



# APOE-MS4A genetic interactions are associated with executive dysfunction and network abnormality in clinically mild Alzheimer's disease

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## ABSTRACT

**Purpose of the research:** Although single nucleotide polymorphisms of membrane-spanning 4A (*MS4A*) (rs670139) and several other susceptibility genes have shown interaction effects on the risk of Alzheimer's disease (AD), little is known about the interaction effects of apolipoprotein E (*APOE*) with *MS4A* (rs670139) on cognitive performances, and the underlying pathogenesis is unclear. The study aimed to investigate the *APOE-MS4A* (rs670139) interaction effects on cognitive performances, cortical volumes, and functional connectivity (FC) in brain networks.

**Principal results:** Cognitive performances were characterized in each genotypic group, and were compared between normal controls and patients in each genotypic group. *APOE-MS4A* interaction effects on memory and executive function scores, cortical volumes, and FC in brain networks were demonstrated. Significant effects of *APOE-MS4A* interactions on FC were observed in executive control network (ECN) (T maxima = 4.99, false discovery rate-corrected  $p < .001$ ), the calculation score (F3, 87 = 6.218;  $p = .015$ ), and the volume in prefrontal (F3, 87 = 4.374;  $p = .039$ ) and orbitofrontal cortices (F3, 87 = 6.022;  $p = .016$ ). The calculation score was correlated with each frontal volume (cc) ( $\rho = 0.304$ ;  $p = .004$ ) and genetic interaction-associated FC in ECN ( $\rho = 0.282$ ;  $p = .008$ ). Variations in genotypes affected the relationship between the calculation score and each frontal volume (cc).

**Major conclusions:** These findings indicate that the genetic interaction effects on FC in ECN might contribute to pathogenic mechanisms underlying the interaction effects of *APOE-MS4A* on calculation ability in AD.

## 1. Introduction

The early symptoms in the evolution of Alzheimer's disease (AD) are memory deficits, followed by a progressive decline in visuospatial, attention, and executive function (Petersen and Edot, 2003). Executive function consists of complex attention, planning, initiation, monitoring, inhibition, and flexibility in goal-oriented behavior (Cahn-Weiner et al., 2007). Executive impairment has been associated with loss of

independence in patients with AD (Boyle et al., 2003). Executive function deficits in the early stage of AD are shown to be associated with a rapid decline in instrumental activities of daily living in later stages of dementia (Cahn-Weiner et al., 2007). In addition, symptomatic treatment of executive dysfunction at the mild dementia phase of AD has been suggested to improve the quality of life of the patients (Martyr and Clare, 2012). The pathophysiological process underlying the severity of executive deficits in AD patients may be associated with

**Abbreviations:** A $\beta$ , amyloid  $\beta$ ; AD, Alzheimer's disease; ANOVA, analysis of variance; apoE, apolipoprotein E; CDR, Clinical Dementia Rating; CVLT, Chinese Version Verbal Learning Test; DLPFC, dorsolateral prefrontal cortex; DMN, default mode network; DSB, Digit Span Backward; ECN, executive control network;  $\epsilon 4 +$  carriers, *APOE- $\epsilon 4$*  carriers;  $\epsilon 4$  non-carriers, *APOE- $\epsilon 4$*  non-carriers;  $\epsilon 4 + /GG$ -carriers, *APOE- $\epsilon 4$*  carriers with *MS4A*-rs670139-GG genotype;  $\epsilon 4 + /T$ -allele-carriers, *APOE- $\epsilon 4$*  carriers with *MS4A*-rs670139-T-allele genotype;  $\epsilon 4 - /GG$ -carriers, *APOE- $\epsilon 4$*  non-carriers with *MS4A*-rs670139-GG genotype;  $\epsilon 4 - /T$ -allele-carriers, *APOE- $\epsilon 4$*  non-carriers with *MS4A*-rs670139-T-allele genotype; FC, functional connectivity; FDR, false discovery rate; GM, grey matter; HWE, Hardy-Weinberg equilibrium; MMSE, Mini-Mental State Examination; MNI, Montreal Neurological Institute; MRI, magnetic resonance imaging; NFTs, neurofibrillary tangles; NCs, normal controls; OFI, orbital frontoinsular; PCC, posterior cingulate cortex; rs-fMRI, resting state-functional MRI; SPM 12, Statistic Parametric Mapping software version 12; TIV, total intracranial volume; TMB, Trail Making Test B

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frontal atrophy (Morgen et al., 2014), decreased functional connectivity (FC) within the executive control network (ECN) (Seeley et al., 2007), and genetic factors (Morgen et al., 2014).

With regards to genetic effects, a meta-analysis demonstrates that apolipoprotein E (*APOE-ε4*) carriers perform significantly worse on executive function tasks than non-carriers do (Wisdom et al., 2011). The potential mechanism underlying the executive impairment is that *APOE-ε4* carriers have lower prefrontal volume than non-carriers do (Wishart et al., 2006; Honea et al., 2009; Bender and Raz, 2012). However, *APOE-ε4* carriers sometimes show better performance on executive function tasks than non-carriers do (Wolk and Dickerson, 2010). Increased FC between the prefrontal cortex and the anterior cingulate cortex has been suggested as a neural basis for better executive performances in *APOE-ε4* carriers than in non-carriers (Machulda et al., 2011); however, a link between *APOE-ε4* carrier genotype and decreased neuronal activity in prefrontal areas, as revealed by fluorodeoxyglucose-positron emission tomography has been observed in the prefrontal areas (Arenaza-Urquijo et al., 2015). These discrepancies may be due to interaction effects of *APOE* with age (Bender and Raz, 2012), years of education (Arenaza-Urquijo et al., 2015), or genes (Morgen et al., 2014) on the influence of *APOE-ε4* carrier status on executive performances, prefrontal volumes, or prefrontal activity.

In addition to *APOE-ε4* carrier status, single nucleotide polymorphisms in nine susceptibility genes—namely *ABCA7* (Reitz et al., 2013), *CR1*, *CLU*, *PICALM*, *BIN1* (Seshadri et al., 2010), *CD2AP*, *CD33*, *EPHA1* (Naj et al., 2011), and membrane-spanning 4A (*MS4A*) (Naj et al., 2011)—have been shown to be associated with the risk of AD. These genes fall into several functional pathways, including immune response, cholesterol metabolism, and synaptic function (Karch et al., 2012; Tanzi, 2012). As ion channel adaptor proteins (Zuccolo et al., 2010), *MS4A* family members are involved in immune response (Antunez et al., 2011) and cellular signal transduction (Tanzi, 2012) in AD. The *MS4A* minor allele T (Hollingsworth et al., 2011) in rs670139 has been found to be associated with increased amyloid  $\beta$  ( $A\beta$ ) plaques and intracellular neurofibrillary tangles (NFTs) (Karch et al., 2012). This minor allele of *MS4A* (rs670139) may have a detrimental effect on clinical severity by affecting the NFT load (Karch et al., 2012); however, *MS4A*-rs670139-T-allele sometimes is shown to have a protective role in the pathogenesis of AD (Naj et al., 2011). Discrepancies between the previous studies suggest the possible effects of gene-environment or gene-gene interactions (epistasis).

Genetic interactions between *APOE* and *PICALM* have been found to be associated with structural and clinical heterogeneity of AD (Morgen et al., 2014). Evidence has revealed the interaction effects of *MS4A* (rs670139) with *CLU* (Lambert et al., 2013) or *CD33* (Lambert et al., 2013; Ebbert et al., 2014) on conferring risk of AD. Even though *MS4A* and *APOE* are both involved in the control of inflammation (Antunez et al., 2011; Dorey et al., 2014), synaptic function (Herz et al., 2009; Tanzi, 2012),  $A\beta$  generation (Karch et al., 2012; Dorey et al., 2014), and NFT phosphorylation (Han et al., 2010; Karch et al., 2012), little is known about the interaction effects of *MS4A* with *APOE* on these biologic mechanisms. Nonetheless, the interaction between *MS4A* and *APOE* is likely to have phenotypic relevance, which would contribute to the heterogeneity of AD in terms of clinical symptoms, cortical volumes, and FC.

In the present study, we characterized the pattern of cognitive performances in 91 CE patients with different genotypes, and we compared the cognitive performances between these AD patients and normal controls (NCs). We investigated the possible interaction effects of *APOE* with *MS4A* (rs670139) on memory and executive function scores, hippocampal and frontal volume, and FC in default mode network (DMN) and ECN. Through these analyses, this study aimed to explore the contribution of these genetic variants to clinical and pathological heterogeneity in AD.

## 2. Materials and methods

### 2.1. Inclusion and exclusion criteria

Ninety-one patients with AD were enrolled from the Department of Neurology of Chang Gung Memorial Hospital from 2011 to 2017. They were diagnosed according to the International Working Group criteria (Dubois et al., 2010) with a clinically typical AD. The diagnostic biomarkers included the medial temporal lobe atrophy on magnetic resonance imaging (MRI), and/or reduced glucose metabolism in temporoparietal regions on fluorodeoxyglucose-positron emission tomography, and/or increased florbetapir retention on amyloid positron emission tomography. The exclusion criteria included a history of clinical stroke, a modified Hachinski ischemic score  $> 4$  (Rosen et al., 1980), or depression. The patients were included on the basis of a consensus of panels composed of neurologists, neuroradiologists, and experts in nuclear medicine. Only mild-stage AD patients with a Clinical Dementia Rating (CDR) score of 0.5 or 1 were included in this study. All AD patients were under stable treatment with acetylcholine esterase inhibitors from the time of diagnosis. NCs were recruited from an outpatient clinic with a 1:1 age-, sex-, and education (years)-matched to AD patients. Twenty-nine included NCs had no underlying neurological or psychiatric disorder, no subjective or objective cognitive deficits, and no neuroimaging abnormalities.

### 2.2. Study design

The study was approved by Chang Gung Memorial Hospital's Institutional Review Committee on Human Research, and all of the participants and their authorized caregivers (when appropriate) provided written informed consent. Cognitive testing and MRI were all performed within a period of four weeks.

### 2.3. Genotyping

Genomic DNA was extracted from blood samples using a commercial kit (Gentra Puregene Blood Kit, Qiagen, USA), followed by genotyping for SNP rs670139 at the *MS4A* gene using the polymerase chain reaction-restriction fragment length polymorphism method (Elias-Sonnenschein et al., 2013). The *APOE* genotype was also determined. Genotyping was conducted by an operator blinded to the clinical data. Patients were classified into two genotypic groups based on the *MS4A* SNP: rs670139-GG carriers (GG-carriers) and rs670139-T-allele carriers (T-allele-carriers). Those with one or two *APOE-ε4* alleles were defined as *APOE-ε4* carriers ( $\epsilon4+$  carriers), and the remainder were classified as *APOE-ε4* non-carriers ( $\epsilon4$  non-carriers). Among the 53  $\epsilon4$  non-carriers, 52 non-carriers were homozygous for the  $\epsilon3$  allele ( $\epsilon3/\epsilon3$ ) and one non-carrier was heterozygous ( $\epsilon2/\epsilon3$ ). In addition, 33  $\epsilon4+$  carriers were heterozygous ( $\epsilon3/\epsilon4$ ), and five carriers were homozygous ( $\epsilon4/\epsilon4$ ). The  $\chi^2$  test was used to assess whether the allele frequencies agreed with expectation in Hardy-Weinberg equilibrium (HWE). Statistical significance was set at  $P < .05$ .

### 2.4. MRI acquisition

MRI scans were acquired on a GE 3 T Signa Excite scanner (GE Medical System, Milwaukee, WI). The scanning protocol of T1-weighted imaging included inversion-recovery-prepared, three-dimensional, spoiled, gradient-recalled acquisition in a steady-state sequence with a repetition time/inversion time of 8600 ms/450 ms,  $240 \times 240$  mm field of view, and 1-mm slice thickness. Resting state-functional MRI (rs-fMRI) scans were performed with the patients' eyes closed using a T2\*-weighted echo-planar imaging sequence (TR 2500 ms, TE 45 ms, FOV  $240 \times 240$  mm, flip angle  $10^\circ$ , thickness 4 mm, and 200 scans of 32 contiguous axial slices) with a total scanning time of 10 min per subject.

## 2.5. Structural MRI pre-processing

The Statistic Parametric Mapping software version 12 (SPM 12) (<http://www.fil.ion.ucl.ac.uk/spm/software/>) was used to pre-process T1 MRI scans, including removal of non-relevant tissue, intensity and spatial normalization to the Montreal Neurological Institute (MNI) space, and tissue segmentation. Using *Segmentation* in SPM 12, the images were segmented into grey matter (GM) and white matter. The raw volume (cc) in hippocampus, orbitofrontal and prefrontal cortices, and total intracranial volume (TIV) (cc) were estimated with surface-based atlas maps in Computational Anatomy Toolbox 12 in SPM12 (Gaser and Dahnke, 2016). TIV-adjusted regional volume was calculated by raw volume (cc) divided by TIV (cc).

## 2.6. Rs-fMRI pre-processing

The first 10 volumes of rs-fMRI were discarded to reduce fluctuations in MRI signals. The steps of pre-processing rs-fMRI included slice time correction, realignment, segmentation, normalization into standard stereotactic MNI spaces, spatial smoothing using a Gaussian Kernel of 6 mm, and resampling to  $2 \times 2 \times 2$  mm<sup>3</sup>. Subsequently, images were detrended and filtered to 0.008 ~ 0.09 Hz. Bad volumes were detected and repaired if global average blood oxygen level dependent signal from scan to scan exceeded 1% variation and/or if framewise displacement exceeded 0.25 mm/TR. White matter and cerebrospinal fluid signals were regressed out from each voxel using an anatomical component-based noise correction method as implemented in CONN toolbox (<http://www.nitrc.org/projects/conn>) (Behzadi et al., 2007; Whitfield-Gabrieli and Nieto-Castanon, 2012).

## 2.7. Neuropsychological assessments

Memory function was examined using Chinese Version Verbal Learning Test (CVVLT) (Chang et al., 2010), assessing number of items retrieved over four learning trials of a 9-word list in free recall after 10 min (CVVLT-10 min). A modified Rey–Osterrieth Complex Figure copy for visuospatial function (Boone, 2000) was also evaluated. The CDR and Mini-Mental State Examination (MMSE) (Folstein et al., 1975; Morris, 1993) were used to assess the general intellectual function. Executive function was evaluated with Digit Span Backward (DSB) and Trail Making Test B (TMB) (Weintraub et al., 2009). In addition, calculation ability was determined, and the genetic and neural basis for calculation ability was investigated in the present study. Early calculation impairment in AD manifests as difficulties with complex calculation procedures, especially with multiplication, followed by complex addition and subtraction, and lastly by simple addition and subtraction (Mantovan et al., 1999). Difficulties in multiplication in AD patients have been suggested to arise from a monitoring deficit, which is one of the executive function components (Cahn-Weiner et al., 2007). Calculation processing has been associated with increased functional activity in the prefrontal cortex, suggesting the role of ECN in the calculation ability (Menon et al., 2000). Although the calculation ability depends on previous math skills, linguistic abilities, and executive function (Jogi and Kikas, 2016), the calculation score might be associated with executive performances after controlling for educational level of each patient. Moreover, evaluating the calculation performance might play an important role in AD because it has been suggested as a tool for cognitive rehabilitation in dementia patients (Kawashima et al., 2005). Our calculation tests comprised two additions, two subtractions, and one multiplication. Each addition and each subtraction consisted of one and two items respectively, and the multiplication is composed of a multiplicand of > 100 and a multiplier of < 100 (e.g.  $214 \times 35$ ). Patients were asked to perform the computations in a written form. Calculation performance was assessed using number of correct answers over the five calculation tests.

## 2.8. Statistical analysis

### 2.8.1. Comparison of clinical and volumetric variables

Clinical and volumetric data were expressed as mean  $\pm$  standard deviation. Based on the study rationale, the patients were further classified into four genotypic groups:  $\epsilon 4+$  carriers with *MS4A*-rs670139-GG genotype ( $\epsilon 4+$ /GG-carriers);  $\epsilon 4+$  carriers with *MS4A*-rs670139-T-allele genotype ( $\epsilon 4+$ /T-allele-carriers);  $\epsilon 4$  non-carriers with *MS4A*-rs670139-GG genotype ( $\epsilon 4-$ /GG-carriers); and  $\epsilon 4$  non-carriers with *MS4A*-rs670139-T-allele genotype ( $\epsilon 4-$ /T-allele-carriers). The Mann-Whitney *U* test for continuous variables and  $\chi^2$  test for dichotomous variables were used to compare among each genotypic group and control group. All statistical analyses for continuous variables were conducted using SPSS software (SPSS version 22 for Windows®, SPSS Inc., Chicago, IL).

### 2.8.2. Interaction effects on cognitive and volumetric variables

Analysis of variance (ANOVA) was used to identify significant effects of the interactions between *APOE* ( $\epsilon 4+$  carriers versus  $\epsilon 4$  non-carriers) and *MS4A* (GG- versus T-allele-carriers) on cognitive function scores and regional volume.

We used partial correlation to analyze the relationship between each frontal volume and each executive function score in all AD patients and AD patients in each genotypic group. We then used Fisher transformation to further analyze the differences in correlation coefficient value of  $\rho$ , which indicates the relation of each executive function score with each frontal volume between different genotype group.

### 2.8.3. Interaction effects on cognition and FC in brain networks

An ANOVA was used to investigate the effects of the interactions between *APOE* and *MS4A* (rs670139) on FC within DMN anchored by the posterior cingulate cortex (PCC) seed (radius 10 mm), and on FC within ECN anchored by the dorsal lateral prefrontal cortex (DLPFC) and those of orbital frontoinsula (OFI) seeds (radius 10 mm). MNI coordinates of PCC seed were  $x = -2$ ,  $y = -36$ ,  $z = 35$  (Margulies et al., 2009), while those of DLPFC seed were  $x = 44$ ,  $y = 36$ ,  $z = 20$  and OFI seed were  $x = 38$ ,  $y = 26$ ,  $z = -10$  (Seeley et al., 2007). The ANOVA was only conducted in AD patients.

The ANOVA was computed in CONN toolbox (Whitfield-Gabrieli and Nieto-Castanon, 2012) with threshold of uncorrected  $p < .001$  at peak-level and false discovery rate (FDR)-corrected  $p < .05$  at cluster-level, using second-level analysis of relative functional inter-regional covariance, the FC in brain networks. The whole-brain correlation maps were converted into z-score maps using a Fisher's *r*-to-*z* transformation. After demonstrating the genetic effect on FC in the brain networks, FC between each seed and each peak cluster was further extracted (Whitfield-Gabrieli and Nieto-Castanon, 2012) to correlate with the cognitive function scores.

Because education (years) had significant correlation with each executive function score ( $p < .05$ ), the correlation of executive function scores with other variables was analyzed using a partial correlation after controlling for education (years).

## 3. Results

### 3.1. Clinical and pathological characteristics

In total, 91 CE patients (32 GG-carriers and 59 T-allele-carriers) and 29 NCs completed the study. AD patients within each genotypic group had significantly lower scores in MMSE, CDR, and CVVLT-10 min than NCs ( $P < .05$ ). AD patients in the genotypic groups except the  $\epsilon 4-$ /GG-carrier group had lower hippocampal volume than NCs ( $P < .05$ ). Other detailed results of comparison between NCs and patients in each genotypic group are presented in Table 1. The distribution of the *MS4A*-rs670139-TT genotype conformed to HWE with  $\chi^2 = 0.006$  and  $P = .939$ , and the distribution of the *APOE*- $\epsilon 4$  carrier genotype

**Table 1**  
Demographic and clinical data in the genotypic groups.

	Normal controls	$\epsilon 4 + /GG$ -carriers	$\epsilon 4 + /T$ -allele-carriers	$\epsilon 4 - /GG$ -carriers	$\epsilon 4 - /T$ -allele-carriers	P value
Sample size (n)	29	17	21	15	38	
Age (years)	69.9 $\pm$ 5.1	70.0 $\pm$ 6.2	72.2 $\pm$ 8.1	70.1 $\pm$ 7.4	72.6 $\pm$ 8.2	0.453
Sex (woman/man)	12/17	9/8	11/10	4/11	15/23	0.510
Education (years)	8.8 $\pm$ 4.3	8.2 $\pm$ 5.5	7.7 $\pm$ 5.3	9.7 $\pm$ 4.4	8.4 $\pm$ 5.0	0.798
MMSE	27.0 $\pm$ 2.3	20.3 $\pm$ 6.5* (< 0.001)	21.9 $\pm$ 4.9* (0.001)	21.8 $\pm$ 6.6* (0.002)	23.1 $\pm$ 5.4* (0.002)	< 0.001
Clinical Dementia Rating	0.0 $\pm$ 0.0	0.6 $\pm$ 0.3* (< 0.001)	0.6 $\pm$ 0.3* (< 0.001)	0.6 $\pm$ 0.2* (< 0.001)	0.5 $\pm$ 0.2* (< 0.001)	< 0.001
Memory function score						
CVVLT-10 min	7.0 $\pm$ 1.6	2.5 $\pm$ 3.4* (< 0.001)	3.2 $\pm$ 3.4* (< 0.001)	5.0 $\pm$ 2.2*† <sup>a</sup> (0.014)	5.3 $\pm$ 2.1*† <sup>b</sup> (0.008)	< 0.001
Visuospatial function score						
Modified ROCF copy	16.7 $\pm$ 0.9	13.6 $\pm$ 5.4*	14.8 $\pm$ 4.1	14.6 $\pm$ 4.4	14.8 $\pm$ 4.8	0.140
Executive function score						
TMB (seconds)	72.7 $\pm$ 35.7	94.8 $\pm$ 37.6* (0.040)	97.1 $\pm$ 34.8* (0.016)	93.1 $\pm$ 29.1 (0.068)	88.7 $\pm$ 34.8 (0.065)	0.097
Digital span backward	4.5 $\pm$ 1.4	3.1 $\pm$ 1.7* (0.004)	3.7 $\pm$ 1.6 (0.077)	4.1 $\pm$ 1.7 (0.466)	3.7 $\pm$ 1.3* (0.028)	0.038
Calculation	4.5 $\pm$ 1.1	3.2 $\pm$ 1.9* (0.002)	4.1 $\pm$ 1.0† <sup>c</sup> (0.358)	4.2 $\pm$ 1.3† <sup>d</sup> (0.560)	3.6 $\pm$ 1.4* (0.008)	0.011
Regional volume (cc)						
Hippocampus	3.7 $\pm$ 0.4	2.8 $\pm$ 0.7* (< 0.001)	3.1 $\pm$ 0.6* (< 0.001)	3.4 $\pm$ 0.6† <sup>e</sup> (0.153)	3.4 $\pm$ 0.6*† <sup>f</sup> (0.022)	< 0.001
Orbitofrontal gyrus	19.5 $\pm$ 2.2	19.1 $\pm$ 2.3	18.6 $\pm$ 1.8	18.4 $\pm$ 2.7	19. $\pm$ 2.6	0.540
Prefrontal gyrus	63.3 $\pm$ 6.5	61.5 $\pm$ 7.0	62.3 $\pm$ 5.4	60.5 $\pm$ 7.8	61.9 $\pm$ 8.1	0.784

Data are presented as mean  $\pm$  standard deviation (P value compared with normal controls); P value denotes significant differences among groups on Mann-Whitney U test for continuous variables, and  $\chi^2$  test for dichotomous variables. \*P < .05, compared with normal controls. †P < .05, compared with  $\epsilon 4 + /GG$ -carriers; P of †<sup>a</sup> = 0.05, †<sup>b</sup> < 0.001, †<sup>c</sup> = 0.037, †<sup>d</sup> = 0.033, †<sup>e</sup> = 0.003, and †<sup>f</sup> = 0.001. CVVLT-10 min, free recall after 10 min in Chinese version of the Verbal Learning Test;  $\epsilon 4 + /GG$ -carriers: *APOE*- $\epsilon 4$  carriers ( $\epsilon 4 +$  carriers) with *MS4A*-rs670139-GG genotype;  $\epsilon 4 + /T$ -allele-carriers:  $\epsilon 4 +$  carriers with *MS4A*-rs670139-T-allele genotype;  $\epsilon 4 - /GG$ -carriers: *APOE*- $\epsilon 4$  non-carriers ( $\epsilon 4 -$  carriers) with *MS4A*-rs670139-GG genotype;  $\epsilon 4 - /T$ -allele-carriers:  $\epsilon 4 -$  carriers with *MS4A*-rs670139-T-allele genotype; MMSE, Mini-Mental State Examination; ROCF, Rey-Osterrieth Complex Figure; TMB, Trail Making Test B.

conformed to HWE with  $\chi^2 = 0.836$  and  $P = .360$ . Allele frequencies did not violate the expectation in HWE.

Because education (years) significantly correlated with the scores in calculation ( $\rho = 0.417$ ,  $p < .001$ ), DSB ( $\rho = 0.324$ ,  $p = .002$ ), and TMB (seconds) ( $\rho = -0.362$ ,  $p < .001$ ), the correlations of the three cognitive scores with other variables were analyzed after controlling for education (years).

To investigate the relationship between the calculation score and executive performances, we correlated the calculation score with DSB and TMB (seconds) scores after controlling for education (years). The calculation score was significantly correlated with TMB (seconds) ( $\rho = -0.373$ ,  $p < .001$ ) and DSB ( $\rho = 0.447$ ,  $p < .001$ ) scores. The results indicated that the calculation ability was associated with executive performances in AD patients.

### 3.2. Effects of genotypic variation on cognitive performances and regional volumes

Dose-dependent gradients were observed in CVVLT-10 min score (Fig. 1A) and hippocampal volumes in the four genotypic groups (Fig. 1B), suggesting the main effect of *APOE* on memory performance and hippocampal volumes. Neither dose-dependent gradient nor interaction effects were observed in modified ROCF copy score (Fig. 1C).

Regarding executive function scores and frontal volumes, this study showed that there were a significant effect of *APOE-MS4A* (rs670139) interaction on the calculation score ( $p = .015$ ; Fig. 1D, Table 2) and a borderline effect of *APOE-MS4A* (rs670139) interaction on the DSB score (Fig. 1E, Table 2), but the interaction effect on the TMB score (seconds) was not significant ( $p > .05$ ; Fig. 1F). Moreover, there were interaction effects of *APOE* with *MS4A* (rs670139) on the volumes in orbitofrontal and prefrontal cortices ( $p < .05$ ; Fig. 1G–H, Table 2).

Because there were genetic interaction effects on calculation and DSB scores, as well as orbitofrontal and prefrontal volumes, we correlated the two executive function scores with each frontal volume. Each volume of interest is shown on left panel of Fig. 2. After controlling for education (years) and TIV (cc), DSB score was correlated with orbitofrontal volume (cc) ( $\rho = 0.258$ ,  $p = .014$ ; Fig. 2) and calculation score had correlation with both orbitofrontal ( $\rho = 0.236$ ,  $p = .026$ ; Fig. 2)

and prefrontal ( $\rho = 0.304$ ,  $p = .004$ ; Fig. 2) volumes (cc).

### 3.3. Genetic interaction effects on FC in brain networks and scores in calculation and DSB

ANOVA showed interaction effects of *APOE* with *MS4A* (rs670139) on FC within DMN anchored by the PCC seed (Table 2), and within ECN anchored by the DLPFC and OFI seeds (Table 2 and Fig. 3A). DLPFC and OFI seeds are shown on top of Fig. 3. The genetic interaction-associated FC in DMN and ECN anchored by the DLPFC seed did not correlate with the calculation or DSB scores ( $p > .05$ ).

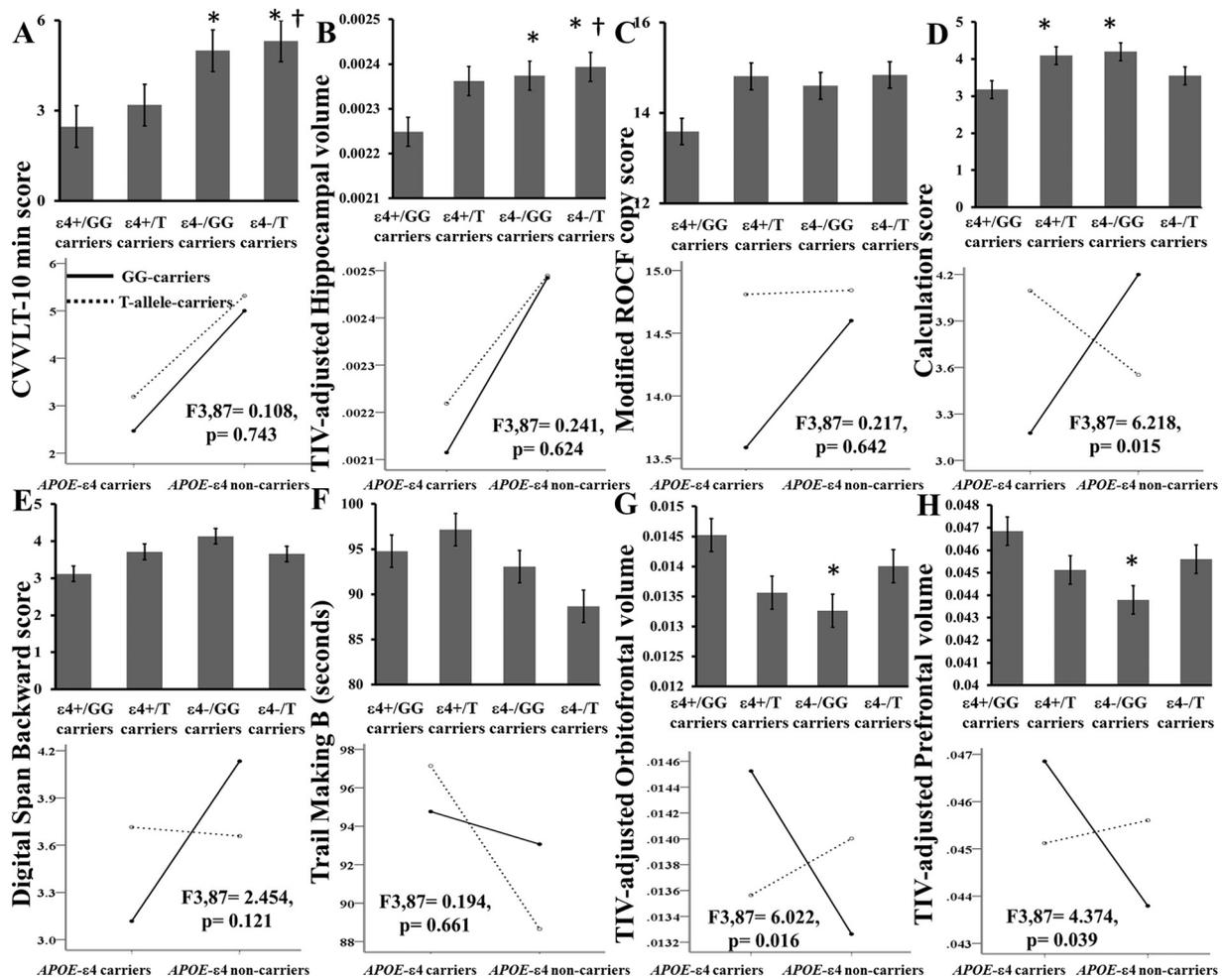
In ECN anchored by the OFI seed, *APOE-MS4A* interaction-associated FC in the left middle frontal gyrus ( $x = -36$ ,  $y = 18$ ,  $z = 42$ ; peak cluster 4 in Fig. 3A) had correlation with the calculation score ( $\rho = 0.286$ ,  $p = .007$ , Fig. 3B), but not with the DSB score ( $p > .05$ ). We further analyzed the association between the calculation score and FC of left middle frontal gyrus (peak cluster 4) with the OFI and DLPFC seeds, respectively. We demonstrated that the calculation score was correlated with FC between the left middle frontal gyrus (peak cluster 4) and the OFI seed ( $\rho = 0.259$ ,  $p = .014$ , Fig. 3C), but not with FC between the left middle frontal gyrus (peak cluster 4) and the DLPFC seed ( $p = .145$ ; Fig. 3D).

We further added GM maps as covariance of no interest to the genetic interaction effects on FC within ECN anchored by the DLPFC and OFI seeds (Fig. 4).

In ECN anchored by the OFI seed, *APOE-MS4A* interaction-associated FC in the left middle frontal gyrus ( $x = -28$ ,  $y = 4$ ,  $z = 50$ ; T maxima = 4.99; Cluster size = 1446; FDR-corrected  $p < .001$ ) had correlation with calculation score ( $\rho = 0.282$ ,  $p = .008$ , Fig. 4). In ECN anchored by the DLPFC seed, *APOE-MS4A* interaction-associated FC in the left supramarginal gyrus ( $x = -46$ ,  $y = -28$ ,  $z = 24$ ; T maxima = 4.70; Cluster size = 527; FDR-corrected  $p = .001$ ) did not correlate with calculation score ( $p = .765$ , Fig. 4).

### 3.4. Genetic interaction on the calculation score, FC, and frontal volume

Genetic interaction-associated FC between left middle frontal gyrus (peak cluster 4 in Fig. 3A and peak cluster in right panel of Fig. 4) and



**Fig. 1.** Interaction effects of APOE with MS4A (rs670139) on cognitive function scores (A, C–F) and TIV-adjusted volume (B, G–H). \* $P < .05$ , compared to the  $\epsilon 4 + / GG$ -carriers. † $P < .05$ , compared with  $\epsilon 4 + / T$ -allele-carriers.  $\epsilon 4 + / GG$ -carriers: APOE- $\epsilon 4$  carriers ( $\epsilon 4 +$  carriers) with MS4A-rs670139-GG genotype;  $\epsilon 4 + / T$ -allele-carriers:  $\epsilon 4 +$  carriers with MS4A-rs670139-T-allele genotype;  $\epsilon 4 - / GG$ -carriers: APOE- $\epsilon 4$  non-carriers ( $\epsilon 4 -$  carriers) with MS4A-rs670139-GG genotype;  $\epsilon 4 - / T$ -allele-carriers:  $\epsilon 4 -$  carriers with MS4A-rs670139-T-allele genotype; GG-carriers: MS4A-rs670139-GG carriers; T-allele-carriers: MS4A-rs670139-T-allele carriers. CVVLT-10 min: free recall after 10 min in Chinese version of the Verbal Learning Test; TIV: total intracranial volume.

the OFI seed had a correlation with calculation score. The *APOE-ε4* carrier genotype had detrimental effects on FC of left middle frontal gyrus with the OFI seed (peak cluster 4 in Fig. 3A and peak cluster in right panel of Fig. 4) and on calculation score (Fig. 1D) in GG-carriers, but not in T-allele-carriers. Collectively, these results indicated that the genetic interaction effects on FC and the calculation score were similar.

In contrast, the interaction effects between *APOE* and *MS4A* on calculation score were different from that on each frontal volume. The  $\epsilon 4 +$  carriers had lower calculation scores but higher frontal volumes than  $\epsilon 4$  non-carriers in GG-carrier group. We further analyzed the differences in  $\rho$  value, which indicated the correlation of the calculation score with each frontal volume between different genotypic groups (Table 3).

A comparison using Fisher transformation showed significant differences in  $\rho$  value, indicating the correlation of the calculation score with prefrontal volume (cc) between  $\epsilon 4 +$  carriers and non-carriers ( $P = .028$ ), between  $\epsilon 4 + / T$ -allele-carriers and  $\epsilon 4 - / GG$ -carriers ( $P = .001$ ), and between  $\epsilon 4 + / T$ -allele-carriers and  $\epsilon 4 - / T$ -allele-carriers ( $P = .006$ ) (Table 3). The results indicated that the genotypic variation affected the correlation of calculation score with each frontal volume (Table 3).

## 4. Discussion

### 4.1. Main findings

This study has three main findings. First, an *APOE-MS4A* (rs670139) interaction effect on calculation score was observed, and the calculation score was correlated with genetic interaction-associated FC in ECN anchored by the OFI seed. Second, there were *APOE-MS4A* (rs670139) interaction effects on orbitofrontal and prefrontal volume and on FC in ECN anchored by the DLPFC and OFI seeds. The genetic interaction effects were found on FC in ECN mainly between DLPFC seed and left supramarginal gyrus (peak cluster 1 in Fig. 3), as well as between the OFI seed and the left middle frontal gyrus (peak cluster 4 in Fig. 3). After controlling for GM maps, the genetic interaction effects on FC in ECN were still significant between DLPFC seed and left supramarginal gyrus and between the OFI seed and the left middle frontal gyrus (Fig. 4). Third, genotypic variation affected the pathological-clinical relationship between the prefrontal volume and the calculation score.

### 4.2. Interaction effects of APOE with MS4A (rs670139) on FC in ECN

Previous studies have found the evidence of the interaction effect of *MS4A* (rs670139) with *CLU* (Lambert et al., 2013) or *CD33* (Lambert et al., 2013; Ebber et al., 2014) on the AD risk. Although *APOE-MS4A*

**Table 2**

Seed-to-voxel analysis reveals brain regions with significant effects of *APOE-MS4A* (rs670139) interactions on cognitive function scores, regional volume, and functional connectivity in brain networks.

Seed	Cluster	MNI (x, y, z)	Cluster size	T	p-FDR of size
PCC	Left frontal lobe	-26, 4, 24	876	5.78	< 0.001
DLPFC	Left supramarginal gyrus	-46, -26, 24	445	4.70	0.003
	Right medial orbitofrontal gyrus	6, 70, -4	1208	4.22	< 0.001
Orbital Frontoinsula	Right middle orbitofrontal gyrus	42, 54, -16	329	4.59	0.009
	Left middle frontal gyrus	-36, 18, 42	1309	4.71	< 0.001

Cognitive function score or regional volume	Main effects	F3,87	p value
Digital Span Backward score	<i>APOE-ε4</i> carrier genotype	1.965	0.165
	<i>MS4A</i> (rs670139)	0.031	0.860
<i>APOE-ε4</i> carrier genotype × <i>MS4A</i> (rs670139)		2.454	0.121
Calculation score	<i>APOE-ε4</i> carrier genotype	0.586	0.446
	<i>MS4A</i> (rs670139)	0.187	0.667
<i>APOE-ε4</i> carrier genotype × <i>MS4A</i> (rs670139)		6.218	0.015
TIV-adjusted orbitofrontal volume	<i>APOE-ε4</i> carrier genotype	1.411	0.238
	<i>MS4A</i> (rs670139)	0.103	0.749
<i>APOE-ε4</i> carrier genotype × <i>MS4A</i> (rs670139)		6.022	0.016
TIV-adjusted prefrontal volume	<i>APOE-ε4</i> carrier genotype	2.312	0.132
	<i>MS4A</i> (rs670139)	0.002	0.964
<i>APOE-ε4</i> carrier genotype × <i>MS4A</i> (rs670139)		4.374	0.039

T maxima, and contiguous voxels of cluster size are shown. The significance clusters are detected with thresholds of FDR-corrected  $P < .05$  at the cluster-level and uncorrected  $P < .001$  at the peak-level. DLPCF, dorsolateral prefrontal cortex; FDR, false discovery rate; MNI (x, y, z), local maxima coordinates on the Montreal Neurological Institute template brain; PCC, posterior cingulate cortex; TIV, total intracranial volume.

interaction effects are not associated with the increased AD risk (Hollingsworth et al., 2011), we found an interaction effect between *APOE* and *MS4A* on FC in ECN, suggesting that the genetic interaction effects are involved in the pathological changes in patients with AD.

Greater FC between the prefrontal cortex and the anterior cingulate cortex has been found in *APOE-ε4* carriers than in non-carriers in cognitively normal elderly individuals (Machulda et al., 2011). However, another study demonstrates that *APOE-ε4* carriers have lower neuronal activity in prefrontal areas than non-carriers do (Arenaza-Urquijo et al., 2015). The present study showed that *APOE-ε4* carriers had higher FC between left middle frontal gyrus and OFI seed than non-carriers did in *MS4A*-rs670139-T-allele carrier group, rather than in *MS4A*-rs670139-GG carrier group. Taken together, these results suggest that modulation effects of *MS4A* genotype might be potentially implicated in the pathogenic mechanisms underlying the discrepant effects of *APOE-ε4* carrier status on frontal activity or FC of the frontal cortices with other cortical regions shown in previous studies.

The epistasis effect may occur because both *APOE* and *MS4A* modulate neuronal synaptic function. *APOE* encodes apolipoprotein E (apoE) that is involved in the neuronal integrity and regeneration process (Ignatius et al., 1986). *MS4A* family members are involved in cellular signaling transduction (Tanzi, 2012). The genotypic interaction effect on synaptic function might be the molecular basis of *APOE-MS4A* interaction effects on FC in brain networks.

#### 4.3. Interaction effects of *APOE* with *MS4A* (rs670139) on executive performances

With regards to the effect of the *APOE-ε4* carrier status on executive performances and its neural basis, the *APOE-ε4* carriers sometimes have better executive performances than non-carriers do (Wolk and Dickerson, 2010), but sometimes *APOE-ε4* carriers show worse executive performances than non-carriers do (Wisdom et al., 2011). Because the present study demonstrated that *APOE-ε4* carriers had lower calculation scores than non-carriers in the *MS4A*-rs670139-GG carrier group, but had better calculation scores than non-carriers in the *MS4A*-rs670139-T-allele carrier group, genetic interaction effects might be the potential neural basis for the discrepant relationships of the *APOE-ε4* carrier status with executive impairment.

ApoE4 encoded by *APOE-ε4* carrier genotype has been shown to modulate both detrimental toll-like receptor and beneficial interleukin-4R-nuclear receptor pathways in chronic neuroinflammation (Tai et al., 2015). *MS4A* family members have been found to be associated with phagocytic function in microglia and immune responses in brain (Karch et al., 2012). Pro-inflammatory cytokines have been demonstrated to be associated with working memory (Yang et al., 2013). The genotypic interaction effects on neuroinflammation might contribute to the pathogenic mechanisms underlying *APOE-MS4A* interaction effects on the executive performances in the calculation tasks.

#### 4.4. Relationship between executive performances and frontal volumes

The association between executive performances and activation in the prefrontal cortex has been attributed to the critical prefrontal cortex involvement in tasks that require encoding action sequences, coordinating action, and maintaining abstract sequential movement plans (Badre et al., 2009). In agreement with the critical role of the frontal lobe in the executive function status as shown in previous studies, the current study indicated that both DSB and calculation scores correlate with the frontal volume.

Moreover, the present study revealed that DSB score was positively correlated with the orbitofrontal volume, and the calculation score was positively correlated with the orbitofrontal and prefrontal volumes. In this regards, previous studies also indicate that orbitofrontal cortex is involved in the manipulation and monitoring of information in working memory (Barbey et al., 2011), and the inferior and middle prefrontal cortices are associated with the calculation process (Menon et al., 2000).

However, the clinicopathological relationships were discrepant between different genotypic groups. Significant differences were observed in the correlation coefficient of calculation scores with the prefrontal volume between *APOE-ε4* non-carriers and *APOE-ε4* carriers, between  $\epsilon4- /GG$ -carriers and  $\epsilon4+ /T$ -allele-carriers, and between  $\epsilon4- /T$ -allele-carriers and  $\epsilon4+ /T$ -allele-carriers.

Previous study has shown that *APOE-ε4* allele dose-dependently modulates the relationship between executive performances and the cortical volume in cognitively normal aging individuals (Cacciaglia et al., 2018). Our results supported the observation and expanded on

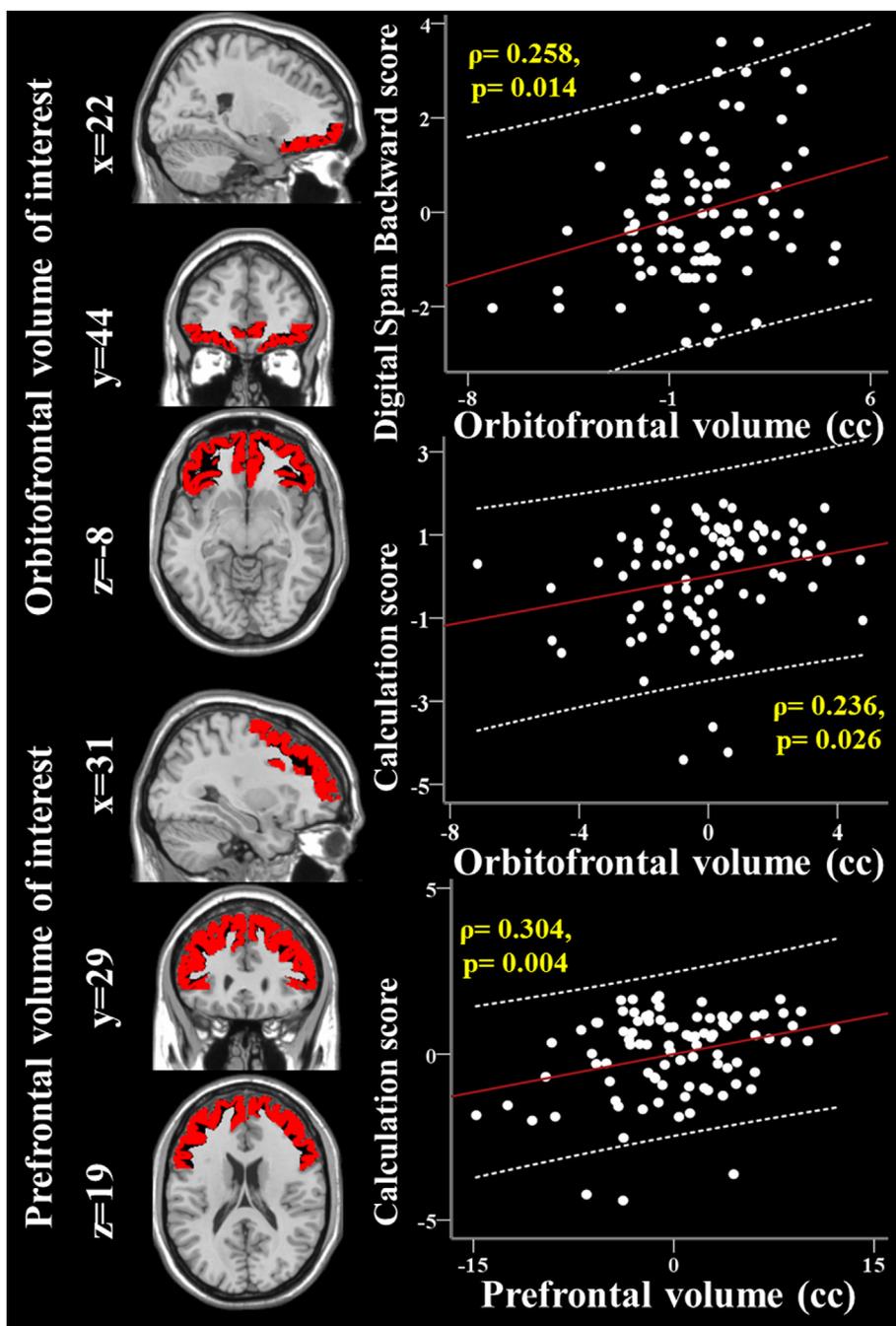
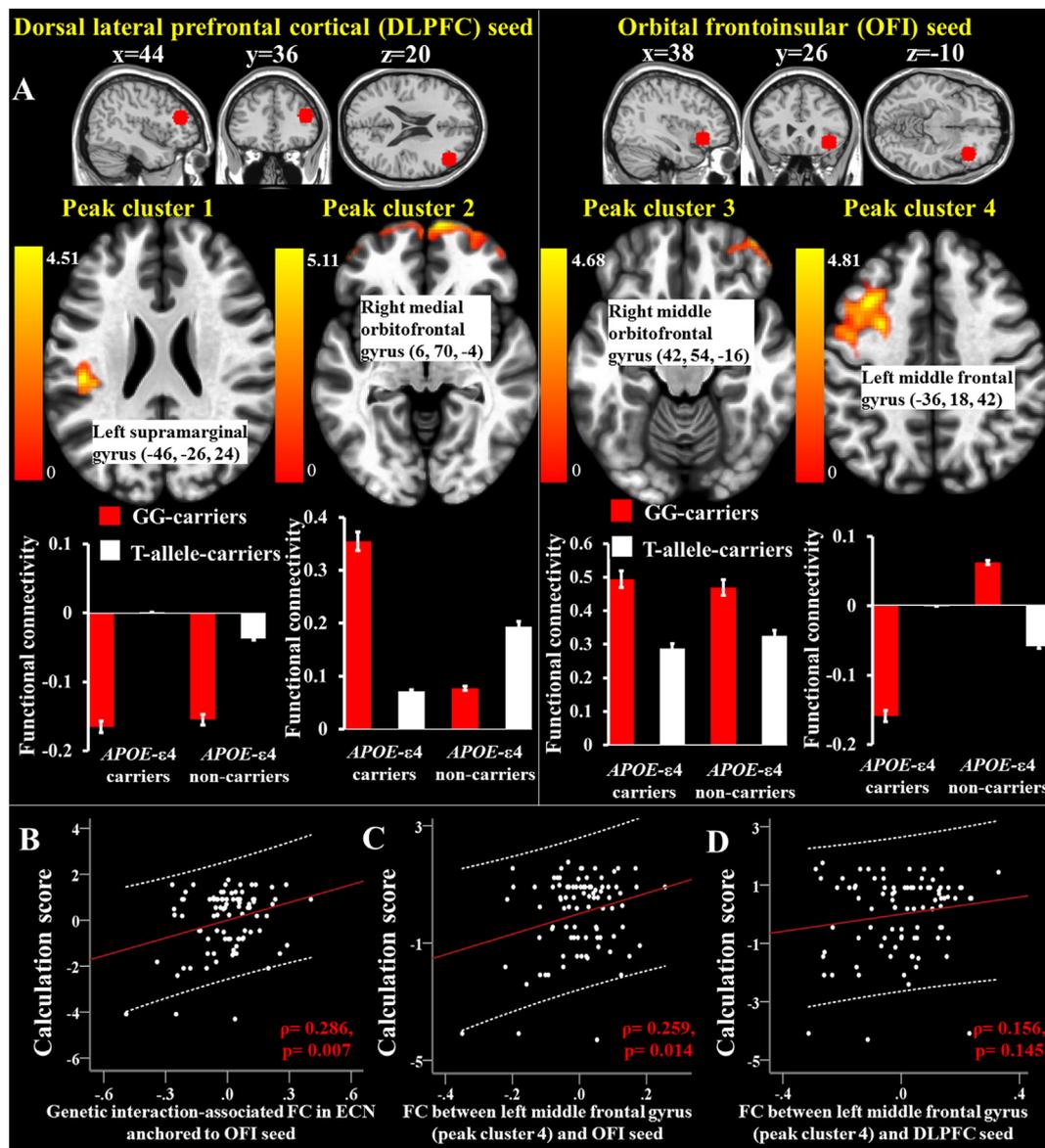


Fig. 2. Each volume of interest rendered on the Montreal Neurological Institute template and partial regression plots indicate the correlation of orbitofrontal and prefrontal volumes (cc) with each cognitive function score. Residuals are plotted for each variable to adjust for the effects of total intracranial volume (cc) and education (years). The 95% confidence interval is the area enclosed by the dashed curves.

some of the previous findings. The present study demonstrated a positive correlation of the calculation score with each frontal volume in the *APOE-ε4* non-carriers with AD, but not in *APOE-ε4* carriers with AD. In addition, the calculation score was correlated with prefrontal volume in *MS4A*-rs670139-GG-carriers with AD, and the score was also correlated with orbitofrontal volume in *MS4A*-rs670139-T-allele carriers with AD. Collectively, these results suggested that both *APOE-ε4* carrier status and *MS4A* (rs670139) genotype modulated the correlation of the calculation score with the prefrontal volume in AD patients.

#### 4.5. Calculation scores and FC between the OFI seed and the left middle frontal gyrus in ECN

The *APOE-MS4A* (rs670139) interaction effects on calculation scores and each frontal volume were different; however, the interaction effects on the calculation score and FC of the OFI seed with the left middle frontal gyrus were similar. The *APOE-ε4* carrier genotype had detrimental effects on the calculation score and FC between left middle frontal gyrus and the OFI seed in the GG-carrier group, but showed protective effects on these variables in the T-allele-carrier group. In addition, the genetic interaction-associated FC was correlated with the calculation score. The genetic interaction effects on FC of the OFI seed with the left middle frontal gyrus might be the neural basis for the



**Fig. 3.** Statistic maps of interaction effects of APOE with MS4A (rs670139) on functional connectivity (FC) in executive control network (ECN). ECN is anchored by the DLPFC and OFI seeds (A), and partial regression plots show correlation between calculation score and FC (B-D). Residuals are plotted for each variable to adjust for the effect of education (years). The 95% confidence interval is the area enclosed by the dashed curves. GG-carriers: MS4A-rs670139-GG carriers; T-allele-carriers: MS4A-rs670139-T-allele carriers.

**APOE-MS4A interaction effect on the calculation performance.**

The relationship between neuronal activity in the middle frontal gyrus and calculation ability has also been demonstrated in one previous study (Menon et al., 2000). Another study further demonstrates that calculation ability is associated with co-activation in the prefrontal and right anterior-insular cortices (Cowell et al., 2000). In consistent with these findings, the present study indicated that the calculation performance was associated with the FC between the OFI seed and middle frontal gyrus.

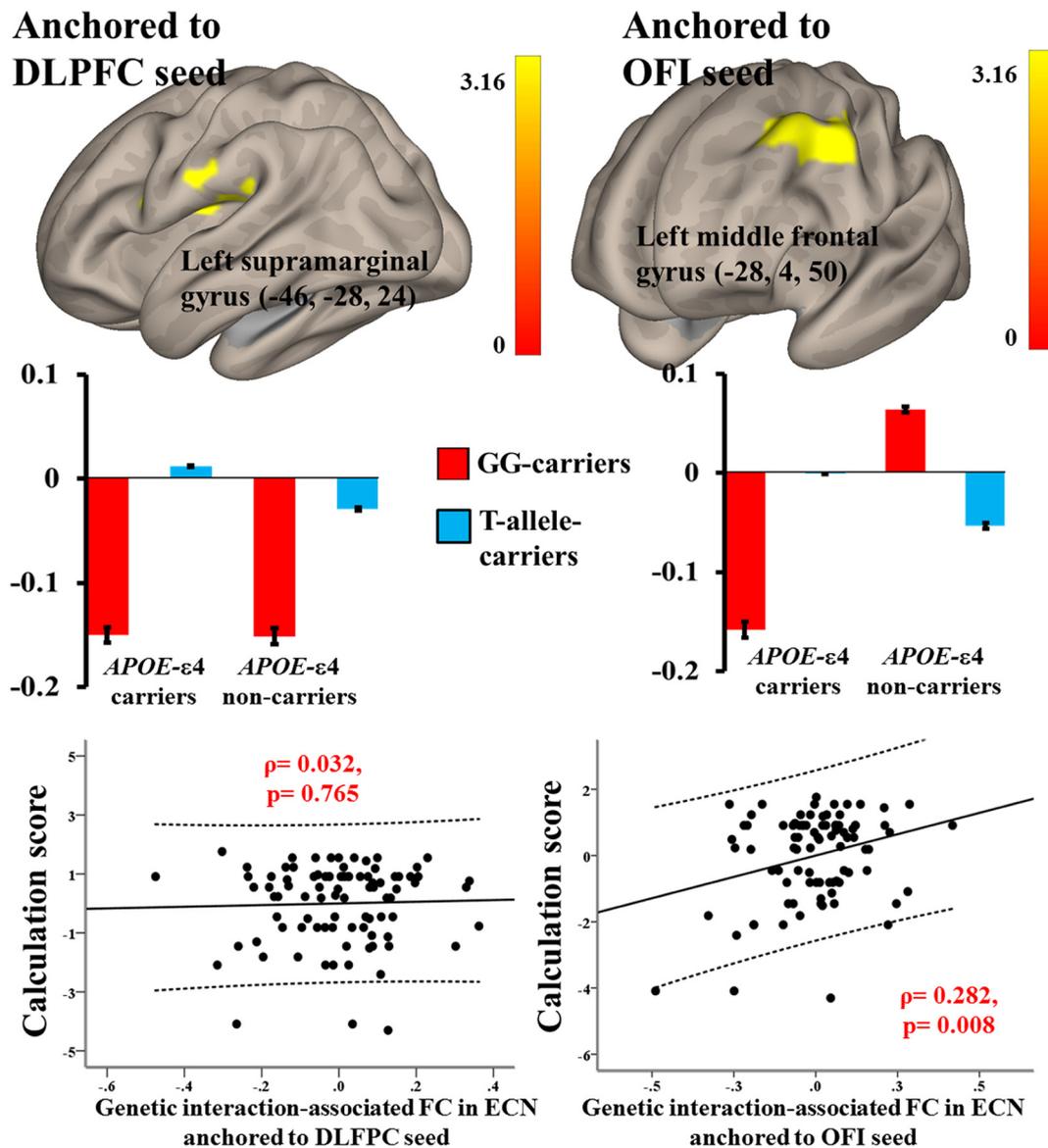
In addition, the DLPFC and inferior parietal gyrus are activated during calculation tasks, as demonstrated by fMRI (Rickard et al., 2000). The present study demonstrated that genetic effects on the calculation score and FC of the DLPFC seed with the left supramarginal gyrus paralleled each other; however, FC of the left supramarginal gyrus with the DLPFC seed did not correlate with the calculation score. Therefore, it would be difficult to determine whether the inferior parietal gyrus is involved in the calculation tasks.

**4.6. Therapeutic consideration in the future**

Identifying the interaction effect of MS4A (rs670139) risk factor with APOE might be useful to illustrate the pathogenesis of cognitive variation in AD and to optimize the treatment strategies for AD.

**4.7. Limitations**

The present study has three limitations. First, although calculation impairment was evident in AD patients, different patterns of calculation dysfunction were not characterized in this study. It is not clear how math skills, linguistic abilities, and executive function affected calculation performance separately. Therefore, to avoid the effects of math skills or linguistic abilities, this present study controlled for the educational level in each correlation analysis. Second, pathological effects of Aβ and NFTs on the genotype-associated variation in the clinical presentation were not elucidated in this study. Further investigation would be needed to determine how pathological depositions influence



**Fig. 4.** Statistic maps of interaction effects of APOE with MS4A (rs670139) on functional connectivity (FC) in executive control network (ECN) after adding grey matter maps as covariance of no interest. Genetic interaction affects FC between the dorsal lateral prefrontal cortical (DLPFC) seed and the left supramarginal gyrus and FC between the orbital fronto-insular (OFI) seed and the left middle frontal gyrus (upper row), and partial regression plots show correlation between calculation score and FC (lower row). Residuals are plotted for each variable to adjust for the effect of education (years). The 95% confidence interval is the area enclosed by the dashed curves. GG-carriers: MS4A-rs670139-GG carriers; T-allele-carriers: MS4A-rs670139-T-allele carriers.

**Table 3**

Correlations of calculation score with each frontal volume in each genotypic groups after controlling for education (years) and total intracranial volume (cc).

	$\epsilon 4 + / \text{GG-carriers}$	$\epsilon 4 + / \text{T-allele-carriers}$	$\epsilon 4 - / \text{GG-carriers}$	$\epsilon 4 - / \text{T-allele-carriers}$
Orbitofrontal volume (cc)	0.163 (0.561)	0.198 (0.415)	0.481 (0.096)	<b>0.349 (0.037)</b>
Prefrontal volume (cc)	0.243 (0.383)	-0.409 (0.082)	<b>0.760 (0.003)*</b>	<b>0.420 (0.011)*</b>
	<i>APOE-<math>\epsilon 4</math> carriers</i>		<i>APOE-<math>\epsilon 4</math> non-carriers</i>	
Orbitofrontal volume (cc)	0.128 (0.458)		<b>0.337 (0.016)</b>	
Prefrontal volume (cc)	-0.006 (0.972)		<b>0.466 (0.001)*</b>	
	<i>GG-carriers</i>		<i>T-allele-carriers</i>	
Orbitofrontal volume (cc)	0.168 (0.374)		<b>0.260 (0.050)</b>	
Prefrontal volume (cc)	<b>0.368 (0.045)</b>		0.259 (0.051)	

Data are represented as  $\rho$  (p) values; bold fonts highlight  $p < .05$ . \*indicates the correlation coefficient with a significant difference from that in the square;  $\epsilon 4 + / \text{GG-carriers}$ : *APOE- $\epsilon 4$  carriers* ( $\epsilon 4 +$  carriers) with *MS4A-rs670139-GG* genotype;  $\epsilon 4 + / \text{T-allele-carriers}$ :  $\epsilon 4 +$  carriers with *MS4A-rs670139-T-allele* genotype;  $\epsilon 4 - / \text{GG-carriers}$ : *APOE- $\epsilon 4$  non-carriers* ( $\epsilon 4 -$  carriers) with *MS4A-rs670139-GG* genotype;  $\epsilon 4 - / \text{T-allele-carriers}$ :  $\epsilon 4 -$  carriers with *MS4A-rs670139-T-allele* genotype; GG-carriers: *MS4A-rs670139-GG* carriers; T-allele-carriers: *MS4A-rs670139-T-allele* carriers.

the genetic interaction effects on cognitive performances, FC in brain networks, and the cortical volume would be needed. Third, the genetic interaction effects on cognitive performances, FC, and cortical volume were only investigated in AD patients. The present analyses were limited to AD patients, due to the small sample size of NC group, to obtain a more genetically and cognitively homogenous study population. Further study would be needed to illustrate how genetic interaction affects pathophysiological changes in NCs.

## 5. Conclusions

We found interaction effects of *APOE* with *MS4A* (rs670139) on calculation performance. The genetic interaction effects on FC of the brain networks might be the neural basis for the *APOE-MS4A* interaction effects on the cognitive performances. Furthermore, this study showed that genotypic variation affected the relationship between the prefrontal volume and the calculation score.

## Ethics approval and consent to participate

The study was approved by Chang Gung Memorial Hospital's Institutional Review Committee on Human Research, and all of the participants and their authorized caregivers provided written informed consent.

## Submission declaration and verification

This work is not published previously, not under consideration for publication elsewhere, and it is approved by all authors.

## Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Competing interests

The authors declare that they have no competing interests.

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## Authors' contributions

YTC participated in the design of the study and drafted the manuscript. YTC and MS performed the statistical analysis. CWH, JJJ and WNC participated in the sequence alignment and clinical evaluation of patients. EM and CCC conceived the study, and participated in its design and coordination, and helped draft the manuscript. All authors read and approved the final manuscript.

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