of interest. Effects were again adjusted for gender, years of education, mean frame-wise displacement, and network GMV.

Longitudinal analyses

Complementing our cross-sectional analyses, we examined the moderating effect of *APOE*-ε4 genotype on these longitudinal changes that were not examinable with cross-section only data. Presence of longitudinal change was a prerequisite for analyses. In the previous study (Ng et al., 2016), we identified significant longitudinal changes (i.e., over time) in intranetwork FCs (DMN, ECN, marginal in SN), one internetwork FC (ECN-DMN), and one cognitive domain (processing speed). Linear mixed-effects models (LMM) with random intercept and slope were used to examine the potential moderation by *APOE*-ε4 genotype on longitudinal changes in each of the four FC measures or processing speed separately (Equation 2). In each model, the measure of interest across multiple time points served as the outcome variables, while genotype (*APOE*-ε4 effect), years from baseline scan (longitudinal ageing/time effect), baseline age (cross-sectional age effect), and interactions among these three factors served as predictors. For each LMM, mean FD, network GMVs, gender, and years of education were included as nuisance covariates.

$$\begin{aligned} Y_{ij} = {} & \beta_{00} + \beta_{01}(\text{Gender}_j) + \beta_{02}(\text{Education}_j) + \beta_{03}(\text{Age}_j) + \beta_{04}(\text{Mean FD}_{ij}) \\ & + \beta_{05}(\text{GMV}_{ij}) + \beta_{06}(APOE_j) + \beta_{07}(\text{Age}_j \times APOE_j) + \beta_{10}(\text{Time}_{ij}) + \\ & \beta_{11}(\text{Age}_j \times \text{Time}_{ij}) + \beta_{12}(APOE_j \times \text{Time}_{ij}) + \beta_{13}(\text{Age}_j \times APOE_j \times \text{Time}_{ij}) \\ & + \mu_{0j} + \mu_{1j}(\text{Time}_{ij}) + r_{ij} \end{aligned} \quad (2)$$

where the effect of each predictor on measure *Y* of individual j at time point i was denoted by the specific β_0s and β_1s. In particular, β_{11}, β_{12}, and β_{13} represented estimated effects due to the longitudinal predictor time and its interactions (terms with ×) with age and genotype; μ_{0j} and μ_{1j} represented individual variability in intercepts and longitudinal slopes (i.e., random effects).

To investigate whether *APOE*-ε4 genotype influences brain–cognition association longitudinally, we conducted a GLM similar to the cross-sectional analysis, in which the individual rate of change in processing speed was the outcome (the only cognitive measure showing a longitudinal decline), with individual rate of change in FC (among those FC metrics influenced by *APOE*-ε4 genotype), age at baseline, *APOE*-ε4 genotype, and their interactions as predictors of interest. The rate of change in network GMVs, gender, and years of education were included as covariates. The rate of change in FC and processing speed used in this GLM were derived from an LMM in which time from baseline scan was the only longitudinal predictor without interacting with baseline age or genotype (Goh, Beason-Held, An, Kraut, & Resnick, 2013; Ng et al., 2016).

TABLE 2 Statistical summary of cross-sectional multiple regression analyses of the internetwork functional connectivity (FC) between the executive control (ECN) and salience network (SN), and visuospatial memory

Regression term	Coefficient	Standard error	*t* value	*p* value
Internetwork ECN-SN FC				
Gender	−0.0045	0.0097	−0.46	.643
Education level	0.0024	0.0013	1.84	.069
GMV_{DMN}	0.3848	0.3707	1.04	.301
GMV_{SN}	−0.0753	0.3463	−0.22	.828
Mean FD	**0.3513**	**0.1032**	**3.40**	**.001**
APOE	**−0.0285**	**0.0120**	**−2.38**	**.019**
Age	0.0010	0.0010	0.96	.341
APOE × Age	0.0004	0.0022	0.20	.844
Visuospatial memory				
Gender	−2.1286	1.9508	−1.09	.279
Education level	0.3681	0.3225	1.14	.258
APOE	−0.9919	2.6819	−0.37	.713
Age	0.1573	0.1840	0.86	.396
***APOE* × Age**	**−0.9256**	**0.4601**	**−2.01**	**.048**

Note. Abbreviations: FD = frame-wise displacement; GMV = grey matter volume.
APOE–ε4 carriers showed less positive coupling (i.e., negative *APOE* coefficient, negligible two-way interaction) of ECN-SN FC over time as compared to the non-carriers. Statistically significant effects ($p < .05$) are highlighted in bold.

To further show that the observations cannot be fully accounted for by motion, we also ran the analyses with functional data with motion scrubbing (FD ≤ 0.2 mm, DVARS ≤ 0.5%). All analyses were performed in R 3.3.0 (https://www.r-project.org/) on OSX 10.9.2.

3 | RESULTS

3.1 | Demographic characteristics

At baseline, the *APOE*-ε4 carriers and noncarriers were comparable in terms of age, gender, education, general cognition (MMSE), and head movement (Table 1).

3.2 | Genotype effects on the cross-sectional differences in functional connectivity and cognition

From the cross-sectional dataset, *APOE*-ε4 genotype yielded statistically significant group differences in the internetwork FC between the ECN and the SN ($p = .019$). Its negative coefficient (−0.285, Table 2) indicated lower internetwork ECN-SN FC in the carriers compared to the noncarriers.

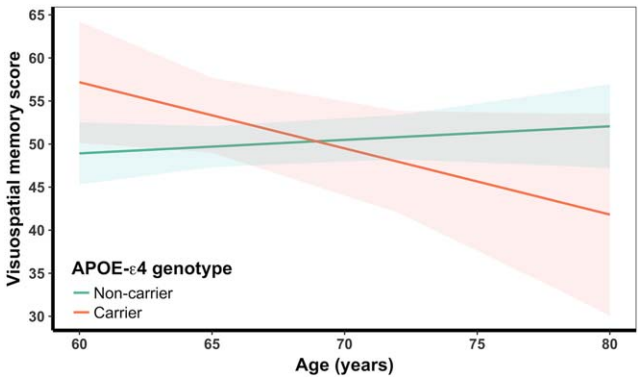

FIGURE 2 *APOE*-ε4 carriers (orange) had larger age-dependent decrease in visuospatial memory scores than noncarriers (green). Shades represent 95% confidence intervals [Color figure can be viewed at wileyonlinelibrary.com]

For cognitive performance, there were no statistically reliable *APOE*-ε4 genotype effects in attention, processing speed, or executive function. There was a statistically significant baseline age × genotype interaction for visuospatial memory ($p = .048$). Compared to noncarriers, there was a more prominent age-related decrease in the memory performance among carriers: older carriers performed worse than younger carriers (Table 2 and Figure 2).

Given the presence of *APOE*-ε4 effects cross-sectionally, we examined whether *APOE*-ε4 allele moderated the brain–cognition association between the internetwork ECN-SN FC and visuospatial memory, but no statistically significant relationship was observed.

TABLE 3 Statistical summary of longitudinal linear mixed effect model of the internetwork functional connectivity between the default mode (DMN) and executive control network (ECN)

Regression term	Coefficient	Standard error	*t* value	*p* value
Gender	0.0062	0.0108	0.57	.568
Education level	−0.0022	0.0017	−1.30	.198
GMV_{DMN}	−0.2561	0.2752	−0.93	.354
GMV_{ECN}	0.0939	0.2721	0.35	.731
Mean FD	−0.0297	0.1029	−0.29	.774
APOE	−0.0058	0.0143	−0.41	.686
Age	−0.0019	0.0011	−1.76	.083
Time	−0.0026	0.0020	−1.27	.209
APOE × Age	0.0045	0.0028	1.61	.113
***APOE* × Time**	**0.0102**	**0.0050**	**2.06**	**.043**
Time × Age	**0.0008**	**0.0004**	**2.21**	**.031**
APOE × Age × Time	−0.0002	0.0011	−0.22	.827

Note: * represents interaction. Abbreviations: FD = frame-wise displacement; GMV = grey matter volume.
APOE-ε4 carriers showed more positive coupling (i.e., positive *APOE* × time coefficient, negligible three-way interaction) of ECN-DMN FC over time as compared to the noncarriers. Statistically significant effects ($p < .05$) are highlighted in bold.

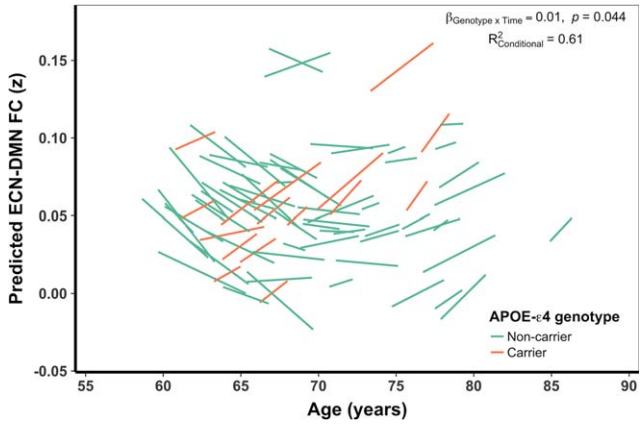

FIGURE 3 *APOE*-ε4 carriers had more rapid loss of functional segregation between the default mode network and the executive control network over time. Spaghetti plot of the predicted individual longitudinal changes in the segregation between the default mode network (DMN) and executive control network (ECN) showed a modulation by *APOE*-ε4 genotype. As indicated by the positive model coefficient (β), *APOE*-ε4 carriers (orange) showed more positive coupling (i.e., increased positive functional connectivity, ECN-DMN FC) over time as compared to the noncarriers (green) regardless of baseline age. R^2 refers to the variance explained by the fixed and random effects of the linear mixed model [Color figure can be viewed at wileyonlinelibrary.com]

Motion scrubbing did not change the results substantially (Supporting Information, Table S5).

3.3 | Genotype effect on the longitudinal changes in functional connectivity

Individual differences across time were substantial in both FC and cognitive measures (Supporting Information, Table S2). *APOE* genotype did not yield any statistically significant group effect in grey matter volume and intranetwork FCs changes over time. For internetwork ECN-DMN FC, in addition to the time × age interaction as identified in our previous work (Ng et al., 2016), there was also a statistically significant time × genotype effect ($p = .043$). The positive coefficient (0.01, Table 3) and the estimated longitudinal patterns (Figure 3) suggested that *APOE*-ε4 carriers had more loss of functional segregation between the DMN and ECN over time than noncarriers. Exploration on the FC measures that did not show a longitudinal change on average in our past study showed no evidence of a longitudinal *APOE*-ε4 genotype effect (Supporting Information, Table S3).

3.4 | Genotype effect on the longitudinal changes in cognition

Genotype did not yield statistically significant moderation on the longitudinal decline in processing speed. Exploration on the cognitive domains that did not show longitudinal decline in our past study also showed no evidence of a longitudinal *APOE*-ε4 genotype effect (Supporting Information, Table S4).

TABLE 4 Statistical summary of longitudinal *APOE* genotype moderating effect on brain–cognition association in healthy older adults

Regression term	Coefficient	Standard error	*t* value	*p* value
Gender	−0.0608	0.1225	−0.50	.621
Education level	−0.0193	0.0189	−1.02	.311
GMV_{ECN}	141.70	155.30	0.91	.365
GMV_{DMN}	−129.70	173.70	−0.75	.458
APOE	−0.0451	0.1772	−0.26	.800
Age	−0.0097	0.0110	−0.88	.382
FC	**−100.20**	**33.23**	**−3.01**	**.004**
APOE × Age	−0.0502	0.0332	−1.51	.136
APOE × FC	196.00	136.30	1.44	.155
FC × Age	−7.5450	7.03	−1.07	.287
***APOE* × Age × FC**	**59.27**	**28.97**	**2.05**	**.045**

Note. Abbreviations: GMV = grey matter volume.
APOE-ε4 genotype interacted with age and the rate of change in inter-network functional connectivity (FC) between the default mode (DMN) and executive control network (ECN) to influence the rate of change in processing speed performance. Statistically significant effects ($p < .05$) are highlighted in bold.

3.5 | Genotype effect on the longitudinal changes in brain–cognition association

We finally examined the moderating effect of the *APOE*-ε4 allele on the association of inter-network ECN-DMN FC changes with the change in processing speed (Ng et al., 2016), given the presence of a genotype effect on the FC. The association between the rate of reduction in ECN-DMN segregation and the rate of decline in processing speed showed a significant *APOE* × age × FC interaction ($p = .045$; Table 4). Plotting the predicted brain–cognition regression slopes at three example ages (Figure 4) suggested that noncarriers showed a negative correlation throughout all older ages, such that faster decline in processing speed was associated with greater loss of ECN-DMN segregation as reported previously (Ng et al., 2016); interestingly, younger *APOE*-ε4 carriers showed a similar negative association, but this association appeared to reverse gradually in the older carriers.

Motion scrubbing did not alter the longitudinal results substantially (Supporting Information, Tables S6 and S7).

4 | DISCUSSION

Our principal objective was to examine cross-sectional and longitudinal changes simultaneously in task-free intra- and internetwork FC between *APOE*-ε4 carriers and noncarriers in a sample of healthy older individuals. Indeed, FC differed cross-sectionally and longitudinally between the two *APOE*-ε4 genotype groups. Cross-sectionally, *APOE*-ε4 carriers had lower functional connectivity between the ECN and SN than carriers; they also had lower visuospatial memory performance in an age-dependent manner. Longitudinally, carriers had more loss of functional segregation between the DMN and ECN than noncarriers over time (Chan et al., 2014; Geerligs et al., 2014); the association between the rate of reduced segregation of ECN-DMN and the rate of declined processing speed was moderated by age and *APOE*-ε4 genotype. Collectively, these findings are consistent with the proposal that *APOE*-ε4 accelerates biological ageing (Bartzokis et al., 2007; Matura et al., 2014). Among other adversities, the *APOE*-ε4 allele would speed up the ageing process through brain network degradation, which may include aberrant changes in functional segregation between core cognitive ICNs; the allele may also exert synergistic effect with age-related changes on such degradation to hamper cognitive performance in healthy old adults.

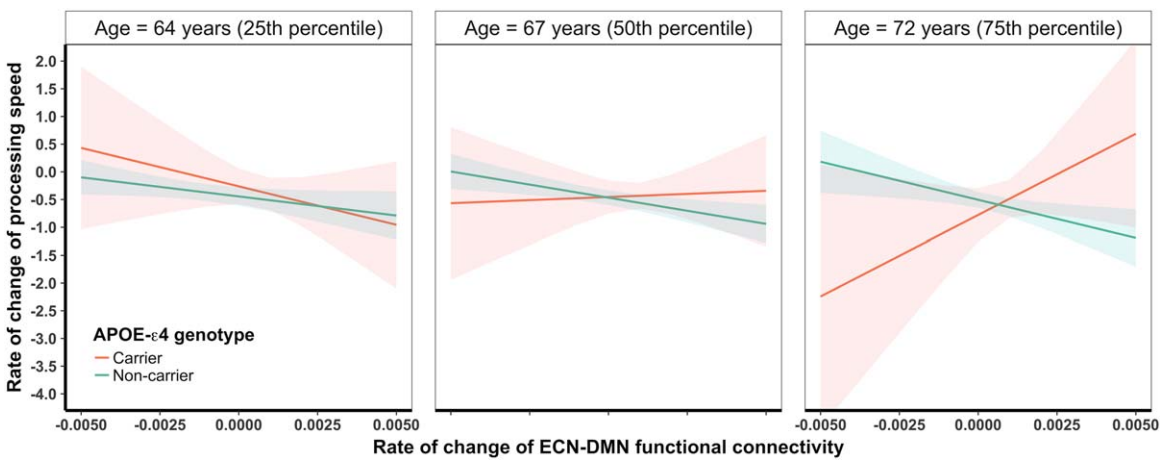

FIGURE 4 *APOE*-ε4 genotype moderated brain–cognition association. The genotype effect in the brain–cognition associations estimated by the multiple regression model is plotted at three example ages, based on the age range at baseline, for *APOE*-ε4 carriers (orange lines) and noncarriers (green lines). While increased coupling between the executive control and default mode network (ECN-DMN functional connectivity, positive is worse) was generally associated with declining processing speed performance (negative is worse) in *APOE*-ε4 noncarriers (all green lines), this trend was present with stronger correlations in younger carriers (orange line, first panel) but gradually reversed in the older carriers (orange line, third panel). Shades represent 95% confidence intervals [Color figure can be viewed at wileyonlinelibrary.com]

4.1 | APOE-ε4 was associated with poor visuospatial memory performance

Neuropsychological findings on *APOE*-ε4 effect have been mixed (Knight et al., 2014; Reinvang et al., 2013), but stronger evidence has pointed toward poorer memory and executive function performance comparing carriers to noncarriers (Small, Rosnick, Fratiglioni, & Backman, 2004). In our cohort, this difference appeared as a stronger age-dependent decrease in the visuospatial memory performance in carriers than noncarriers cross-sectionally.

4.2 | APOE-ε4 was associated with lesser coupling between task-positive intrinsic connectivity networks but not within-networks across individuals

We found suggestive evidence of a cross-sectional internetwork ECN-SN FC difference between the *APOE*-ε4 carriers and noncarriers. While as distinct functional networks, the ECN and the SN tended to share positive FC (He et al., 2013), consistent with their increased activation during cognitive tasks compared to the deactivated DMN. Past studies on healthy ageing have demonstrated the importance of the connectivity profile with the SN (Chand, Wu, Hajjar, & Qiu, 2017; Onoda, Ishihara, & Yamaguchi, 2012), for instance, by improving the classification success on episodic memory performance and executive function between young and old participants (La Corte et al., 2016). Lower ECN-SN FC may suggest that *APOE*-ε4 carriers had aberrant communication between networks critical for externally oriented cognition compared to noncarriers. While we did not observe a reliable linear relationship between the cross-sectional changes in the ECN-SN FC and visuospatial memory performance, both networks subserve functions related to cognitive control processes that are important for carrying out cognitive tasks (Beason-Held, Hohman, Venkatraman, An, & Resnick, 2017; Suri et al., 2017). Therefore, one possible hypothesis is that carriers have altered regulation of control processes that subsequently influence their memory performance.

4.3 | APOE-ε4 was associated with reduced internetwork segregation but not intranetwork specialization in aging adults over time

Interestingly, we found that *APOE*-ε4 carriers suffered more loss in ECN-DMN functional segregation than noncarriers over time. While noncarriers showed a gradual loss of segregation after a period of maintained segregation as a function of baseline age, *APOE*-ε4 carriers demonstrated a consistently steeper reduction of segregation across all baseline ages. Decreasing network segregation is argued to be a key feature of brain ageing (Chan et al., 2014; Geerligs et al., 2014), thus *APOE*-ε4 may compromise normal ageing process by accelerating it. Multimodal neuroimaging findings may lend support to this possible linkage. *APOE*-ε4 genotype effects on task-free FC have been attributed to different neurodevelopmental courses between carriers and noncarriers (Dowell et al., 2016; Ferreira et al., 2016; Trachtenberg et al., 2012). In parallel, task-based fMRI studies had revealed less deactivation in the DMN in carriers than noncarriers during task performance, implicating a disrupted balance between the DMN and task-positive networks (Lind et al., 2006; Persson et al., 2008). Middle-aged *APOE*-ε4 carriers also showed activation patterns that were more similar to those of the elderly than their noncarrier cohort during attention and memory tasks (Evans et al., 2014). *APOE*-ε4 allele has also been linked to accelerated atrophy in various limbic and cortical areas (Hostage et al., 2013) and higher baseline Aβ deposition (Resnick et al., 2015). As a result of accelerated ageing, *APOE*-ε4 carriers might be more vulnerable to pathology. One possibility may lie in its close link with Aβ, which has been shown to disrupt local metabolism and FC already in clinically normal participants (Fouquet, Besson, Gonneaud, La Joie, & Chetelat, 2014; Kang et al., 2017; Schultz et al., 2017). *APOE*-ε4 may promote Aβ deposition, exacerbating its detrimental impact on brain function and structure (Ba et al., 2016; Resnick et al., 2015).

Unexpectedly, despite previous cross-sectional findings on the differences in intranetwork FC between *APOE*-ε4 groups (Machulda et al., 2011; Wu et al., 2016), we did not find *APOE*-ε4-related intranetwork FC changes over time (nor cross-sectionally). Age-related decrease in intranetwork FC, a sign of compromised specialized processing, is also a common phenomenon in aging (Damoiseaux, 2017). The lack of evidence of the influence of *APOE*-ε4 allele on intranetwork FCs could be due to methodological difference between network level and regional FC (Dennis and Thompson, 2014) or cohort differences. Nevertheless, it might also suggest higher vulnerability of internetwork segregation, which is argued to mature later in life (Tian, Ma, & Wang, 2016) and is extensively affected in healthy ageing (Ferreira et al., 2016), to risk factors such as genetic predisposition.

4.4 | APOE-ε4 moderated the association between loss of functional segregation and declining processing speed

Importantly, we found that the correlation between the rate of reduced ECN-DMN segregation and the rate of declined processing speed reported previously (Ng et al., 2016) was modulated by age and *APOE* genotype in an intriguing fashion. This brain–cognition association between poorer functional segregation and poorer processing speed was expressed in both *APOE*-ε4 noncarriers and younger carriers; however, this was gradually reversed in older *APOE*-ε4 carriers. A possible scenario is that younger old individuals may be able to maintain functional segregation (internetwork FC) to compensate for their decline in functional specialization (intranetwork FC) of the core ICNs but they fail to regulate this segregation later in life (Ng et al., 2016). In contrast, *APOE*-ε4 carriers appear to exhaust this compensatory process faster (i.e., in younger carriers). For instance, Ye et al. (2016) examined the longitudinal changes in the FC between the left hippocampus and the right frontal regions in *APOE*-ε4 carriers and noncarriers who were cognitively normal or MCI. Over 35 months, normal noncarriers showed maintained FC, MCI noncarriers showed increased FC, normal carriers showed increased FC, and MCI carriers showed decreased FC. Thus, the FC changes in *APOE*-ε4 carriers (normal-to-MCI) appeared as an advanced version (increase-to-decrease) of the noncarriers (stable-to-

increase), which is qualitatively similar to the internetwork FC changes we observed. Furthermore, the longitudinal increase in hippocampal-right inferior frontal FC in their study was associated with better episodic memory, suggesting a compensatory change (Gregory et al., 2017). Indeed, according to the Default to Executive Coupling Hypothesis of Aging (DECHA), coupling between the DMN and ECN could reflect an adaptation process, in which externally oriented goal-directed neural processes (ECN) are assisted by stored knowledge (DMN) (Turner and Spreng, 2015). Therefore, the reversed brain–cognition association (i.e., increased coupling, slower decline in processing speed) extrapolated to the older carriers might indicate such adaptation to keep their cognitive normality.

Of note, the association between *APOE*-ε4 and AD risk has been found to be weaker in the oldest population (83 years old and above), due to reasons such as the increasing potency of other pathological factors and survivor effect (Gardner et al., 2013), future studies and more detailed statistical models are needed to better understand the difference in FC–cognition association between the older elderly ε4 carriers and noncarriers. In addition to insights from the interplay between cohort (cross-sectional) and individual (longitudinal) changes, such studies should also track carriers and noncarriers from middle age to older age, and track them longitudinally for a longer period to provide a more complete picture about within-individual linear and nonlinear trajectory. They should also include task-based data for further clarifying the functional relevance (e.g., if FC changes reflect adaptation or degradation) of this age-dependent modulation (Campbell and Schacter, 2017). Taken together, our findings supported the potential capability of *APOE*-ε4 to accelerate ageing on brain network dynamics.

4.5 | Limitations

We acknowledge a few limitations. First, the number of *APOE*-ε4 carriers in our longitudinal cohort is still relatively small although not atypical (Yang et al., 2014), thus our findings warrant replications in larger samples. Second, the spatial specificity of our findings is relatively low due to the averaging across multiple ROIs within each network. Given the moderate reliability (Supporting Information, Table S2), these functional connectivity measures may have limited sensitivity to detect cognition- or genetic-related associations. Future studies need to improve sensitivity by enhancing longitudinal sampling and considering the complex relationships between more regionally defined functional connectivity (Razlighi et al., 2014). Third, our study focused on linear functional brain changes. Future studies should explore the possible nonlinear age-dependent influence of brain function and structure on brain and cognitive ageing (Hong et al., 2015). Finally, with increasing data availability, the possible differential influence of *APOE*-ε2 allele (Shu et al., 2016) and other important risk factors that are hypothesized to synergize or counteract with *APOE* genotype to impact ageing, such as amyloid disposition (Buckley et al., 2017; Schultz et al., 2017), should be examined.

5 | CONCLUSIONS

To our best knowledge, our study is among the first longitudinal studies to demonstrate greater longitudinal decline in the functional segregation of large-scale brain networks (ECN-DMN) in cognitively intact, older individuals possessing the *APOE*-ε4 allele compared to their counterparts without such allele. Furthermore, both cross-section and longitudinal results pointed to the impact of the *APOE*-ε4 allele on the coupling between these networks. Such results suggest that *APOE*-ε4 may promote more rapid brain ageing in terms of brain network degradation. *APOE*-ε4 also interacted with the age-related longitudinal change in functional segregation to influence cognitive performance in healthy elderly. These detrimental changes in brain and cognition may possibly lead to the higher risk for dementia in *APOE*-ε4 carriers.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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