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ABSTRACT

BACKGROUND AND PURPOSE: *APOE4* is the best-documented genetic risk factor for sporadic AD. Previous research showed that *APOE4* is associated with increased risk of occurrence and earlier onset of AD in a gene dose–dependent manner. However, the specific role of *APOE4* in processing of brain functions requires further investigation. Investigators have used fMRI to measure brain activity on the basis of the blood oxygen level–dependent contrast. This study investigates the effects of *APOE4* on fMRI during *n*-back WM tasks in healthy middle-aged adults.

MATERIALS AND METHODS: From 110 participants, 81 individuals without objective or subjective cognitive impairment underwent *APOE* genotyping. Nine *APOE4* carriers and 9 age- and sex-matched non-*APOE4* controls were recruited for fMRI examinations during WM tasks.

RESULTS: Both groups displayed increased brain activation in response to increases in WM loads. During low-WM-load tasks, the *APOE4* carriers recruited significantly greater additional processing resources than the non-*APOE4* carriers. During moderate- and high-WM-load tasks, the *APOE4* carrier group displayed fewer increases in activation than the non-APOE4 carrier group.

CONCLUSIONS: *APOE* genetic polymorphisms may affect brain functioning in subjects without dementia. The patterns of brain activation during different levels of WM load suggest possible subclinical impairment of WM capacity in *APOE4* carriers (ClinicalTrials.gov registration: NCT01287819).

ABBREVIATIONS: AD = Alzheimer disease; APOE4 = *Apolipoprotein E ε*4 allele; BOLD = blood oxygen level–dependent; MMSE = Mini-Mental State Examination; WM = working memory

A D is the most common cause of dementia in the world. Although the specific pathophysiology of AD remains unclear, compelling evidence has shown that genetic factors play an important role in its occurrence.[1] There are 3 genes linked to familial AD, including *amyloid precursor protein*, *presenilin-1*, and *presenilin-2*.[2] *APOE4* is the best-documented genetic risk factor for sporadic AD.[3] The genetic polymorphisms for the *APOE* gene include 3 alleles: ε2, 3, and 4. Previous research identified *APOE4* as associated with increased risk of occurrence and earlier onset of AD in a gene dose–dependent manner.[4,5] *APOE4* carriers present with more severe memory impairment and greater reductions in regional brain volume and metabolism, in comparison with non-*APOE4* carriers.[6-9] These findings indicate that *APOE4* is important for brain functioning, though the mechanisms by which it exerts its effects need further clarification. Genetic background can thus confer changes in brain structure, metabolism, and functioning, or even behaviors.

Investigators extensively use fMRI to evaluate brain function. Most studies use an indirect-measurement method based on the blood oxygen level–dependent contrast.[10,11] Under normal physiologic conditions, the amplitude of the BOLD signal is linearly connected to neural activity and can provide task-specific information concerning neural functions and networks.[12-14]

WM has the ability to actively keep information for further use by prioritizing, modifying, and protecting it from interference.[15]

Therefore, the operations of WM consist of memory itself and retention of active information in a stable yet flexible manner.[16] Most patients with AD present with progressive impairment of episodic memory, and such dysfunction constitutes the core diagnostic criterion of probable AD.[17] However, several findings in the literature also unveil deficits in WM and executive function in patients with AD and mild cognitive impairment.[18-20] Previous studies of fMRI documented that WM emerges from interactions among higher sensory, attentional, and mnemonic functions, with separable neural bases.[21-25] WM has a fundamental cognitive function, which can be evaluated by fMRI, and thus provides a good domain for testing the differences among individuals with various genetic backgrounds. fMRI studies of *APOE4* carriers have shown decreased neural activity in the medial temporal lobe and increased (probable compensatory) neural activity in prefrontal and parietal regions under tasks of encoding and retrieval of episodic memory.[26,27] Neural activity alteration in WM in subjects at risk of AD is not conclusive.[28] An initial study by Burggren et al[29] by using a digit-span task showed no difference in activation patterns between elderly *APOE4* carriers and non-*APOE4* carriers. They concluded that additional cognitive effort in persons at genetic risk for AD is specific to episodic encoding. Later studies by using an *n*-back WM paradigm showed no difference in activation patterns between *APOE4* carriers and non-*APOE4* carriers in young adults but increased activation in the frontal and parietal lobe in elderly *APOE4* carriers.[30,31] To the best of our knowledge, there is no publication to date about neural activity alteration in WM in middle-aged subjects at risk of AD, especially with different WM loads.

MATERIALS AND METHODS
Participants
One hundred ten subjects who underwent physical examinations at Taipei Medical University–Shuang Ho Hospital participated in this study. All participants provided written informed consent, and this study was approved by the hospital institutional review board. The participants self-completed the Alzheimer's Disease 8 (AD8) questionnaire for subjective cognitive impairment and were assessed with the MMSE for objective cognitive impairment by a neuropsychologist.[32,33] Participants with AD8 >2 or MMSE <26 were excluded. Eighty-one participants without subjective or objective cognitive impairment were selected for further genetic analysis, from which 12 *APOE4* carriers, all ε3/4 heterozygous, and 69 non-*APOE4* carriers were found. Nine *APOE4* carriers underwent fMRI analysis. Nine age-and sex-matched controls were randomly selected from the non-*APOE4* carriers.

Working Memory Task
WM capacity can be tested by using a variety of tasks. A commonly used measure in fMRI is the *n*-back task. The participant is required to monitor a series of stimuli and to respond whenever a stimulus is presented that is the same as the one presented *n* trials previously. Parametric designs, comparing *n* = 1, *n* = 2, and *n* = 3 trials are often used. Some studies have also used a 0-back control condition. In this study, *n*-back WM tasks were presented by using E-Prime, Version 2.0 (http://www.pstnet.com/eprime.cfm). At the beginning, a task guide describing the rules of the study was

shown. The *n*-back tasks comprised 4 conditions, 0-, 1-, 2-, and 3-back. The 0-back control condition had a minimal WM load, and individuals were asked to decide whether the current letter matched a single target letter that was specified before the condition began. During the 1-back condition, they were asked to decide whether the current letter matched the previous one. During the 2-back and 3-back conditions, the participants were asked to decide whether the number currently presented matched the number that had been presented 2 and 3 trials back in the sequence. Each condition was conducted in a single run, which consisted of 3 epochs. Each epoch contained 30 seconds of presentation with numbers and 30 seconds of fixation on a crosshair. Twelve numbers were presented in the 30-second period, and each consisted of a 0.5-second appearance of a black number on the screen against a white background and 2 seconds of a fixation cross. All stimuli were projected onto an overhead screen, which participants could view through a mirror located on the scanner's head coil. Participants were instructed to press a response pad with the right index finger when the currently presented number was a target or to press the middle finger when the currently presented number was not a target. The number of correct and incorrect responses was recorded.

Conditions were counterbalanced among participants: Each condition was equally often preceded and followed by each of the other conditions. Before the scans, each participant was instructed on the entire experimental procedure and practiced the tasks outside the scanner to reduce anxiety. To further reduce any group effect independent of WM processes, all participants were trained before scanning to ensure that they could perform the 1-back condition to an accuracy criterion of 70%.

Imaging Methods
Imaging was performed on a 3T MR imaging system (Discovery MR750; GE Healthcare, Milwaukee, Wisconsin). An 8-channel head coil was used for signal reception. Care was taken to minimize the effects of movement by instructing participants to remain still and by placing foam padding around the head. Functional data were collected by using an EPI sequence (TR = 3000 ms, TE = 35 ms, flip angle = 90°, FOV = 230 mm^2, matrix = 64 × 64, 40 sections, section thickness = 3 mm, and intersection gap = 1 mm) for a 3.5-minute period resulting in 70 volumes. Two dummy scans, which enabled the MR signal to achieve a steady state, were applied in each scan. A T1-weighted anatomic dataset was obtained from each subject by using brain-volume imaging (TR = 8.2 ms, TE = 3.2 ms, TI = 450 ms, flip angle = 12°, FOV = 240 mm^2, matrix = 256 × 256, 160 sections, voxel size = 0.9375 × 0.9375 × 1 mm^3). T2-weighted images were acquired by using fast recovery fast spin-echo (TR = 5700 ms, TE = 100 ms, FOV = 230 mm^2, matrix = 416 × 416, 20 sections, section thickness = 5 mm, intersection gap = 2 mm, and NEX = 1).

Data Analysis
fMRI data were preprocessed by using SPM5 (Wellcome Department of Imaging Neuroscience, London, UK) implemented in Matlab, Version 7.9 (MathWorks, Natick, Massachusetts). To reduce motion artifacts, we realigned each participant's functional

Demographic information, cognitive function, and accuracy rates of *n*-back WM in participants

	Total (*n* = 110)	ApoE4- (*n* = 9)	ApoE4+ (*n* = 9)	ApoE4- versus ApoE4+ t	ApoE4- versus ApoE4+ p
Sex (male/female)	53:57	4:5	4:5	–	–
Mean age (SD) (yr)	57.60	42.78 (9.63)	42.11 (9.13)	0.53	.34
Mean MMSE score (SD)	28.49 (2.65)	29.56 (0.73)	28.89 (1.17)	0.42	.38
Mean AD8 score (SD)	0.54 (1.20)	0.44 (0.73)	0.11 (0.33)	0.09	.76
0-Back accuracy rate		90% (17)	92% (15)	−0.19	.85
1-Back accuracy rate		93% (6)	89% (17)	0.50	.63
2-Back accuracy rate		80% (11)	85% (9)	−0.82	.43
3-Back accuracy rate		80% (9)	85% (13)	−0.86	.41

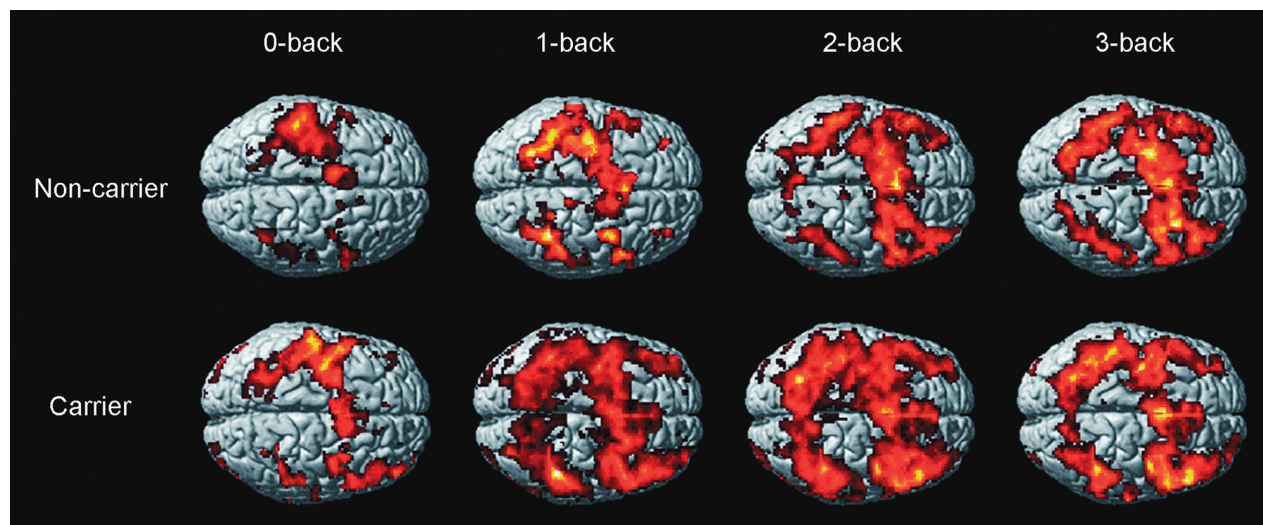**Note:**—*APOE4+* indicates an *APOE4* carrier; *APOE4−*, a non-*APOE4* carrier.

FIG 1. Activation maps of *n*-back task of *APOE4* carriers and non-*APOE4* carriers in a surface-rendered projection displayed on a standardized brain atlas (display threshold, $P < .01$; extent, 3 voxels). Increased activation in bilateral frontal and parietal regions is noted, consistent with activation of WM circuitry in both groups.

images with the first volume of the series by using the rigid-body transformation procedure and resectioned them by using fourth-degree B-spline function interpolation while adjusting for residual motion-related signal changes. All motion-corrected images were spatially normalized to the EPI template of the Montreal Neurological Institute. The normalization process involved minimizing the residual sum of squared differences among the images to be normalized. Before modeling the fMRI signal changes, normalized EPI images were spatially smoothed by convolution with a 3D Gaussian filter of $6 \times 6 \times 6 \text{ mm}^3$ full width at half maximum to increase the signal-to-noise ratio, validate the statistical analysis, and reduce anatomic variations among participants.

Statistical Analysis

Demographic and neuropsychological test data for both groups were compared by using 2-tailed *t* tests or χ^2 tests. For each *n*-back condition, the datasets were analyzed by modeling the experimental conditions by using boxcar functions convolved with a hemodynamic response function in the context of the general linear model used by SPM5. To obtain the brain-activation patterns for each group, we entered smoothed normalized scans for all participants into this general linear model and created contrast images for between 0- and 1-back (1 > 0), 1- and 2-back (2 > 1), and 2- and 3-back (3 > 2) for each group. A 1-sample *t* test was

applied for within-group analyses. The probability threshold value was set at .01 uncorrected, with a minimum cluster extent of 3 contiguous voxels. The generated contrast images for 1- > 0-back, 2- > 1-back, and 3- > 2-back were then used for the second-level multiparticipant/between-group random effects analyses, to obtain the activation differences between *APOE4* carriers and noncarriers. The 2-sample *t* test was applied for between-group analyses. The probability threshold value was set at .01 uncorrected, with a minimum cluster extent of 3 contiguous voxels.

RESULTS

The Table describes the basic demographic characteristics of both groups. The groups did not differ significantly regarding sex, age, and years of education. Group accuracies for the *n*-back task did not differ among *APOE4* carriers and non-*APOE4* carriers. There was, however, a significant trend of increasing WM load associated with declines in performance accuracy in both groups ($P < .001$).

Figure 1 shows activation maps, during the *n*-back task, of *APOE4* carriers and controls in a surface-rendered projection displayed on a standardized brain atlas (display threshold, $P < .01$; extent, 3 voxels). Both groups displayed increased activation in
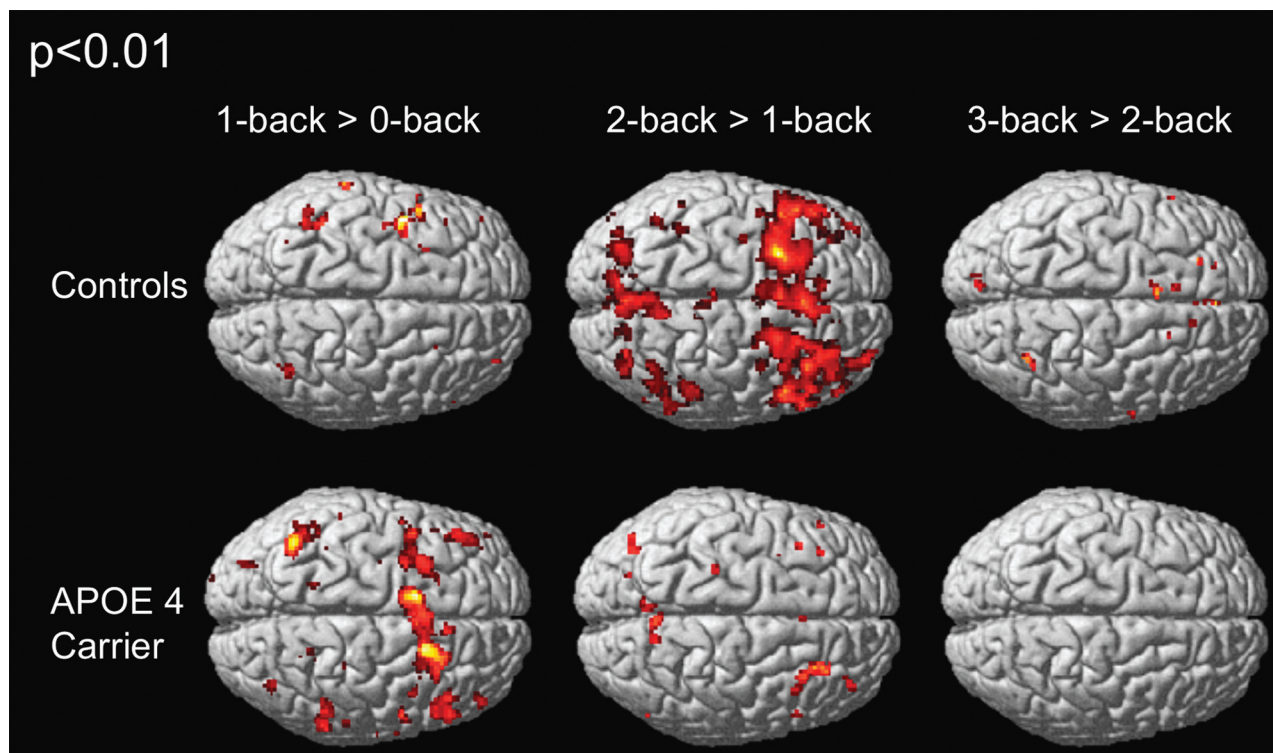
FIG 2. Activation maps of 1-back > 0-back, 2-back > 1-back, and 3-back > 2-back conditions of *APOE4* carriers and non-*APOE4* carriers in a surface-rendered projection displayed on a standardized brain atlas (display threshold, $P < .01$; extent, 3 voxels). In both groups, an increase in brain activation occurred in response to each increase in WM load. The degree of increased brain activation was different in both groups. It was greatest in 2-back > 1-back conditions in non-*APOE4* carriers, and greatest in 1-back > 0-back conditions in *APOE4* carriers. A visual comparison of the between-group difference in 1-back > 0-back conditions shows less extent of activation in the noncarrier group. In 2-back 1-back and 3-back > 2-back conditions, less increase in activation is seen in the carrier group.

bilateral frontal and parietal regions, consistent with activation of WM circuitry.

Figure 2 displays activation maps, during 1-back > 0-back, 2-back > 1-back, and 3-back > 2-back conditions, of *APOE4* carriers and non-*APOE4* carriers. Both groups displayed increased brain activation in response to each increase in WM load. The degree of increase in brain activation differed among the groups and was greatest in the 1-back > 0-back conditions in the *APOE4* carriers and in the 2-back > 1-back conditions in the non-*APOE4* carriers. Visual comparison of the between-group differences in the 1-back > 0-back conditions shows lesser activation in the non-*APOE4* carrier group. In 2-back > 1-back and 3-back > 2-back conditions, the *APOE4* carrier group demonstrated fewer increases in activation than the non-*APOE4* carriers.

DISCUSSION

APOE4 carriers displayed more rapid memory decline and mesial temporal lobe atrophy than non-*APOE4* carriers.[34-41] The present study recruited individuals without subjective or objective cognitive impairment, hence subjects with normal cognition, to test the effects of *APOE4* on the brain functioning of WM by using BOLD fMRI. Performance of *n*-back WM was not different between groups ($P = .41–.85$), similar to the findings of a previous publication.[42] fMRI analysis showed that during the low-WM-load task, functional activation significantly increased to a greater extent in the *APOE4* carriers than in the non-*APOE4* carriers. As the WM load increased, the non-*APOE4* carriers maintained their

ability to further increase activation. In contrast, the *APOE4* carriers showed fewer increases in activation during the moderate- and high-WM-load conditions. One possible explanation for this phenomenon is mild impairment of WM capacity in the *APOE4* carriers. In the low-WM load (1-back > 0-back conditions), the *APOE4* carriers recruited additional processing resources to compensate for processing inefficiencies. In contrast, the non-*APOE4* carriers were not impaired or challenged by this low-WM load and, therefore, required relatively fewer increases in processing resources to perform the task. As the WM load increased, the non-*APOE4* carriers continued to show increasing activation, presumably because they still had processing reserves available to be drawn on with increasing WM demand. The *APOE4* carriers, however, had already recruited most of their (assuming a finite amount) available resources, and thus little additional activation occurred.

In this study, the accuracy rate of 0-back, 2-back, and 3-back WM tasks appeared insignificantly better in the *APOE4* carrier group. Among the *APOE4* carriers, the accuracy rates did not dramatically decline along with the increase of memory loads when the brain activation recruitment had reached the plateau. It is speculated that there is a ceiling effect of working memory in this study. Continuing increases in memory load might enable the detection of the decline of performance in *APOE4* carriers. Dickerson et al[43] demonstrated increased hippocampal activation in mild cognitive impairment compared with normal aging and AD

under fMRI scanning during face-name associative encoding tasks. They hypothesized that there is a phase of increased medial temporal lobe activation early in the course of prodromal Alzheimer disease. Our findings of increased functional activation under low WM load in *APOE4* carriers may agree with their hypothesis and may be applied to the cognitively normal high-risk subjects. Explanations of such functional alteration include a compensatory mechanism for regional neuronal loss and disruption of the intrinsic connectivity of memory networks.[44,45] Filbey et al[46] also found compensatory neural activation in the medial frontal and parahippocampal gyrus in young and elderly *APOE4* carriers during a 9-square-grid visual WM task, similar to our findings in low-WM load.

The relationship between the WM fMRI findings and underlying pathology is not clear at present. It could reflect a hereditary pattern in *APOE4* carriers or could be a consequence of functional decline of the subclinical brain. Using resting-state fMRI, Sheline et al[47] showed that *APOE4* alters the neural network in individuals with normal cognition without amyloid burden, as detected by using Pittsburgh compound B–amyloid PET. However, the long-term effects of these types of brain functional changes remain unknown.

There are a few limitations to this study. First, amyloid pathology was not completely excluded. Numerous studies have proved that amyloid burden can occur many years before objective or subjective cognitive declines, especially among *APOE4* carriers.[8,48,49] In the present study, amyloid pathology, which results in brain subclinical dysfunction, might potentially have contributed to the increase in BOLD. Second, the sample size of this study was small. *APOE4* carrier rates are low in Asian populations relative to whites. This study recruited only 9 *APOE4* carriers and 9 non-*APOE4* carriers and was able to achieve statistical significance. Third, the insignificantly better performance of some *n*-back WM tasks in the *APOE4* carrier group was unexpected and was not perfectly compatible with the fMRI presentation. Future large-scale research investigations, by using amyloid imaging and long-term follow-ups, are, therefore, warranted.

CONCLUSIONS

In summary, fMRI BOLD significantly increased in the *APOE4* carriers during low-WM-load tasks but did not increase during moderate- and high-WM loads, which occurred in non-*APOE4* carriers. Based on previous research and our findings, these differences could have a hereditary basis. The phenomenon could reflect an impaired WM processing resource associated with subclinical brain dysfunction. This study, therefore, also provided further evidence to confirm the effects of genetic background on brain functioning. Further studies are required to fully elucidate the underlying mechanisms.

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