

## ORIGINAL ARTICLE

# Sex-Dependent Effects of the APOE $\epsilon$ 4 Allele on Behavioral Traits and White Matter Structures in Young Adults

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## Abstract

The APOE  $\epsilon 4$  allele is associated with a risk of Alzheimer's disease in the elderly, with the association being pronounced in females. Conversely, findings of the effects of the APOE  $\epsilon 4$  allele in young adults are mixed. Here, we investigated the sex–genotype interaction effects of the APOE  $\epsilon 4$  allele on cognitive functions as well as brain structures among 1258 young adults. After adjusting for multiple comparisons, there were significant effects of the interaction between sex and the number of APOE  $\epsilon 4$  allele on some speed tasks (e.g., simple processing speed tasks and the reverse Stroop task) as well as on regional white matter volume (rWMV). The observed sex–genotype interaction conferred better cognitive performance and greater rWMV in the anterior frontal and precentral white matter areas in females having more APOE  $\epsilon 4$  alleles and reduced rWMV in the same areas in male having more APOE  $\epsilon 4$  alleles. These findings support the long-debated antagonistic pleiotropic effects of the APOE  $\epsilon 4$  allele in females.

**Key words:** APOE genotype, imaging genetics, sex interaction effects, white matter structures, young adults

## Introduction

Apolipoprotein E (APOE) is a serum protein as well as a major constituent of chylomicrons, very-low-density lipoprotein, and some subforms of high-density lipoprotein. It is involved in the transport of cholesterol and fatty acids as well as neuroplasticity and neural repair (Boyles et al. 1989). It is encoded by APOE, which has three common alleles ( $\epsilon 2$ ,  $\epsilon 3$ , and  $\epsilon 4$ ) (Das et al. 1985). Several studies have shown that the APOE  $\epsilon 4$  allele is a vital risk factor of Alzheimer's disease in the elderly, with this association being particularly higher in females than in males during the earlier old age (Neu et al. 2017). Additionally, this allele has a relationship with lower cognitive functions in healthy elderly individuals, and some studies have indicated stronger effects in females than in males (Beydoun et al. 2012), although this finding has not been evaluated by meta-analyses. This type of APOE  $\epsilon 4$ –sex interaction has been shown in a biomarker that distinguishes Alzheimer's disease (tau pathology), and stronger relationships between APOE  $\epsilon 4$  allele and greater values in biomarkers of Alzheimer's disease are observed in females (Altmann et al. 2014). This APOE  $\epsilon 4$ –sex interaction might be moderated by estrogen because one study reported that females with the APOE  $\epsilon 4$  allele who underwent estrogen replacement therapy had a reduced risk of Alzheimer's disease (Rippon et al. 2006).

It is noteworthy that some studies have indicated that the APOE  $\epsilon 4$  allele has a relationship with better cognitive performance in young adults, particularly in tasks that need attention instead of those that require memory (for reviews, see Han and Bondi 2008; Rusted and Carare 2015). With the existing literature in mind, an “antagonistic pleiotropy” hypothesis has been suggested, wherein the APOE  $\epsilon 4$  allele has differential effects on cognitive performance, depending on age (Han and Bondi 2008). Nonetheless, a recent meta-analysis of the effects of the APOE  $\epsilon 4$  allele on cognitive functions in young adults has demonstrated that there is a trend of improved executive functions among young adults and no other differences in other areas across both males and females (Weissberger et al. 2018).

Several neuroimaging studies have assessed the effects of the APOE  $\epsilon 4$  allele on brain structure among healthy people. Numerous such studies in healthy elderly individuals have indicated that this allele has a relationship with greater atrophy (as shown by lower regional brain volumes as well as deficits in other neuroimaging measures) (Cherbuin et al. 2007). Moreover, some of these studies have described stronger effects in females (Sampedro et al. 2015). However, structural studies, including young adults, have shown mixed effects of the APOE  $\epsilon 4$  allele.

These studies have particularly focused on the medial temporal lobe structures. A smaller-scale study has described the APOE  $\epsilon 4$  allele's relationship with a lower quantitative index (cortical thickness) in the medial temporal lobe (Shaw et al. 2007), whereas a recent study with a large sample size has shown that there are no relationships between APOE genotypes and quantitative index (volume) in the medial temporal lobe (Khan et al. 2014). Another study that included a small sample size has described the relationships between APOE genotypes and white matter structures (Heise et al. 2011).

Briefly, there have been various studies on the effects of APOE genotypes. In studies of the elderly, the main effects of APOE genotypes (regardless of sex) as well as the effects of interaction between sex and APOE genotypes on the risk of Alzheimer's disease, cognitive functions, and neural mechanisms were explored. In studies of young adults, basically, the main effects of APOE genotypes (regardless of sex) on both cognitive functions and neural mechanisms were also explored.

Even with a great number of studies, whether APOE genotypes differentially affect both cognitive and neural mechanisms in young males and females (who show fully increased estrogen levels) has not been explored in a large sample, which detects subtle interaction effects. The aim of this study is to address this issue using a large sample. Considering the robust interaction of APOE genotypes with sex in the elderly, we have hypothesized the effects of the interaction between APOE genotypes and sex in young adults as well. Because there is a strong genetic association between APOE genotypes and Alzheimer's disease, it is important to have an understanding on how genotypes interact with sex to affect individual cognitive functions as well as the underlying neural mechanisms.

## Material and Methods

### Subjects

The present study is involved in a current project, aiming to explore the relationships among brain imaging metrics, cognitive functions, and a variety of modifying factors (including genetic factors). The present study included 1258 healthy, right-handed subjects (741 males and 517 females) with sufficient data to explore the influence of APOE genotypes using whole-brain voxel-based morphometry (VBM). The mean age of the subjects was 20.7 years (standard deviation, 1.8; range, 18–27 years). The subjects included university students, postgraduates, or university graduates for less than 1 year. All subjects

had normal vision and had no history of neurological or psychiatric illness. Handedness was assessed using the Edinburgh Handedness Inventory (Oldfield 1971).

For additional details on subjects' information, see [Supplemental Methods](#). A written informed consent was acquired from all subjects, and this study was approved by the Ethics Committee of the Tohoku University. The descriptions in this study are from our prior study which is in relation to the same project (Takeuchi et al. 2019b).

### Genotyping of Subjects

APOE genotype was acquired via a standard protocol using the polymerase chain reaction. APOE alleles correspond to allele combinations at single-nucleotide polymorphisms rs429358 and rs7412. The individual genotypes at the two sites were combined to make a single standard APOE genotype.

The APOE genotype distribution in the 1258 subjects was as follows:  $\epsilon 2/\epsilon 2$  (male,  $n = 1$ ; female,  $n = 1$ , total,  $n = 2$ , 0.2%),  $\epsilon 2/\epsilon 3$  (male,  $n = 57$ ; female,  $n = 38$ ; total,  $n = 95$ , 7.6%),  $\epsilon 3/\epsilon 3$  (male,  $n = 547$ ; female,  $n = 363$ ; total,  $n = 910$ , 72.3%),  $\epsilon 3/\epsilon 4$  (male,  $n = 129$ ; female,  $n = 110$ ; total,  $n = 239$ , 19.0%), and  $\epsilon 4/\epsilon 4$  (male,  $n = 7$ ; female,  $n = 5$ ; total,  $n = 12$ , 1.0%). Subjects with an undetermined genotype, whether  $\epsilon 1/\epsilon 3$  or  $\epsilon 2/\epsilon 4$ , and subjects with an undetermined genotype for other reasons were not part of this study. Thus, in this study, there are no subjects having a genotype of  $\epsilon 1/\epsilon 3$  or  $\epsilon 2/\epsilon 4$ . The frequency of approximately 1.0% of the  $\epsilon 4/\epsilon 4$  genotype is the same as that of the prior study with a young Japanese huge sample (Katsuya et al. 2002). Weinberg equilibrium has shown no deviations from the expected genotype distribution ( $P > 0.05$ ).

In this study, we have divided the subjects based on the number of  $\epsilon 4$  alleles (0, 1, 2) in accordance with the additive effects model. This strategy is the same with previous larger-scale studies ( $N > 1000$ ) (Acevedo et al. 2010) and is consistent with the finding that the risk of Alzheimer's disease obviously increased with the increase in the number of  $\epsilon 4$  allele (Neu et al. 2017). However, other studies have used other models (Cherbuin et al. 2007). For results and notes regarding the model that compared the subjects with  $\epsilon 4$  carriers and non- $\epsilon 4$  carriers as well as the model which added the covariate of the existence of  $\epsilon 2$  allele (there were only 2 subjects with 2  $\epsilon 2$  alleles; thus, the covariate evaluated only whether subjects had at least one allele) in addition to the number of  $\epsilon 4$  alleles (0, 1, 2), see the [Supplemental Methods](#) section. Basically, using these other models did not change the overall conclusion of the study. Comparisons between subjects with  $\epsilon 2/\epsilon 2$  and  $\epsilon 2/\epsilon 3$  genotypes were also indicated in the [Supplemental Methods](#), [Supplemental Results](#), and [Supplemental Discussion](#) sections.

### Psychological Measures

Subjects underwent the following neuropsychological tests. The descriptions in this subsection are from the prior study that employed the same methods (Takeuchi et al. 2015).

[A] A (computerized) digit span task for the evaluation of working memory (Takeuchi et al. 2011).

[B] Raven's advanced progressive matrix test (Raven 1998): a nonverbal reasoning task and a representative measure of general intelligence.

[C] Tanaka B-type intelligence test (TBIT) (Tanaka et al. 2003): a nonverbal mass intelligence test used for third-year junior high school and older examinees. This test determines psychometric intelligence based on multiple forms of speed tasks. It

does not include story problems but uses figures, single numbers, and letters as stimuli. In all the subtests, the subjects have to solve as many problems as possible within a certain period (a few minutes) of time. For details on these subtests, see Takeuchi et al. (2013a).

[D]: The perception factor of TBIT: the subscore focusing on simple processing speed (PS).

[E] Word-Color and Color-Word tasks: the measures of simple PS.

[F] Reverse Stroop task and Stroop task: the measures of the executive function or inhibition. Tasks [E] and [F] are part of the Hakoda's version of the Stroop task (matching-type). Unlike the traditional oral naming Stroop task, Hakoda's version is a matching-type Stroop task that requires subjects to check whether the answers that they have chosen are right. The test comprises two control tasks (Word-Color and Color-Word): a Stroop task and reverse Stroop task. For details on these subtests, see Takeuchi et al. (2014).

[G] The S-A creativity test: a measure for creativity by divergent thinking. Subjects have to create as many notions as possible to the given tasks within 5 min. For details, see our previous study (Takeuchi et al. 2010).

In various periods of the projects, somewhat different sets of psychological measures were collected from subjects because of the limited time for the experiment and the temporal focus on specific study purposes, leading to a number of subjects in each analysis as shown in [Table 1](#).

### Image Acquisition and Preprocessing

All MRI data were obtained with a 3-T Philips Achieva scanner. High-resolution T1-weighted structural images were acquired for VBM. Diffusion-weighted data were obtained with the use of a spin-echo echo-planar imaging sequence to acquire fractional anisotropy (FA) maps. The parameters for these acquisitions were as described in the prior study and are provided in the [Supplemental Methods](#) section (Takeuchi et al. 2013b).

The preprocessing of T1-weighted structural data used the Statistical Parametric Mapping 12 software (SPM12; Wellcome Department of Cognitive Neurology, London, UK) implemented in Matlab (Mathworks Inc., Natick, MA, USA).

Using the new segmentation algorithm and the diffeomorphic anatomical registration through exponentiated Lie algebra (DARTEL) registration process implemented in SPM12, the T1-weighted structural images of each individual were segmented and normalized to the Montreal Neurological Institute (MNI) space to obtain images with  $1.5 \times 1.5 \times 1.5 \text{ mm}^3$  voxels. In addition, we performed a volume change correction (modulation) (Ashburner and Friston 2000). Subsequently, the generated rGMV and rWMV images were smoothed by convolving them with an isotropic Gaussian kernel of 8-mm full width at half maximum (FWHM). The descriptions in this paragraph were mostly reproduced from our previous study using the exact same methods (Takeuchi et al. 2018).

The preprocessing of the diffusion data was performed using SPM8, which is implemented in Matlab. First, with the use of the prior validated (Takeuchi et al. 2013b) twisted two-step segmentation process, methods of the new segmentation algorithm and DARTEL-based registration process were altered to account for the signal distribution of FA within the white matter, FA maps were normalized, and these normalized FA images were then masked using a custom mask image, which is most likely the white matter and smoothed. Details of these preprocessing

**Table 1** Psychometric scale scores for each number of APOE ε4 alleles with statistical values

	ε4=0 (male) (N = 605) (20.8 ± 1.9 y.o.)	ε4=1 (male) (N = 129) (20.9 ± 1.9 y.o.)	ε4=2 (male) (N = 7) (19.6 ± 1.5 y.o.)	ε4=0 (female) (N = 402) (20.5 ± 1.5 y.o.)	ε4=1 (female) (N = 110) (20.8 ± 1.6 y.o.)	ε4=2 (female) (N = 5) (21.6 ± 1.9 y.o.)	Main effect P value <sup>a</sup> (uncorrected, FDR)	Sex interaction effect <sup>b</sup> [P (uncorrected, FDR), r (males, females)]
Digit span (N = 1252)	36.83 ± 6.97	37.55 ± 8.00	29.83 ± 4.67	34.85 ± 6.42	35.19 ± 6.30	34.00 ± 4.82	1.000, 0.758	0.804, 0.732 –2.0 × 10 <sup>–4</sup> , 0.014
RAPM (N = 1258)	28.81 ± 3.76	28.48 ± 4.13	23.57 ± 3.33	27.98 ± 3.89	28.14 ± 3.56	28.4 ± 2.87	0.097, 0.153	0.765, 0.732 –0.084, 0.019
Total score of TBIT (N = 1136)	114.6 ± 12.1	112.6 ± 11.5	107.3 ± 8.3	109.5 ± 11.5	111.0 ± 11.0	115.0 ± 11.9	1.000, 0.758	0.004, 0.014* –0.081, 0.062
Perception factor of TBIT (N = 1136)	49.51 ± 7.43	48.64 ± 6.65	45.86 ± 3.52	48.92 ± 6.61	49.80 ± 6.53	52.5 ± 7.53	0.272, 0.338	0.002, 0.009* –0.062, 0.068
Word-Color task (items) (N = 1256)	71.25 ± 8.18	70.71 ± 7.37	67.14 ± 5.3	70.00 ± 7.28	72.27 ± 7.19	74.20 ± 6.05	0.211, 0.288	<0.0002 <sup>c</sup> , 0.001* –0.043, 0.136
Color-Word task (items) (N = 1256)	52.38 ± 7.18	51.39 ± 6.05	50.71 ± 3.37	52.77 ± 6.31	53.85 ± 6.89	55.00 ± 3.41	0.643, 0.732	0.011, 0.030* –0.058, 0.075
Reverse Stroop task (items) (N = 1255)	59.90 ± 8.51	59.18 ± 7.66	52.43 ± 6.65	59.54 ± 7.70	61.87 ± 7.93	63.80 ± 8.84	0.804, 0.732	<0.0002 <sup>c</sup> , 0.001* –0.066, 0.130
Stroop task (items) (N = 1256)	48.34 ± 7.88	47.32 ± 7.75	46.14 ± 3.52	49.63 ± 6.60	49.94 ± 6.25	51.20 ± 3.66	0.046, 0.105	0.101, 0.153 –0.055, 0.027
S-A creativity test (N = 1258)	37.18 ± 10.59	37.82 ± 9.97	46.71 ± 11.56	39.07 ± 9.69	39.81 ± 10.71	45.2 ± 10.78	0.059, 0.115	1.000, 0.758 0.057, 0.051

Notes: RAPM, Raven's Advanced Progressive Matrix

<sup>a</sup>P values for main effects (entire cohort) of the APOE ε4 allele

<sup>b</sup>P value for the sex and the APOE ε4 allele interaction

<sup>c</sup>For the calculation of FDR-adjusted P values, uncorrected P values < 0.0002 were treated as 0.0002 (1/5000, once in 5000 iterations).



procedures as well as their validation were shown in prior study (Takeuchi et al. 2013b). For more detailed descriptions and rationales of these procedures, please see the [Supplemental Methods](#) section.

### Statistical Group-Level Analysis of Imaging and Psychological Data

Behavioral data were assessed using the R software, version 4.0.0 (Team-R-Core 2014). The main effects of the APOE  $\epsilon$ 4 allele (the number of alleles) on psychological measures among the entire cohort and the interaction between the number of APOE  $\epsilon$ 4 alleles and sex were evaluated with the analyses of covariance (ANCOVAs). As seen in these analyses, sex was a fixed factor, and both age and the number of APOE  $\epsilon$ 4 alleles (0, 1, and 2) were covariates. *P* values that were not corrected for multiple comparisons were evaluated with permutation (5000 iterations)-ANCOVAs using the *lmPerm* package (Wheeler 2010) of R because of the relatively small number of subjects with the  $\epsilon$ 4/ $\epsilon$ 4. We used permutation tests because permutation tests are distribution-free and robust to the small sample size in each group (Tanizaki 2006). In psychological analyses, the statistical results having a threshold of  $P < 0.05$ , which is corrected for false discovery rate (FDR) with the two-stage sharpened method (Benjamini et al., 2006), were statistically significant. This multiple comparison correction method was used in the results of the abovementioned ANCOVAs. Because nine psychological measures were observed, analyses of the main effects and the sex interaction effects had 18 *P* values for multiple comparison correction.

Second-level statistical analyses were conducted using the SPM8 software. In group-level imaging analyses, we tested for the main effects of the number of APOE  $\epsilon$ 4 alleles in the entire cohort as well as the interaction effects between the number of APOE  $\epsilon$ 4 alleles and sex on regional brain structures.

In the analyses of regional gray matter volume (rGMV) and regional white matter volume (rWMV), only voxels with a signal intensity of  $>0.05$  were part for all the subjects. The analyses of FA were limited to the white matter mask made as previously mentioned.

Whole-brain analyses were performed using voxel-wise ANCOVA with sex difference as a grouping factor which employs the full factorial option of SPM8. All analyses involved the age and the number of APOE  $\epsilon$ 4 alleles as covariates. These covariates were modeled so that each had a unique relationship with imaging measures for each sex. In these analyses, the centering option was utilized for centering the interactions. The main effects of the number of APOE  $\epsilon$ 4 alleles [contrasts of (the effects of the number of APOE  $\epsilon$ 4 alleles for males and females) defined as (1 1) or (−1−1)] and the interaction between sex and the number of APOE  $\epsilon$ 4 alleles [contrasts of (the effect of the number of APOE  $\epsilon$ 4 alleles for males, the effect of the number of APOE  $\epsilon$ 4 alleles for females) defined as (−1 1) or (1−1)] were evaluated using *t*-contrasts. We have utilized *t*-contrasts to evaluate the interaction between both males and females. This procedure was used in our previous neuroimaging studies and other studies investigating sex interaction effects (Yamasue et al. 2008; Yang et al. 2014; Takeuchi et al. 2019a, 2019b). However, the statistical design was not identical to those who employed psychological analyses as are the cases of our previous studies (Takeuchi et al. 2019a, 2019b). This is because to our knowledge, neither R nor SPSS can adopt the

abovementioned statistical model of sex-interaction analyses employed in neuroimaging analyses using SPM.

A multiple comparison correction was performed with threshold-free cluster enhancement (TFCE) (Smith and Nichols 2009) and randomized (5000 permutations) nonparametric testing with the use of the TFCE toolbox (<http://dbm.neuro.uni-jena.de/tfce/>). We used a threshold of family-wise error rate which is corrected at  $P < 0.05$ .

## Results

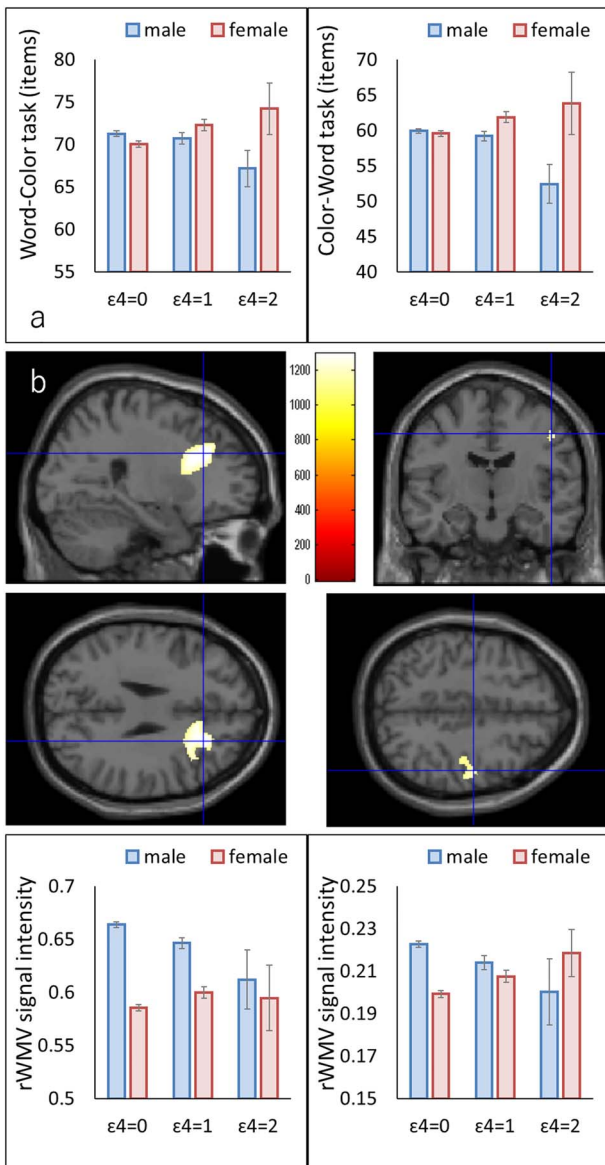
### Main and Interaction Effects of APOE $\epsilon$ 4 Genotypes on Psychological Metrics

ANCOVAs have demonstrated no significant main effects of APOE  $\epsilon$ 4 genotypes (the number of alleles) on psychological test scores, even at the uncorrected level. After correction for multiple comparisons (FDR), ANCOVAs have shown no significant interaction effects between sex and the number of APOE  $\epsilon$ 4 alleles on the total TBIT score (a measure of psychometric intelligence using speed tasks), the perception factor of TBIT, Word-Color task, Color-Word task (all measures of simple PS), and reverse Stroop task (a measure of executive function and inhibition). In the case of Word-Color task and reverse Stroop task, the interactions were primarily regulated by a significant positive correlation with the number of APOE  $\epsilon$ 4 alleles in females (Word-Color task,  $r = 0.136$ ,  $P = 0.002$ ; reverse Stroop task,  $r = 0.130$ ,  $P = 0.003$ , simple linear regression analyses) rather than a negative correlation in males (e.g., Word-Color task,  $r = -0.043$ ,  $P = 0.241$ ; reverse Stroop task,  $r = -0.066$ ,  $P = 0.075$ ). However, for other significant results, the strength of negative correlations in males and the positive correlations in females were not substantially different (Table 1).

### Main and Interaction Effects of APOE $\epsilon$ 4 Genotypes on Brain Structures

Whole-brain ANCOVAs of rGMV have shown no significant main effects of APOE  $\epsilon$ 4 genotypes and no interaction effect between sex and the number of APOE  $\epsilon$ 4 alleles on rGMV. Alternatively, whole-brain ANCOVAs have shown that there is a significant effect of the interaction between sex and APOE  $\epsilon$ 4 genotypes on two, broad white matter clusters: one below the right precentral gyrus and the other underneath the right lateral prefrontal cortex/right anterior cingulate cortex, spreading across the genu and body of the corpus callosum, anterior limb of the right internal capsule, right anterior and superior corona radiata, right external capsule, and right superior fronto-occipital fasciculus (Fig. 1b). Within both the clusters, the number of APOE  $\epsilon$ 4 alleles have shown that there is a significant negative correlation with mean rWMV in males (the former cluster,  $r = -0.113$ ,  $P = 0.002$ ; the latter cluster,  $r = -0.106$ ,  $P = 0.004$ ; simple linear regression analyses) and a significant positive correlation in females (the former cluster,  $r = 0.097$ ,  $P = 0.028$ ; the latter cluster,  $r = 0.120$ ,  $P = 0.006$ ). The whole-brain ANCOVAs of rWMV have shown that there are no significant main effects of the APOE  $\epsilon$ 4 allele on rWMV.

Finally, according to FA's whole-brain ANCOVAs, there are no significant main effects and no effects of the interaction between sex and the APOE  $\epsilon$ 4 allele on imaging measures.



**Figure 1.** APOE  $\epsilon 4$  genotype–sex interaction effects on cognitive function as well as regional brain volumes in young adults. (a) Effects on cognitive test performance. The left and right panels show the results of the Word-Color task, which is a measure of simple processing speed, and reverse Stroop task, which is a measure of executive function or inhibition, respectively. Error bars constitute the standard errors of mean. (b) Effects on rWMV. The left panels show an rWMV cluster underneath the right lateral prefrontal cortex and close to the anterior cingulate cortex, whereas the right panels show an rWMV cluster below the right precentral gyrus. The upper panels show the areas that are remarkably influenced by the APOE  $\epsilon 4$  allele–sex interaction. The results were acquired using a threshold for TFCE of  $P < 0.05$  based on 5000 permutations. Findings are overlaid on a “single-subject T1” image from SPM8. The color bar constitutes the TFCE value. The lowest two panels are the profiles of rWMV in the areas of significant clusters in both males and females of each number of the APOE  $\epsilon 4$  allele.

### The Associations between rWMV of Significant Clusters and Significant Behavioral Measures

Post hoc partial correlation analyses have shown that after controlling age, sex, and the APOE  $\epsilon 4$  allele, the rWMV of the previously mentioned significant cluster underneath the right

precentral gyrus (cluster No.2 in Table 2) has shown a significant positive correlation with the performance of Word-Color task ( $P = 0.017$ , partial correlation coefficient = 0.068), performance of Color-Word task ( $P = 0.017$ , partial correlation coefficient = 0.068), and performance of reverse Stroop task ( $P = 0.037$ , partial correlation coefficient = 0.059) but not with the total and perception scores of TBIT ( $P > 0.1$ ). Further, post hoc partial correlation analyses have indicated that after controlling age and sex rWMV of another larger significant cluster (cluster No.1 in Table 2), a significant positive correlation with performance of Word-Color task ( $P = 0.008$ , partial correlation coefficient = 0.074), performance of Color-Word task ( $P = 0.016$ , partial correlation coefficient = 0.068), and performance of reverse Stroop task ( $P = 0.002$ , partial correlation coefficient = 0.089) was shown but not with the total and perception score of TBIT ( $P > 0.1$ ).

### Discussion

To our knowledge, this is the first study that has assessed the sex-dependent effects of APOE genotypes on cognitive functions and regional brain structures using a very large sample of young adults (age during which females show fully increased estrogen levels). Partially consistent with our original hypothesis, the genotype–sex interaction affected the performance of an executive function task (reverse Stroop task) and simple PS tasks as well as an intelligence test, which comprise speed cognitive tasks (total score of TBIT). Specifically, females have indicated a significant positive correlation between the number of APOE  $\epsilon 4$  alleles and performance. Furthermore, partially consistent with our hypothesis, we have seen that there is an effect of the sex–genotype interaction on rWMV just below the right lateral prefrontal cortex and the right anterior cingulate cortex as well as underneath the right precentral gyrus. Precisely, males exhibited significant negative correlations between the number of APOE  $\epsilon 4$  alleles and rWMV, whereas females exhibited significant positive correlations.

Females with a greater number of APOE  $\epsilon 4$  alleles demonstrated superior performance in some speed tasks, which is consistent with the “antagonistic pleiotropy” hypothesis of this gene (in the elderly). These results are also in accordance with the general finding that young APOE  $\epsilon 4$  allele carriers repeatedly exhibit a superior performance, specifically in tasks that require attention rather than memory (Rusted and Carare 2015). Additionally, the effects of the observed sex interaction might explain why previous meta-analyses could not show a strong antagonistic pleiotropic effect of the APOE  $\epsilon 4$  allele in young adults (Weissberger et al. 2018).

The sex–genotype interaction affects rWMV of areas related to attention, executive function, and motor function; therefore, this interaction may underlie the observed behavioral findings. Significant interaction effects were seen in the white matter areas which are underneath the right lateral prefrontal cortex/right anterior cingulate cortex (which also included the genu of the corpus callosum) and underneath the right precentral gyrus. It has been proposed that the anterior cingulate cortex is a part of the task-relevant control of attention and/or response selection in the presence of conflicting information (MacLeod and MacDonald 2000). Conversely, the lateral prefrontal cortex is implied in both executive function and selective attention (Baddeley 2003). The genu of the corpus callosum connects the bilateral lateral prefrontal cortex (Barbas and Pandya 1984) and therefore might mediate interhemispheric information transfer

**Table 2** Regional brain volumes significantly influenced by the interaction between sex and the APOE  $\epsilon 4$  allele

No	Included large bundles** (number of significant voxels in the left and right side of each anatomical area)	x	y	z	TFCE value	Corrected P value (TFCE, FWE)	Cluster size (voxel)
1	The genu of the corpus callosum (54)/the body of the corpus callosum (39)/the anterior limb of the internal capsule (R:75)/the anterior corona radiata (R:674)/the superior corona radiata (R:66)/the external capsule (R:14)/the superior fronto-occipital fasciculus (R:33)	25.5	27	28.5	1294.65	0.032	2071
2	None	48	-12	45	1152.56	0.043	238

Note: \*\*The anatomical labels and significant clusters of major white matter fibers were determined using the ICBM DTI-81 Atlas (<http://www.loni.ucla.edu>).

which involves the lateral prefrontal cortex. Together with the white matter areas of the precentral cortex that are a part of motor function, these changed white matter structures might be the basis for some of the behavioral changes that are observed among APOE  $\epsilon 4$  allele carriers (e.g., in simple PS and executive attention tasks). Consistent with this idea, our prior study has indicated that simple PS and Stroop task performance are positively correlated with rWMV of widespread white matter areas (Magistro et al. 2015), and our additional analyses have shown that rWMV of clusters of significant effects of APOE  $\epsilon 4$  allele–sex interaction have a significant positive correlation with the performance of simple PS and Stroop task performance (behavioral measures of significant effects of APOE  $\epsilon 4$  allele–sex interaction). Although it is tempting to speculate that the observed relationships between APOE  $\epsilon 4$  allele and brain structures lead to the relationships between APOE  $\epsilon 4$  allele and functional connectivity that involve relevant regions (such as the hippocampus and the right DLPFC), in the present sample, there are no such associations in supplemental analyses (see [Supplemental Methods](#) and [Supplemental Results](#) for details). This may be because of the lower associations of resting state fMRI measures with the genotypes (Elliott et al. 2018); however, future studies with a larger sample size may have to further assess this concern.

The cross-sectional study design does not permit the exploration of the precise mechanisms that underlie the APOE  $\epsilon 4$  allele–sex interaction effect among young adults. Here, several possibilities were proposed. First, estrogen and the APOE  $\epsilon 4$  allele may behave synergistically in the elderly. For instance, estrogen regulates the expression of APOE (Srivastava et al. 1997), and estradiol promotes synaptic sprouting in response to injury via an APOE-dependent mechanism (Stone et al. 1998). Next, the APOE genotype–sex interaction in the elderly can be mediated by lacking estrogen levels (Yaffe et al. 2000). Conversely, the mechanisms that underlie the antagonistic pleiotropic effects of the APOE  $\epsilon 4$  allele in young adults are still not clear. APOE influences brain activity via multiple mechanisms (Liu et al. 2013). One hypothesis is that APOE is not only involved in the clearance of amyloid beta but also in neuronal remodeling. We have seen that young males and females that have greater neuronal plasticity than another gene have shown the evidence

of less neural tissues (Takeuchi et al. 2020). This may be partially mediated by the pattern that subjects with the genotype of greater neural plasticity tend to show the signs of less neural tissues with conditions of low exercise levels which are widespread in the modern world (Takeuchi et al. forthcoming). Similar mechanisms might be the cause of low rWMVs in young adults with the APOE  $\epsilon 4$  allele. However, this is highly theoretical, and future studies need to show these mechanisms.

The absence of the effects of the APOE  $\epsilon 4$  allele on brain structure independent of sex is not consistent with the effects that are observed in various prior studies, possibly because of methodological differences, for instance, image preprocessing. For example, a prior study (Heise et al. 2011) exploring FA values among 34 young adults and 37 older adults has reported that the APOE  $\epsilon 4$  allele is related to reduced FA across age groups, whereas the present study with 1258 subjects could not detect the effects of the APOE  $\epsilon 4$  allele on FA in young adults. The small sample size used might lead to several false positives or publication bias (Button et al. 2013). In a similar manner, a small-scale study of young adults has shown that there are significant effects of the APOE  $\epsilon 4$  allele on hippocampal volume (O'Dwyer et al. 2012); however, a study with a large sample size ( $N > 1400$ ) could not show such effects (Khan et al. 2014), which is consistent with the present negative findings that are related to rGMV.

The current study has a number of limitations. First, the focus of the study was on young adults; thus, it is not clear whether or how the APOE  $\epsilon 4$  allele influences cognitive functions in children or middle-aged adults. Although the effects of APOE genotypes are firmly established in the elderly, the effects in young adults are still not clear (Rusted and Carare 2015). In fact, even in this study, effects on white matter areas were marginally significant and thus need to be replicated. However, limiting the sample to young adults is also a strength of this study, considering that the age dependence of the effects of the APOE genotype could prevent more subtle interactions with sex and other factors from being detected. Additionally, similar to the case of studies on college units, we have been focusing on educated samples. However, we used a large sample and increased statistical power, and the lack of robust simple association between APOE genotype and cognitive functions across sexes is similar to the findings of



the meta-analysis (Weissberger et al. 2018). Conversely, focusing on the educated sample might decrease the range of cognitive test scores relative to a more representative sample, thereby diminishing the detection of simple relationships among cognition and the genotype across the sexes. Future studies might need to confirm this idea.

This is the first study to assess the effects of interaction between sex and APOE genotype, which has a strong relationship with the risk of Alzheimer's disease in elderly individuals, on cognitive traits, and brain structure in a large sample of young adults. Although this interaction is firmly established in elderly individuals, the effects of the APOE  $\epsilon$ 4 allele in younger adults remain controversial, and it has been stressed in a previous review that it is necessary to focus on the effects of interaction between sex and APOE genotype in young adults (Rusted and Carare 2015). The current study shows that the APOE  $\epsilon$ 4 allele is related to a better performance in speed tasks, including simple PS tasks and executive function tasks as well as enhanced white matter volumes in the anterior frontal and precentral areas in females, whereas it is the opposite in the white matter volumes in males. Collectively, these findings support the antagonistic pleiotropic effects of the APOE  $\epsilon$ 4 allele in females.

## Supplementary Material

Supplementary material can be found at *Cerebral Cortex* online

## Notes

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## References

- Acevedo SF, Piper BJ, Craytor MJ, Benice TS, Raber J. 2010. Apolipoprotein E4 and sex affect neurobehavioral performance in primary school children. *Pediatr Res*. 67:293.
- Altmann A, Tian L, Henderson VW, Greicius MD, Investigators AsDNI. 2014. Sex modifies the APOE-related risk of developing Alzheimer disease. *Ann Neurol*. 75:563–573.
- Ashburner J, Friston KJ. 2000. Voxel-based morphometry—the methods. *Neuroimage*. 11:805–821.
- Baddeley A. 2003. Working memory: looking back and looking forward. *Nat Rev Neurosci*. 4:829–839.
- Barbas H, Pandya DN. 1984. Topography of commissural fibers of the prefrontal cortex in the rhesus monkey. *Exp Brain Res*. 55:187–191.
- Benjamini Y, Krieger AM, Yekutieli D. 2006. Adaptive linear step-up procedures that control the false discovery rate. *Biometrika*. 93:491–507.
- Beydoun MA, Boueiz A, Abougergi MS, Kitner-Triolo MH, Beydoun HA, Resnick SM, O'Brien R, Zonderman AB. 2012. Sex differences in the association of the apolipoprotein E epsilon 4 allele with incidence of dementia, cognitive impairment, and decline. *Neurobiol Aging*. 33:720–731. e724.
- Boyles JK, Zoellner CD, Anderson LJ, Kosik LM, Pitas RE, Weisgraber K, Hui D, Mahley R, Gebicke-Haerter P, Ignatius M. 1989. A role for apolipoprotein E, apolipoprotein AI, and low density lipoprotein receptors in cholesterol transport during regeneration and remyelination of the rat sciatic nerve. *J Clin Invest*. 83:1015–1031.
- Button KS, Ioannidis JP, Mokrysz C, Nosek BA, Flint J, Robinson ES, Munafò MR. 2013. Power failure: why small sample size undermines the reliability of neuroscience. *Nat Rev Neurosci*. 14:365–376.
- Cherbuin N, Leach LS, Christensen H, Anstey KJ. 2007. Neuroimaging and APOE genotype: a systematic qualitative review. *Dement Geriatr Cogn Disord*. 24:348–362.
- Das H, McPherson J, Bruns G, Karathanasis S, Breslow J. 1985. Isolation, characterization, and mapping to chromosome 19 of the human apolipoprotein E gene. *J Biol Chem*. 260:6240–6247.
- Elliott LT, Sharp K, Alfaro-Almagro F, Shi S, Miller KL, Douaud G, Marchini J, Smith SM. 2018. Genome-wide association studies of brain imaging phenotypes in UK Biobank. *Nature*. 562:210–216.
- Han SD, Bondi MW. 2008. Revision of the apolipoprotein E compensatory mechanism recruitment hypothesis. *Alzheimers Dement*. 4:251–254.
- Heise V, Filippini N, Ebmeier K, Mackay C. 2011. The APOE  $\epsilon$ 4 allele modulates brain white matter integrity in healthy adults. *Mol Psychiatry*. 16:908–916.
- Katsuya T, Baba S, Ishikawa K, Mannami T, Fu Y, Inamoto N, Asai T, Fukuda M, Higaki J, Ogata J. 2002. Epsilon 4 allele of apolipoprotein E gene associates with lower blood pressure in young Japanese subjects: the Suita study. *J Hypertens*. 20:2017–2021.
- Khan W, Giampietro V, Ginestet C, Dell'Acqua F, Bouls D, Newhouse S, Dobson R, Banaschewski T, Barker GJ, Bokde AL. 2014. No differences in hippocampal volume between carriers and non-carriers of the ApoE  $\epsilon$ 4 and  $\epsilon$ 2 alleles in young healthy adolescents. *J Alzheimers Dis*. 40:37–43.
- Liu C-C, Kanekiyo T, Xu H, Bu G. 2013. Apolipoprotein E and Alzheimer disease: risk, mechanisms and therapy. *Nat Rev Neurol*. 9:106–119.
- MacLeod CM, MacDonald PA. 2000. Interdimensional interference in the Stroop effect: uncovering the cognitive and neural anatomy of attention. *Trends Cogn Sci*. 4: 383–391.
- Magistro D, Takeuchi H, Nejad KK, Taki Y, Sekiguchi A, Nouchi R, Kotozaki Y, Nakagawa S, Miyauchi CM, Iizuka K, et al. 2015. The relationship between processing speed and regional white matter volume in healthy young people. *PLoS One*. 10:e0136386.
- Neu SC, Pa J, Kukull W, Beekly D, Kuzma A, Gangadharan P, Wang L-S, Romero K, Arneric SP, Redolfi A. 2017. Apolipoprotein E genotype and sex risk factors for Alzheimer disease: a meta-analysis. *JAMA Neurol*. 74:1178–1189.
- O'Dwyer L, Lamberton F, Matura S, Tanner C, Scheibe M, Miller J, Rujescu D, Prvulovic D, Hampel H. 2012. Reduced hippocampal volume in healthy young ApoE4 carriers: an MRI study. *PLoS One*. 7:e48895.
- Oldfield RC. 1971. The assessment and analysis of handedness: the Edinburgh inventory. *Neuropsychologia*. 9:97–113.



- Raven J. 1998. *Manual for Raven's progressive matrices and vocabulary scales*. Oxford: Oxford Psychologists Press.
- Rippon GA, Tang M-X, Lee JH, Lantigua R, Medrano M, Mayeux R. 2006. Familial Alzheimer disease in Latinos: interaction between APOE, stroke, and estrogen replacement. *Neurology*. 66:35–40.
- Rusted J, Carare RO. 2015. Are the effects of APOE  $\epsilon$ 4 on cognitive function in nonclinical populations age- and gender-dependent? *Neurodegener Dis Manag*. 5:37–48.
- Sampedro F, Vilaplana E, De Leon MJ, Alcolea D, Pegueroles J, Montal V, Carmona-Iragui M, Sala I, Sánchez-Saudinos M-B, Antón-Aguirre S. 2015. APOE-by-sex interactions on brain structure and metabolism in healthy elderly controls. *Oncotarget*. 6:26663.
- Shaw P, Lerch JP, Pruessner JC, Taylor KN, Rose AB, Greenstein D, Clasen L, Evans A, Rapoport JL, Giedd JN. 2007. Cortical morphology in children and adolescents with different apolipoprotein E gene polymorphisms: an observational study. *Lancet Neurol*. 6:494–500.
- Smith SM, Nichols TE. 2009. Threshold-free cluster enhancement: addressing problems of smoothing, threshold dependence and localisation in cluster inference. *Neuroimage*. 44:83–98.
- Srivastava RAK, Srivastava N, Averna M, Lin RC, Korach KS, Lubahn DB, Schonfeld G. 1997. Estrogen upregulates apolipoprotein E (apoE) gene expression by increasing apoE mRNA in the translating pool via the estrogen receptor  $\alpha$ -mediated pathway. *J Biol Chem*. 272:33360–33366.
- Stone DJ, Rozovsky I, Morgan TE, Anderson CP, Finch CE. 1998. Increased synaptic sprouting in response to estrogen via an apolipoprotein E-dependent mechanism: implications for Alzheimer's disease. *J Neurosci*. 18:3180–3185.
- Takeuchi H, Taki Y, Hashizume H, Sassa Y, Nagase T, Nouchi R, Kawashima R. 2011. Failing to deactivate: the association between brain activity during a working memory task and creativity. *Neuroimage*. 55:681–687.
- Takeuchi H, Taki Y, Nouchi R, Hashizume H, Sekiguchi A, Kotozaki Y, Nakagawa S, Miyauchi CM, Sassa Y, Kawashima R. 2013a. Effects of working memory-training on functional connectivity and cerebral blood flow during rest. *Cortex*. 49:2106–2125.
- Takeuchi H, Taki Y, Nouchi R, Hashizume H, Sekiguchi A, Kotozaki Y, Nakagawa S, Miyauchi CM, Sassa Y, Kawashima R. 2014. Effects of multitasking-training on gray matter structure and resting state neural mechanisms. *Hum Brain Mapp*. 35:3646–3660.
- Takeuchi H, Taki Y, Nouchi R, Yokoyama R, Kotozaki Y, Nakagawa S, Sekiguchi A, Iizuka K, Yamamoto Y, Hanawa S. 2019a. The effects of family socioeconomic status on psychological and neural mechanisms as well as their sex differences. *Front Hum Neurosci*. 12:543.
- Takeuchi H, Taki Y, Nouchi R, Yokoyama R, Kotozaki Y, Nakagawa S, Sekiguchi A, Iizuka K, Yamamoto Y, Hanawa S, et al. 2018. Allergic tendencies are associated with larger gray matter volumes. *Sci Rep*. 8:3694.
- Takeuchi H, Taki Y, Sassa Y, Hashizume H, Sekiguchi A, Fukushima A, Kawashima R. 2010. Regional gray matter volume of dopaminergic system associate with creativity: evidence from voxel-based morphometry. *Neuroimage*. 51:578–585.
- Takeuchi H, Taki Y, Thyreau B, Sassa Y, Hashizume H, Sekiguchi A, Nagase T, Nouchi R, Fukushima A, Kawashima R. 2013b. White matter structures associated with empathizing and systemizing in young adults. *Neuroimage*. 77:222–236.
- Takeuchi H, Tomita H, Taki Y, Kikuchi Y, Ono C, Yu Z, Nouchi R, Yokoyama R, Kotozaki Y, Nakagawa S, et al. 2019b. A common CACNA1C gene risk variant has sex-dependent effects on behavioral traits and brain functional activity. *Cereb Cortex*. 29:3211–3219.
- Takeuchi H, Tomita H, Taki Y, Kikuchi Y, Ono C, Yu Z, Sekiguchi A, Nouchi R, Kotozaki Y, Nakagawa S. 2015. Cognitive and neural correlates of the 5-repeat allele of the dopamine D4 receptor gene in a population lacking the 7-repeat allele. *Neuroimage*. 110:124–135.
- Takeuchi H, Tomita H, Taki Y, Kikuchi Y, Ono C, Yu Z, Sekiguchi A, Nouchi R, Kotozaki Y, Nakagawa S, et al. 2020. Effects of interaction between the BDNF Val66Met polymorphism and daily physical activity on mean diffusivity. *Brain Imaging Behav*. 14:806–820.
- Tanaka K, Okamoto K, Tanaka H. 2003. *Manual of New Tanaka B type intelligence test*. Tokyo: Kaneko Syobo.
- Tanizaki H. 2006. On small sample properties of permutation tests: a significance test for regression models. *Kobe University Economic Review*. 52:27–40.
- Team-R-Core. 2014. *R: A language and environment for statistical computing*. Vol 2014. Vienna, Austria: R Foundation for Statistical Computing.
- Weissberger GH, Nation DA, Nguyen CP, Bondi MW, Han SD. 2018. Meta-analysis of cognitive ability differences by apolipoprotein e genotype in young humans. *Neurosci Biobehav Rev*. 94:49–58.
- Wheeler B. 2010. lmpPerm: permutation tests for linear models. In: R package version 1.1-2.
- Yaffe K, Haan M, Byers A, Tangen C, Kuller L. 2000. Estrogen use, APOE, and cognitive decline: evidence of gene-environment interaction. *Neurology*. 54:1949–1954.
- Yamasue H, Abe O, Suga M, Yamada H, Inoue H, Tochigi M, Rogers M, Aoki S, Kato N, Kasai K. 2008. Gender-common and-specific neuroanatomical basis of human anxiety-related personality traits. *Cereb Cortex*. 18:46–52.
- Yang W, Liu P, Wei D, Li W, Hitchman G, Li X, Qiu J, Zhang Q. 2014. Females and males rely on different cortical regions in raven's matrices reasoning capacity: evidence from a voxel-based morphometry study. *PLoS One*. 9:e93104.