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RESEARCH ARTICLE

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APOE influences working memory in non-demented elderly through an interaction with *SPON1* rs2618516

Zhen Liu[1,2*] | Xiangwei Dai[1,2*] | Wuhai Tao[1,2*] | Huilan Liu[3*] | He Li[2,4] | Caishui Yang[1,2] | Junying Zhang[1,2] | Xin Li[1,2] | Yaojing Chen[1,2] | Chao Ma[1,2] | Jing Pei[1,2] | Haohao Mao[1,2] | Kewei Chen[2,5] | Zhanjun Zhang[1,2]

[1]State Key Laboratory of Cognitive Neuroscience and Learning & IDG/McGovern Institute for Brain Research, Beijing Normal University, Beijing, 100875, People's Republic of China

[2]BABRI Centre, Beijing Normal University, Beijing, 100875, People's Republic of China

[3]The Third Affiliated Hospital of Beijing University of Chinese Medicine, Beijing, 100029, People's Republic of China

[4]Institute of Basic Research in Clinical Medicine, China Academy of Chinese Medical Sciences, Beijing, 100700, People's Republic of China

[5]Banner Alzheimer's Institute, Phoenix, Arizona

Correspondence
Zhanjun Zhang, MD, State Key Laboratory of Cognitive Neuroscience and Learning, and BABRI Centre, Beijing Normal University, Beijing 100875, China.
Email: zhang_rzs@bnu.edu.cn

Zhen Liu, Xiangwei Dai, Wuhai Tao, and Huilan Liu contributed equally to this study.

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Abstract

Exploring how risk genes cumulatively impair brain function in preclinical phase (i.e., in cognitively normal elderly) could provide critical insights into the pathophysiology of Alzheimer's disease (AD). Working memory impairment has always been a considerable cognitive deficit in AD, which is likely under complex genetic control. Though, the *APOE* ε4 allele could damage the working memory performance in normal elderly, dissociable results have been reported. This allele may exert specific effects in contexts with other genetic variants. The rs2618516 in the spondin 1 gene (*SPON1*) has been associated with AD risk and brain structure in the elderly. *SPON1* may interact with *APOE* through processing the amyloid precursor protein and suppressing amyloid-β levels. Using neuropsychological tasks from 710 individuals, we found significant *SPON1* × *APOE* genotype interactions in working memory and executive function performances. Moreover, such interaction was also found in regional brain activations based on functional magnetic resonance imaging data with the n-back working memory task performed in a sub-cohort of 64 subjects. The effects of ε4 allele on activation of right inferior frontal gyrus, triangular part (IFGtriang.R) were modulated by rs2618516 in a working memory task. Furthermore, lower IFGtriang.R activation was associated with better cognitive functions. Moreover, the IFGtriang.R activation could mediate the impacts of *SPON1* × *APOE* interactions on working memory performance. These findings suggested the importance of weighing *APOE* effects on brain activation under the working memory task within the context of the *SPON1* genotype.

KEYWORDS
activation, Alzheimer's disease, APOE, SPON1, working memory

1 | INTRODUCTION

Cognitive impairment accounts for most clinical symptoms in Alzheimer's disease (AD) (Ferri et al., 2005), but it remains poorly understood and largely difficult to treat. Working memory dysfunction is a key cognitive deficit in AD (Becker, 1988). It is reportedly influenced by a number of factors, such as age. Evidence from a previous study found that healthy older adults showed deteriorated working memory compared with younger adults and teenagers (Fandakova, Sander, Werkle-Bergner, & Shing, 2014). Otherwise, genetic variations are also among the influencing factors for working memory, including the apolipoprotein E (*APOE*) gene

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(Chen et al., 2013), which is the major genetic risk factor for AD (Corder et al., 1993). Previous studies tend to connect worse performance in working memory with the ε4 allele during normal aging (Greenwood, Espeseth, Lin, Reinvang, & Parasuraman, 2014; Reynolds et al., 2006). In keeping with this, there is also evidence that the ε4 allele was associated with an increased rate of decline in working memory (Boyle, Buchman, Wilson, Kelly, & Bennett, 2010). Nevertheless, other researchers discovered dissociable effects of *APOE* on memory in AD, reporting that ε4 carriers displayed greater impairment in memory retention while non-carriers showed more impairment in working memory (Wolk et al., 2010). These results suggest to us that the effects of *APOE* on working memory may be stronger and more predictable in the context of other factors, such as genetic variants that influence *APOE* function.

The spondin 1 gene (*SPON1*) on chromosome 11 encodes the developmentally regulated protein F-spondin, which has been reported to be a putative ligand for the amyloid precursor protein (APP) (Hoe & Rebeck, 2008). The F-spondin would bind to the extracellular domain of APP and inhibit β-secretase cleavage of APP to yield the peptide amyloid-β (Aβ), a core AD neuropathological hallmark (Ho & Sudhof, 2004). In mice, F-spondin overexpression suppressed Aβ levels and improved memory performance (Hafez et al., 2012). Previous longitudinal studies of Aβ and *APOE* demonstrate the importance of interaction between these two contributors in promoting working memory decline in healthy older adults (Mormino et al., 2014; Thai et al., 2015). In consideration of the effect of *SPON1* on APP processing, there may be a modulated influence of *SPON1* on the effect of *APOE* on working memory and task related brain function. Recently, a common single nucleotide polymorphism (SNP) located on 11p15.2 of the *SPON1* gene, named rs2618516, was shown to influence the brain and risk for AD in a genome-wide association study (GWAS) (Jahanshad et al., 2013). Thus, investigation the joint effects of *SPON1* rs2618516 and *APOE* genotype on neural functions during working memory could help our understanding of cognitive deficit during normal aging as well as the preclinical phase of AD. To our knowledge, however, none studies have explored the interactive effect of *SPON1* and *APOE* from the aspects of working memory performance and task-related activation.

Here, we used functional magnetic resonance imaging (fMRI) techniques to provide the first evidence for an interactive effect of *SPON1* and *APOE* on brain activation during working memory performance in Chinese non-demented elderly. Additionally, we investigated the relationship between the activation of the involved brain regions and working memory performance. Based on previous findings that the T allele of rs2618516 exerted a protective effect with respect to dementia severity (Jahanshad et al., 2013), we hypothesized that the rs2618516 T allele would ameliorate the working memory performance and brain activation during working memory tasks of the *APOE* ε4 allele.

2 | METHODS

2.1 | Participants

Subjects were recruited from the Beijing Aging Brain Rejuvenation Initiative Study Group (BABRI), an ongoing longitudinal study examining the brain and cognitive decline in community-dwelling elderly individuals (Li et al., 2013). All enrolled participants were Han Chinese. Participants were qualified for our study if they met the following criteria: right-handed; native Chinese speakers; at least 50 years old; no history of neurological or psychiatric disorders; and a successful blood sample for the genotyping analysis. Specifically, the status "clinically non-demented" was determined by using the DSM IV, Petersen's dementia criteria, and Clinical Dementia Rating (score = 0). Accordingly, 710 non-demented subjects were included in the present study. Demographic information for each group is presented in Table 1. The study was followed by the principles of the Declaration of Helsinki and was approved by the Institutional Review Board of the Beijing Normal University Imaging Center for Brain Research. Written informed consent was obtained from each participant.

2.2 | Neuropsychological testing

All participants were subjected to a battery of neuropsychological tests that assessed several cognitive domains. As mentioned previously, the comprehensive neuropsychological battery comprised the following 7 cognitive domains (the tests used to assess each domain are in parentheses): 1, general mental status (the Mini-Mental-Status Examination-Chinese version [MMSE], Zhang et al., 1990); 2, episodic memory (the Auditory Verbal Learning Test [AVLT], Rosenberg, Ryan, & Prifitera, 1984) and the Rey–Osterrieth Complex Figure test [ROCF] (recall), Rey, 1941); 3, working memory (the Digit Span test, which was a sub-test of the Wechsler Adults Intelligence Scale-Chinese revision); 4, spatial processing (ROCF-copy, Rey, 1941) and the Clock-Drawing Test [CDT], Rouleau, Salmon, Butters, Kennedy, & McGuire, 1992); 5, language (the Category Verbal Fluency Test [CVFT] and the Boston Naming Test [BNT], Guo, 2006); 6, processing speed (the Trail Making Test [TMT] A, Reitan, 1958 and the Symbol Digit Modalities Test [SDMT], Sheridan et al., 2006); and 7, executive function (the TMT-B, Reitan, 1958 and the Stroop Color and Word Test C [SCWT], Guo et al., 2005).

2.3 | Analysis of genotyping

SPON1 rs2618516 was genotyped using TaqMan allele-specific assays on the 7900HT Fast Real-Time PCR System (Applied Biosystems, Foster City, CA). Another two SNPs, rs429358 and rs7412, which collectively form the *APOE* ε2 (with the haplotype of rs429358-rs7412: T/T), *APOE* ε3 (G/T), and *APOE* ε4 alleles (G/G), were also genotyped. The sample success rate for all three SNPs was 100% (i.e., no failures across participants to "call" the polymorphisms) and the reproducibility of all the genotyping was 100% according to a duplicate analysis of at least 10% of the genotypes. Given the risk of the T allele, we combined the CT and TT genotypes into T allele carriers. Thus, according to the rs2618516 genotyping, all participants were divided into two groups: 319 CC and 391 T allele carriers.

2.4 | Experimental paradigm

A block periodic design that incorporated alternating 0-back, 1-back, and 2-back tasks was used during the n-back working memory task.

TABLE 1 Demographics and neuropsychological test results of all participants

	SPON1 CC (*N* = 319)		*SPON1* T-carriers (*N* = 391)				
	APOE ε4 non-carriers (*N* = 270)	*APOE* ε4 carriers (*N* = 49)	*APOE* ε4 non-carriers (*N* = 321)	*APOE* ε4 carriers (*N* = 70)	$F/X^2_{SPON1\times APOE}$ (*p*)	F/X^2_{SPON1} (*p*)	F/X^2_{APOE} (*p*)
Age	64.43 ± 6.87	65.00 ± 7.64	64.26 ± 7.26	64.63 ± 6.78	0.019(.890)	0.141(.708)	0.423(.516)
Gender (male/female)	94/176	16/33	109/212	26/44	0.302(.583)	0.048(.827)	0.216(.642)
Education	11.30 ± 3.15	11.47 ± 3.42	11.19 ± 3.24	11.24 ± 3.36	0.031(.861)	0.263(.608)	0.123(.726)
General mental status							
MMSE	27.89 ± 1.77	27.31 ± 2.37	27.79 ± 1.78	27.63 ± 2.05	1.272(.260)	0.408(.523)	4.107(.043)*
Episodic memory							
AVLT-delay	5.99 ± 2.49	4.94 ± 2.57	5.69 ± 2.55	5.39 ± 2.80	2.620(.106)	0.145(.703)	7.155(.008)**
AVLT-total	30.82 ± 9.04	27.53 ± 8.82	30.62 ± 9.33	29.43 ± 10.28	1.600(.206)	1.101(.294)	5.877(.016)*
ROCF-delay	13.42 ± 6.12	12.08 ± 6.40	13.57 ± 6.45	12.86 ± 6.91	0.227(.634)	0.575(.449)	2.559(.110)
Working memory							
Forward digit span	7.58 ± 1.28	7.02 ± 1.28	7.28 ± 1.31	7.54 ± 1.53	10.164(.001)**	0.865(.353)	1.413(.235)
Backward digit span	4.38 ± 1.29	4.06 ± 1.07	4.26 ± 1.28	4.36 ± 1.33	2.503(.114)	0.571(.450)	0.825(.364)
Spatial processing							
ROCF-copy	33.34 ± 3.30	31.90 ± 6.32	33.29 ± 3.85	32.86 ± 3.50	1.674(.196)	1.560(.212)	6.237(.013)*
CDT	24.79 ± 3.52	24.61 ± 3.06	24.76 ± 3.59	24.70 ± 3.73	0.027(.869)	0.022(.882)	0.141(.708)
Language							
CVFT	45.36 ± 8.84	43.51 ± 11.06	45.07 ± 8.51	44.99 ± 7.91	1.119(.291)	0.579(.447)	1.174(.279)
BNT	23.17 ± 3.64	22.43 ± 4.04	23.26 ± 3.46	23.47 ± 4.21	1.599(.206)	2.751(.098)	0.653(.419)
Processing speed							
SDMT	35.74 ± 11.34	32.98 ± 11.32	35.23 ± 10.69	32.33 ± 10.96	0.002(.962)	0.315(.575)	7.620(.006)**
TMTa time (s)	56.65 ± 19.16	65.41 ± 30.01	58.30 ± 20.80	59.33 ± 23.01	3.449(.064)	1.113(.292)	5.186(.023)*
Executive function							
SCWT C-B time (s)	38.50 ± 15.62	41.92 ± 30.58	38.84 ± 17.12	37.57 ± 18.83	1.810(.179)	1.220(.270)	0.224(.636)
SCWT-C time (s)	76.54 ± 20.96	80.39 ± 31.92	77.47 ± 21.50	76.09 ± 21.29	1.701(.193)	0.671(.413)	0.180(.672)
TMTb time (s)	170.56 ± 63.22	202.57 ± 95.50	177.66 ± 73.15	180.67 ± 71.76	4.760(.029)*	1.239(.266)	6.583(.011)*
TMTb-a time (s)	113.91 ± 54.66	137.16 ± 78.14	119.32 ± 63.94	121.34 ± 60.88	3.247(.072)	0.785(.376)	4.379(.037)*

Values are mean ± standard deviation. The comparison of gender was performed using Wald Chi-square test. Multivariate analysis of covariance was used to determine the interactive effect of *SPON1* × *APOE*, and the main effects of *SPON1* and *APOE* on the neuropsychological tests (age, gender, and years of education as covariates). *$p < .05$, ** $p < .01$.

Abbreviation: *SPON1*, spondin 1 gene; *APOE*, apolipoprotein E; MMSE, Mini-Mental State Examination; AVLT, Auditory Verbal Learning Test; ROCF, Rey–Osterrieth Complex Figure; CDT, Clock-Drawing Test; CVFT, Category Verbal Fluency Test; BNT, Boston Naming Test; SDMT, Symbol Digit Modalities Test; TMT, Trail Making Test; SCWT, Stroop Color and Word Test.

Participants viewed single digits (0–9, displayed in white on a black background) inpseudorandom order. During the 0-back task, the participants were asked to decide whether the target digit (e.g., 1) on the screen was the preassigned digit. During the n-back (n = 1 or 2) task, the participants required to press the button when a digit appeared which matched the one presented n positions ago in the sequence. The stimuli were presented on a personal computer using E-Prime (version 1.0; Psychology Software Tools, Inc., Pittsburgh, Pennsylvania). To ensure that each participant understood the instructions and performed the task correctly, the participants were asked to practice all blocks of the task for 10–15 min before the experiment.

The experiment consisted of 3 blocks for each of the 3 working memory load conditions presented in a random order, with 20 stimulus digits presented per block. At the beginning of each block, the instruction for the condition was presented on the screen for 10 s. Each digit was presented for 1,000 ms with a blank display for 1,000 ms. The entire fMRI session took approximately 8 min.

2.5 | MRI data acquisition

To investigate the interaction of *SPON1* × *APOE* on brain activation during a working memory task, we also acquired MRI data from a subcohort (n = 64) of the study participants using a SIEMENS TRIO 3T scanner in the Imaging Center for Brain Research, Beijing Normal University. There were no differences between the whole sample and the imaging subsample in terms of age, gender and education. Working memory task images were acquired using an echo planar imaging (EPI) sequence (33 axial sections, TE = 30 ms, TR = 2,000 ms, flip angle = 90°, slice thickness = 3.5 mm, acquisition matrix = 64 × 64, field of view = 200 × 200 mm^2). For each participant, 235 image volumes

were obtained. Supporting Information Table S1 provides further details of the imaging sub-sample.

2.6 | Data processing and analysis

Functional data were preprocessed and statistically analyzed with the SPM8 package (http://www.fil.ion.ucl.ac.uk/spm/software/spm8). The preprocessing procedures included slice timing correction, within-subject interscan realignment to correct for possible movement, spatial normalization to a standard brain template in the Montreal Neurological Institute coordinate space, resampling to 3 × 3 × 3 mm, and smoothing with an 8 mm full-width half-maximum Gaussian kernel. Two subjects were excluded because of unacceptable head movement (translation > 3 mm or rotation > 3°) during fMRI scanning. Thus, 62 subjects were entered into a subsequent functional image analysis. On the single-subject level, the data were analyzed according to a fixed effects model (SPM8). The 6 head movement parameters were included in the model as confounding factors. Considering that the 2-back task is a more difficult one for elderly, contrast images were created only by subtracting the 0-back images from the 1-back images. In the second-level analysis, a full factorial analysis of covariance (ANCOVA) (2 × 2) was conducted with *SPON1* rs2618516 (T carriers vs. CC genotype) and *APOE* genotype (ε4 carriers vs. non-carriers) as independent factors for each contrast in SPM 8 (age, gender, and years of education were included as covariates). The statistical threshold was set at $p < .001$ using AlphaSim correction ($p < .01$, >92 voxel cluster size).

In addition to the voxel-wise analyses, several regions of interest (ROI) were identified based on the significant results from the voxel-wise ANCOVA. Spherical regions (6 mm radius) were defined around each of these peak activations. Signal intensities (beta weights) from the significantly activated voxels for each ROI were then calculated for 1-back versus 0-back contrast.

2.7 | Statistical analysis

The Hardy-Weinberg test was completed using PLINK software (Purcell et al., 2007). For the demographic factor of gender, logistic regressions were used to assess the effects of the *SPON1* rs2618516 polymorphism and *APOE*. For age and years of education, a univariate analysis of variance was used to assess the effects of the *SPON1* rs2618516 polymorphism and *APOE*. For neuropsychological assessments and n-back task performance, a multivariable analysis of covariance was conducted, with age, gender, and years of education as covariates. In addition, for the activities of the brain areas and cognitive performances showing significant interactive effects of *SPON1* × *APOE*, and n-back task performance, we further calculated the correlations between them using Pearson partial correlation analyses, after controlling for the influences of age, gender, and years of education, in all subjects to increase statistical power.

2.8 | Mediation analysis

After examining the *SPON1* × *APOE* genotype interaction on working memory performances and task related brain activation, we performed a mediation analyses to identify if the working memory task related brain activation could mediate the interaction of *SPON1* × *APOE* in working memory changes. Thus, in the present study, the *SPON1* × *APOE* genotype interaction was the independent variable, changed working memory performances was the dependent variable and altered task related brain activation was the mediator variables. Therefore, we would assume that whether the association between genotype and working memory is mediated by the altered brain activation. The mediation analyses were performed with Mplus 7 software.

3 | RESULTS

3.1 | Demographic, neuropsychological results, and n-back task performances

The SNPs rs2618516, rs429358, and rs7412 did not show deviations from Hardy-Weinberg equilibrium ($p > .05$). For all participants, the interactions of *SPON1* × *APOE*, *SPON1* effects and *APOE* effects were not significant for age, gender, and years of education (Table 1). These results were the same in the imaging sub-sample (Supporting Information Table S1). All of the subsequent analyses were adjusted for age, gender, and years of education.

For the neuropsychological tests, the interactions of *SPON1* × *APOE* were significant for working memory (Forward digit span, $p = .001$) and executive function (TMTb, $p = .029$) in all participants. The *APOE* effects were significant for general mental status (MMSE, $p = .043$), episodic memory (AVLT-delay, $p = .008$, and AVLT-total, $p = .016$), spatial processing (ROCF-copy, $p = .013$), processing speed (SDMT, $p = .006$, and TMTa, $p = .023$) and executive function (TMTb, $p = .011$, and TMTb-a, $p = .037$) in all participants. However, the *SPON1* effects were not significant for any neuropsychological tests in all participants (Table 1). We found similar but broader results in the imaging sub-sample, that is, the interactions of *SPON1* × *APOE* were significant for working memory (Forward digit span, $p = .029$), executive function (TMTb, $p = .015$, and TMTb-a, $p = .043$), and processing speed (SDMT, $p = .006$, and TMTa, $p = .015$). The *SPON1* effects and *APOE* effects were not significant for all neuropsychological tests and n-back task performances in the imaging sub-sample (Supporting Information Table S1).

3.2 | APOE main effect and SPON1 main effect on brain activations

The *APOE* ε4 carriers and non-carriers exhibited activation in regions associated with working memory tasks, such as the middle frontal gyrus, superior frontal gyrus, inferior parietal lobule, inferior frontal gyrus, precentral gyrus, superior parietal gyrus, and supplementary motor areas in the 1-back versus 0-back contrasts (Supporting Information Figure S1). The *SPON1* CC genotype and T allele carriers activated similar regions during the working memory task (Supporting Information Figure S2). *APOE* and *SPON1* did not show significant main effects in the brain activation analysis.

3.3 | Interactive effect of SPON1 × APOE on brain activations

All four groups exhibited working memory task-related brain activations. The activated regions included the middle frontal gyrus, medial

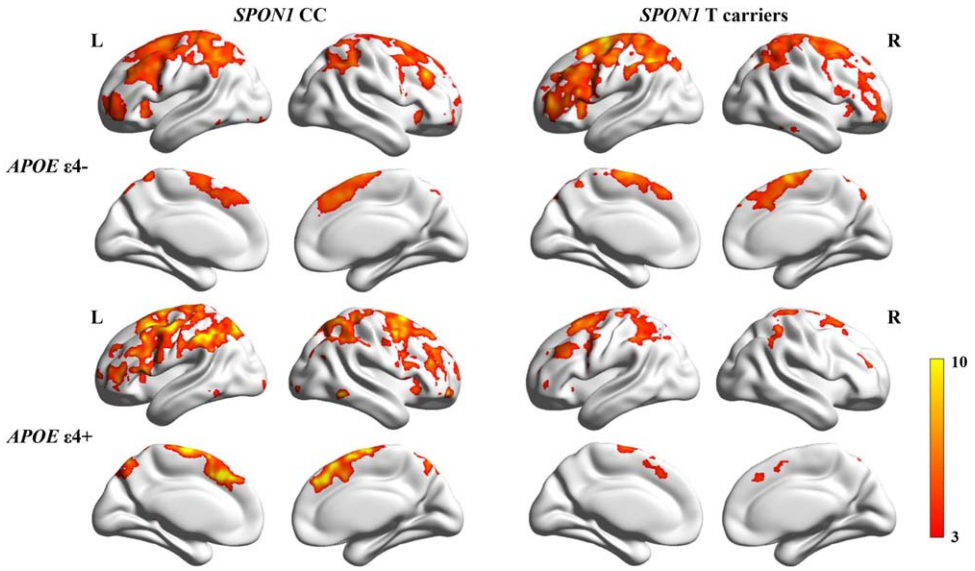

FIGURE 1 The spatial maps show the brain activation under working memory task for the four genotype groups separately

superior frontal gyrus, precentral gyrus, superior parietal gyrus, and inferior parietal gyrus (Figure 1). For the 1-back versus 0-back contrast, the significant interaction effect of *SPON1* × *APOE* was revealed in the right inferior frontal gyrus, triangular part (IFGtriang.R) ($x = 54$ mm, $y = 24$ mm, $z = 21$ mm; voxel size = 98, $p_{\text{AlphaSim-corrected}} < .001$, Figure 2). For T allele carriers, the IFGtriang.R activation in the ε4 carriers was significantly lower than the activation in the ε4 non-carriers ($F = 10.38$, $p = .002$). However, the opposite pattern was found for the *SPON1* CC genotype, that is the IFGtriang.R activation in the ε4 carriers was significantly higher than the activation in the ε4 non-carriers ($F = 5.78$, $p = .019$). Additionally, the IFGtriang.R activation in the *SPON1* CC genotype-ε4 allele non-carriers group was significantly lower than the activation in the *SPON1* T allele carriers-ε4 allele non-carriers group ($F = 6.33$, $p = .015$).The IFGtriang.R activation in the *SPON1* CC genotype-ε4 allele carriers group was significantly higher than the activation in the *SPON1* T allele carriers-ε4 allele carriers group ($F = 10.84$, $p = .002$).

3.4 | Correlations between brain activity and behaviors

To assess whether the brain activity differences could explain behavioral differences, we correlated the activity of IFGtriang.R with cognitive performances of significant interactive effects, and with n-back task performances in all subjects. Pearson correlation analyses indicated that IFGtriang.R activity was associated with response time in the 1-back task ($r = .299$, $p = .023$), MMSE ($r = -.286$, $p = .028$), TMTa ($r = .310$, $p = .017$), and SCWT-C ($r = 0.294$, $p = .024$) (Figure 3). Notably, no significant correlation was found after multiple comparisons ($p_{\text{FDR-corrected}} < .05$).

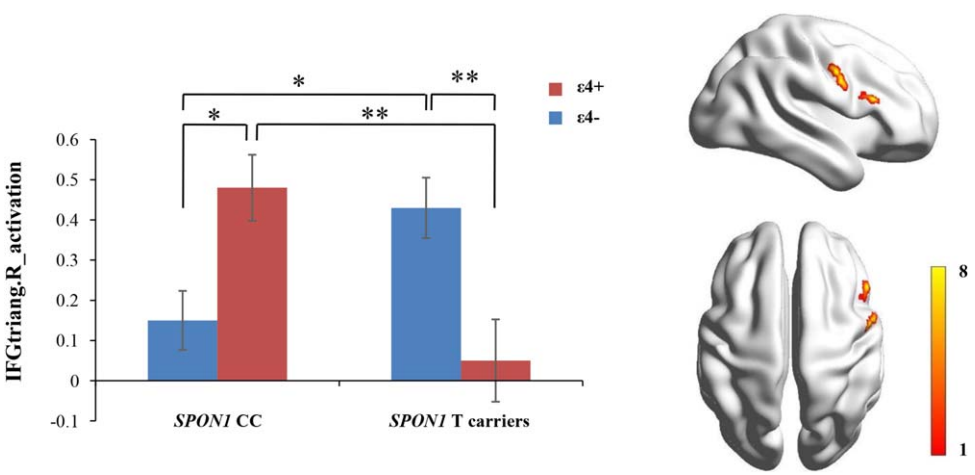

FIGURE 2 The significant interaction of *SPON1* × *APOE* showed in the IFGtriang.R in the 1-back versus 0-back condition ($p_{\text{AlphaSim-corrected}} < .001$). The bar graph showed group mean contrast estimate for the IFGtriang.R of the four genotype groups separately. *$p < .05$, **$p < .01$. IFGtriang.R, right inferior frontal gyrus, triangular part

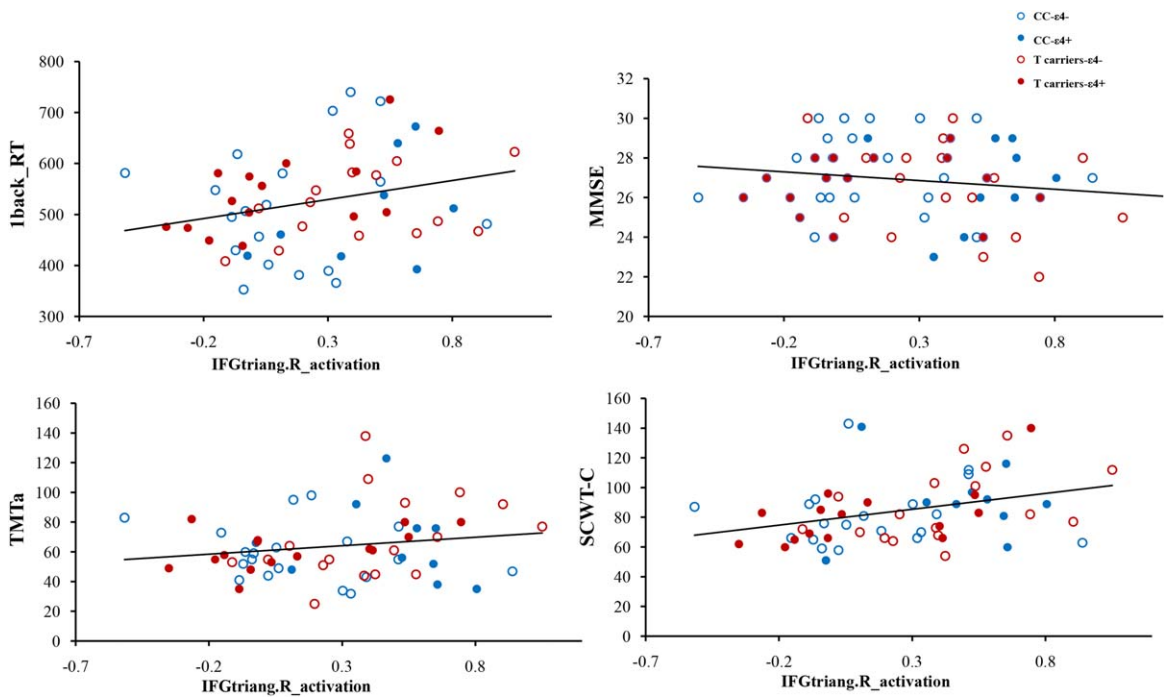

FIGURE 3 Correlations between activity of IFGtriang.R and n-back task performance and neuropsychological tests in all subjects. IFGtriang.R, right inferior frontal gyrus, triangular part; RT, response time; MMSE, Mini-Mental State Examination; TMT, trail-making test; SCWT, Stroop Color and Word Test

3.5 | Mediation analysis

In the mediation analysis, the independent factor was the *SPON1* × *APOE* genotype interaction and dependent variables were changed working memory performances. The proposed mediators were the altered task related brain activation. As shown in Figure 4, mediation analysis indicated that the activation of IFGtriang.R mediated the interactive effect of *SPON1* × *APOE* on response time in 1-back task ($Z = 2.043$, $p = .041$). No other significant mediation effects were found.

4 | DISCUSSION

For the first time in our knowledge, the present study found that the *SPON1* rs2618516 polymorphism moderated the effects of *APOE* on

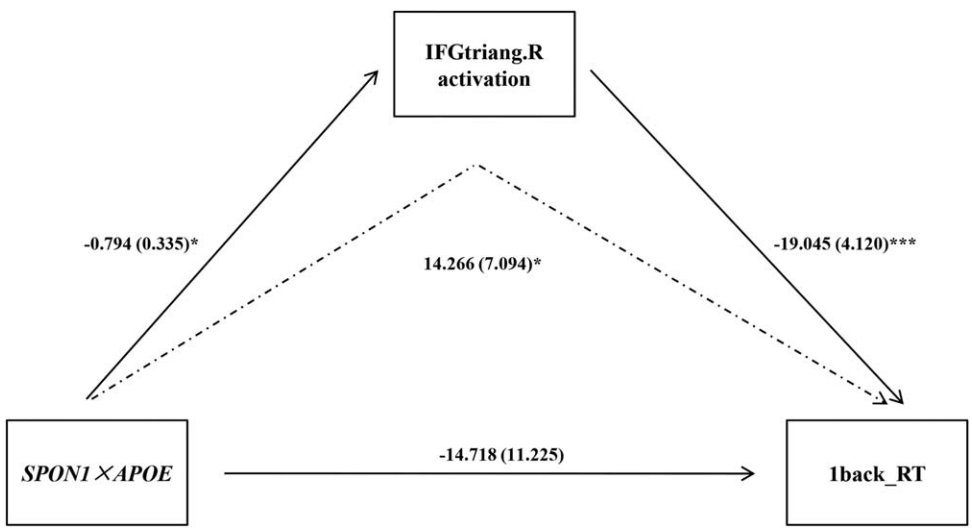

FIGURE 4 The mediation model delineating the direct effect of interaction of *SPON1* × *APOE* on IFGtriang.R activation, the direct effect of interaction of *SPON1* × *APOE* on response time in the 1-back task, the direct effect of IFGtriang.R activation on response time in the 1-back task, and the mediating effect of IFGtriang.R activation on the association between interaction of *SPON1* × *APOE* and response time in the 1-back task. As indicated by the path coefficients and *p* values. $^{*}p < .05$, $^{***}p < .001$. IFGtriang.R, right inferior frontal gyrus, triangular part; RT, response time

working memory in terms of both in behaviors and in the level of brain activations. Behaviorally, we found that *APOE* ε4 carriers performed worse with working memory and executive function if they also harbored the *SPON1* CC genotype, whereas the opposite was true in *SPON1* T allele carriers-ε4 allele carriers. Moreover, the brain activation in IFGtriang.R under working memory tasks was affected by the *SPON1* × *APOE* interaction. Thirdly, the linkage between behavior and brain activation was revealed by the negative correlation between activation in IFGtriang.R and n-back task performance. Further mediation analysis showed that the activation of IFGtriang.R could mediate the interactive effect of *SPON1* × *APOE* on working memory performance.

We first found interactive effects of *SPON1* × *APOE* on working memory and executive function performances. Previous studies have identified the *APOE* ε4 allele as a risk factor for episodic memory, executive function, and global cognitive ability in cognitively normal adults (Wisdom, Callahan, & Hawkins, 2011). Results from longitudinal data showed that memory test performance declined more in *APOE* ε4 carriers (Caselli et al., 2004). However, until recently, few studies have directly investigated the association between the *SPON1* rs2618516 polymorphism and cognitive functions in humans. Limited evidence has demonstrated that the rs2618516 T allele was associated with decreased clinical dementia rating (CDR) scores (Jahanshad et al., 2013). A previous animal study found that the overexpression of F-spondin, a ligand for APP (Hoe & Rebeck, 2008), was related to elevated memory and reduced Aβ deposition (Hafez et al., 2012). Moreover, ample evidence from animal and human studies has confirmed that increased Aβ deposition is linked to poorer cognitive performance on working memory and processing speed (Hsiao et al., 1996; Rodrigue et al., 2012). Thus, one could speculate that some variants in *SPON1* may influence the cognitive process by interacting with *APOE*. Nevertheless, there are few studies that have investigated the interactive effects of *SPON1* × *APOE* on cognitive performance in healthy elderly. Further neuroimaging evidence is also needed to verify these results.

Considering that cognitive performance is strongly correlated with brain activation under the working memory task, we then conducted a brain activation analysis. Working memory deteriorates in older adults compared with younger counterparts and teenagers (Fandakova et al., 2014), more so in AD patients (Yetkin, Rosenberg, Weiner, Purdy, & Cullum, 2006). Previous studies showed inconsistent views about the effect of the *APOE* ε4 allele on working memory performance (Reynolds et al., 2006; Wolk et al., 2010). Regarding the relationship between *APOE* genotype and brain activation during working memory, both positive and negative findings have been reported (Burggren, Small, Sabb, & Bookheimer, 2002; Filbey, Chen, Sunderland, & Cohen, 2010; Wishart et al., 2006). In the current study, we did not find any significant main effects of *APOE* on brain activation under the working memory task. This may suggest that the effects of *APOE* on brain activation under the working memory task could be modulated by other genetic variants. Therefore, we calculated the effects of the interaction of *SPON1* × *APOE* on brain activation under the working memory task and found significant results. Within the *SPON1* rs2618516 CC genotype, ε4 carriers were associated with higher activation of IFGtriang.R under the working memory task, whereas this pattern was opposite in *SPON1* rs2618516 T allele carriers-ε4 allele carriers. A recent study demonstrated that *APOE* disrupted working memory performance through an interaction with other genetic mutations in mice linked to dementia (Biundo, Ishiwari, Del Prete, & D'adamio, 2015). Nevertheless, the effects of *SPON1* on brain activation under the working memory task are still poorly understood. In the present study, we found that the association between the *APOE* ε4 allele and working memory task-related activation was affected by *SPON1* rs2618516, which would provide a reference for future studies about the combination effects of genetic factors on the brain activation under the working memory task.

Further examination of the relationship between brain activation and cognitive functions in all subjects revealed that activation in the IFGtriang.R was negatively related to working memory performances and other cognitive functions, in that higher activation was associated with worse cognitive functions. Coincidentally, the overactivity of the dorsolateral prefrontal cortex in patients with mild cognitive impairment may represent a compensatory mechanism which would enable them to perform normally (Gigi, Babai, Penker, Hendler, & Korczyn, 2010). We also found the effect of the *SPON1* × *APOE* interaction on working memory performance (response time in the 1-back task) was mediated by the activation in the IFGtriang.R using a mediation analysis. As corollaries, these neuroimaging measures, such as brain activation under the working memory task, could be biomarkers to predict the effect of variants in cognition.

The current study had some limitations. First, there is a need to examine the interactive effects of *APOE* ε4 allele with other possible AD risk genes on cognitive performance and brain function. Second, the significant correlations between neuroimaging measurements and cognitive performances reported in the present study should be regarded as exploratory in nature, due to no correlation survived $p < .05$ after FDR correction for multiple comparisons. Thus, it needs to confirm them in future additional studies. Overall, the findings of this study should be interpreted with these limitations in mind.

In summary, the present study suggested the interactive effects of *SPON1* × *APOE* on working memory performance and on the level of activation at IFGtriang.R under a working memory task in non-demented Chinese elderly. This finding highlights the importance of combining different genetic polymorphisms when examining candidate genes that affect cognitive function. Further studies with a larger sample size and longitudinal design are needed to confirm our results.

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CONFLICT OF INTEREST

The authors declare that they have is no conflicts of interest.

ORCID

Zhanjun Zhang http://orcid.org/0000-0001-7266-4218

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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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