ORIGINAL ARTICLE

Sex modulates the apolipoprotein E ε4 effect on white matter and cortical functional connectivity in individuals with amnestic mild cognitive impairment

H. Lin[a,*], Y. Sun[a,*], M. Li[b,c], Y. Zhan[d], L. Lin[a], Z. Ding[b,e,f] and Y. Han[a,g,h]

[a]*Department of Neurology, Xuanwu Hospital of Capital Medical University, Beijing, China;* [b]*Vanderbilt University Institute of Imaging Science, Vanderbilt University, Nashville, TN;* [c]*Department of Radiology and Radiological Sciences, Vanderbilt University Medical Center, Nashville, TN, USA;* [d]*School of Mechanical, Electrical and Information Engineering, Shandong University, Jinan, China;* [e]*Department of Electrical Engineering and Computer Science, Vanderbilt University, Nashville, TN;* [f]*Department of Biomedical Engineering, Vanderbilt University, Nashville, TN, USA;* [g]*National Clinical Research Center for Geriatric Disorders, Beijing; and* [h]*Center of Alzheimer's Disease, Beijing Institute for Brain Disorders, Beijing, China*

Keywords:
apolipoprotein E, functional magnetic resonance imaging, functional connectivity, mild cognitive impairment, sex factors, white matter

Received 15 February 2020
Accepted 18 March 2020

European Journal of Neurology 2020, **0:** 1–7

doi:10.1111/ene.14226

Background and purpose: Recent studies from the Alzheimer's Disease Neuroimaging Initiative show that, in the USA, 75% of patients with Alzheimer's disease are female. To date, there have rarely been any attempts to analyze data by sex or gender, which limits the potential for discovering the effects of sex or gender on disease. Little evidence is available regarding the effect of gender and apolipoprotein E (APOE) ε4 on white matter (WM) connection from the functional perspective due to the lack of appropriate techniques for detecting blood-oxygen-level-dependent signals in WM.

Methods: We took advantage of a new framework known as functional tensor imaging to investigate the effect of sex and APOEε4 on WM cortical functional connectivity throughout the brain.

Results: In a group of female patients with amnestic mild cognitive impairment, we found a significantly reduced functional connectivity in the left posterior limb of the internal capsule, left superior fronto-occipital fasciculus, bilateral temporopolar area and right somatosensory association cortex in APOEε4 carriers in contrast to non-carriers. We also found a significant APOEε4 by sex interaction effect on the right somatosensory association cortex, left temporopolar area and left superior temporal gyrus. The clinical Montreal Cognitive Assessment score was significantly negatively associated with the right somatosensory association cortex with APOEε4 by sex interaction in males.

Conclusions: These results indicate that increased APOE-related risk in women may be associated with decreased activity in both gray matter and WM in patients with amnestic mild cognitive impairment compared with men. The finding suggests accounting for sex differences in neuroimaging biomarkers, diagnostics and treatment strategy.

Introduction

Alzheimer's disease (AD) is a highly prevalent, progressive neurodegenerative disorder. The overall duration of the pre-clinical, prodromal and dementia stages of AD varies from 15 to 24 years [1]. Early identification and intervention are crucial during the prodromal stage of AD rather than attempts to

Correspondence: Y. Han, Department of Neurology, Xuanwu Hospital of Capital Medical University, 45 Changchun Street, Beijing, 100053, China (tel.: +86 13621011941; fax: +86 83167306, e-mail: hanying@xwh.ccmu.edu.cn).
*These authors contributed equally to this work.

reverse AD at dementia stage. Amnestic mild cognitive impairment (aMCI) is a prodromal stage of AD in which about 10–15% of cases will eventually progress to AD. Apolipoprotein E (APOE) ε4 allele is a genetic risk factor for AD. A commonly ignored critical feature of APOEε4 is that females are more susceptible to APOEε4-related pathogenic changes in aMCI compared with males [2,3]. Most studies in the field of clinical AD treated sex or gender as a covariate in the statistical analysis, which limits the potential for discovering the effects of sex or gender on disease [4]. Moreover, recent studies from the Alzheimer's Disease Neuroimaging Initiative show that 75% of patients with AD are female in the USA and females appear to progress to AD more often than males [5].

Further research has been carried out to assess the effect of sex and APOE4 on specific brain changes, but the mechanisms remain controversial. A tau positron emission tomography imaging study reported that female APOEε4 carriers exhibited a significant tau deposition with mild cognitive impairment (MCI) compared with males [3]. Baseline hippocampal volume and APOE4 status were the most important predictors in women with MCI converting to AD [6]. By contrast, two clinical cohort studies (Alzheimer's Disease Neuroimaging Initiative and Karolinska imaging dementia study) reported that male APOEε4 carriers with aMCI had a greater risk than female carriers for the contribution of white matter (WM) microbleeds to AD [7]. A multimodal neuroimaging study indicated no significant interaction between gender and APOEε4 on the default mode network in cognitively normal older adults [8].

In particular, little evidence is available regarding the effect of gender and APOEε4 on WM connection from the functional perspective. It is well known that the nature of WM nourishes much less oxygenated blood and generates much weaker blood-oxygen-level-dependent (BOLD) signals than gray matter (GM) [9–11]. Recently, there have been a growing number of studies describing reliably detected BOLD signals in WM [12,13]. Ding *et al.* [12] proposed a new graph-based framework to measure WM functional connectivity by constructing functional tensors and segmenting WM bundles with specific parcellated GM volumes. Further to this, some research found WM functional connectivity and BOLD signal changes due to task loading or disease conditions [11,14]. Furthermore, WM functional networks were reliably identified in a recent report [15]. In addition, independent component analysis showed increased expression in WM functional activity during the resting state in migraine with aura [16]. These results demonstrated the detectability of WM BOLD signals and, more importantly, reveal the reliability of the analysis method in exploring WM neural activity.

In the present study, we took advantage of the new graph-based imaging analyses framework to investigate the effect of sex and APOEε4 on WM cortical functional connectivity throughout the brain. This study provides new insights suggesting that a full-scale functional framework of the entire brain may be of help in understanding AD progression and improving AD clinical trials.

Methods

Participants

All participants in the current study were collected from Xuanwu Hospital of Capital Medical University. Inclusion criteria for patients with aMCI were as follows [17,18]: (i) concern about subjective memory decline; (ii) abnormal memory performance in single or multiple domains including memory based on review of test (≤1.5 SD); (iii) total Clinical Dementia Rating score of 0.5 or higher; and (iv) independent activities of daily living. The exclusion criteria were as follows: (i) Hamilton Depression Rating Scale score ≥24 points; (ii) suffered from stroke; (iii) other diseases causing cognitive impairment; (iv) contraindications for magnetic resonance imaging; and (v) a history of psychosis or congenital mental growth retardation.

All individuals underwent a standard examination including a clinical screening survey, series of neuropsychological tests, blood test and functional and structural magnetic resonance imaging scans. APOE genotyping was performed as described previously [19]. APOEε4 carriers (APOEε4+) were defined as subjects with at least one ε4 allele (either ε4/ε4 or ε4/ε3). Non-carriers (APOEε4−) were defined as individuals with an ε3 homozygote (ε3/ε3). Individuals with the ε2 allele were excluded. We obtained a sample of 75 subjects. The study was registered on ClinicalTrials.gov (identifier NCT02225964). The protocol was approved by the medical research ethics board of Xuanwu Hospital of Capital Medical University. Written informed consent was completed from all subjects before study.

Region of interest definitions and image analysis

Image acquisition and pre-processing are available in Appendix S1. In this study, WM and GM regions of interest definitions were based on the framework proposed by Ding *et al.* [12]. A total of 48 bundle regions were constructed from the JHU ICBM-DTI-

81 WM atlas [20], including 21 bundles in each hemisphere and six commissure bundles (Table 1). Meanwhile, the GM was segmented into 82 regions (41 in each hemisphere) according to Brodmann's definitions. Furthermore, all bundle regions were restricted within each subject's WM mask threshold tightly at 0.95, for the sake of avoiding partial volume effects from nearby GM regions. Finally, across voxels within each of the WM bundles and GM regions defined above, BOLD signals were averaged to produce a mean time series, which was subsequently used to derive pairwise temporal correlations between these WM bundles and GM regions.

Statistical analysis

All statistical analyses were carried out with SPSS (v24.0, IBM, Armonk, NY, USA). Demographic variables and clinical cognitive scores were compared between males and females using two-tailed *t*-test or chi-squared test. A stratification analysis was used to explore the sex differences in APOEε4 risk in correlation coefficients of BOLD signals in the WM bundle and GM region. A two-way ANCOVA was then performed to detect the sex by APOE interaction in functional connectivity in WM bundles and GM regions with age, years of education and scanner type as covariates. A partial correlation analysis was conducted between the imaging data and neuropsychiatric variables. $P < 0.05$ was considered significant.

Table 1 Abbreviation list of white matter (WM) bundles

Abbreviation	Full name of WM bundles
CST	Corticospinal tract
ML	Medial lemniscus
ICP	Inferior cerebellar peduncle
SCP	Superior cerebellar peduncle
CP	Cerebral peduncle
ALIC	Anterior limb of internal capsule
PLIC	Posterior limb of internal capsule
RLIC	Retrolenticular part of internal capsule
ACR	Anterior corona radiata
SCR	Superior corona radiata
PCR	Posterior corona radiata
PTR	Posterior thalamic radiation (includes optic radiation)
SS	Sagittal stratum (includes inferior longitudinal fasciculus and inferior fronto-occipital fasciculus)
EC	External capsule
CGC	Cingulum (cingulate gyrus)
CGH	Cingulum (hippocampus)
Fx/ST	Fornix (cres)/stria terminalis (cannot be resolved with current resolution)
SLF	Superior longitudinal fasciculus
SFO	Superior fronto-occipital fasciculus (could be a part of anterior internal capsule)
UNC	Uncinate fasciculus
TAP	Tapetum
BCC	Body of corpus callosum
SCC	Splenium of corpus callosum
Fx	Fornix (column and body of fornix)

Results

Behavioral results

A total of 75 patients with aMCI were obtained, including 38 females (ε4 carriers vs. non-carriers: 20 vs. 18) and 37 males (ε4 carriers vs. non-carriers: 18 vs. 19). There were no significant differences in age, gender, years of education or APOEε4 prevalence between females and males in patients with aMCI (all $P > 0.1$). There were also no significant differences in Mini Mental State Examination and Montreal Cognitive Assessment (MoCA) scores (all $P > 0.1$). Detailed information is summarized in Table 2.

Effect of sex differences in apolipoprotein E on functional connectivity in white matter bundles

In the group of males with aMCI, there were no significant differences in WM tract functional signals across the whole brain between APOEε4 carriers and non-carriers (Figs 1a–d and 2a, all $P > 0.05$). However, in the group of females with aMCI, aMCI with APOEε4 allele showed decreased signals in the left inferior cerebellar peduncle, external capsule, uncinate fasciculus, genu of corpus callosum, posterior limb of internal capsule, superior fronto-occipital fasciculus, right cingulum and uncinate fasciculus (Figs 1e–h and 2b, all $P < 0.05$). When a more stringent correction was applied [21], we still observed a significant reduction in

Table 2 Demographic and neuropsychological assessments for all patients with amnestic mild cognitive impairment (aMCI)

aMCI	Male (n = 37)	Female (n = 38)	*t*-test/chi-squared test value	*P*-value
Age (years)	67.73 ± 8.63	66.84 ± 8.98	0.436	0.664[a]
Education (years)	11.73 ± 3.45	10.79 ± 3.91	1.102	0.274[a]
APOEε4 (+/−)	18/19	20/18	0.119	0.730[b]
MMSE score	25.40 ± 0.44	24.82 ± 0.43	0.869	0.354[c]
MoCA score	20.70 ± 0.55	19.58 ± 0.54	2.086	0.153[c]

[a]Two-sample *t*-test; [b]Pearson chi-squared test; [c]One-way ANCOVA.

the left posterior limb of internal capsule and left superior fronto-occipital fasciculus in APOEε4 carriers compared with non-carriers. However, the interaction of sex by APOEε4 was not found.

Effect of sex differences in apolipoprotein E on functional connectivity in gray matter regions

There were no significant differences in cortical functional connectivity between male APOEε4 carriers and non-carriers (Fig. 2c, corrected). Nevertheless, in the group of females with aMCI, the difference of cortical functional connectivity was significant in APOEε4 carriers compared with non-carriers (Fig. 2d, corrected). The findings demonstrated that the cortical connectivity was decreased in the bilateral temporopolar area and superior temporal gyrus and left primary somatosensory cortex, subgenual area, ventral entorhinal cortex, right auditory cortex, associative visual cortex, visuo-motor coordination and somatosensory association cortex in APOEε4 carriers compared with non-carriers. The differences in the bilateral temporopolar area and right somatosensory association cortex were still significant after correction.

We then further detected an interaction for gender and APOE genotype in GM and the result showed a significant interaction effect in the right somatosensory association cortex, right temporopolar area and left superior temporal gyrus. *Post hoc* comparison showed that APOEε4+ females with aMCI had reduced cortical functional connectivity relative to male ε4− and ε4+ and female ε4− (Fig. 3a–c).

Relationship between cortical function and neuropsychological variables

In patients with aMCI, there was a significant negative correlation between MoCA score and the right somatosensory association cortex in males ($r = -0.474$, $P = 0.003$). However, there was no significant correlation between MoCA score and the right somatosensory association cortex in females ($r = -0.285$, $P = 0.082$).

Discussion

The sex-stratified analyses showed that the effect of APOEε4 on functional connectivity of GM and WM was not significant in men, whereas it was significant in women who were prominently influenced by APOEε4 genotype. The interaction of sex and APOEε4 revealed that the cortical functional connectivity was reduced in female APOEε4 carriers with aMCI, suggesting that female APOEε4 carriers are at greater risk of AD progress.

Some studies have reported a higher incidence of AD among women [22,23] Clinical studies have reported that female APOEε4 carriers had a higher risk of AD than male carriers [24]. In addition, an autopsy study found that women had more senile plaques and the association was stronger among ε4 carriers [25]. Another study reported that the association of APOEε4 with neurofibrillary tangles was weaker in men than in women, whereas the association with senile plaques was significant in both sexes [26]. A recent study suggested that women are more susceptible to APOEε4-associated accumulation of neurofibrillary tangles in MCI compared with men [3]. Overall, different associations of APOEε4 and AD pathologic biomarkers were seen in females and males in accordance with previous evidence.

The sex-dependent role of APOEε4 in the risks of developing MCI and in MCI conversions to AD has recently been investigated and there is evidence that women are at greater risk than men [2,27]. However, only a few studies have directly investigated the risk

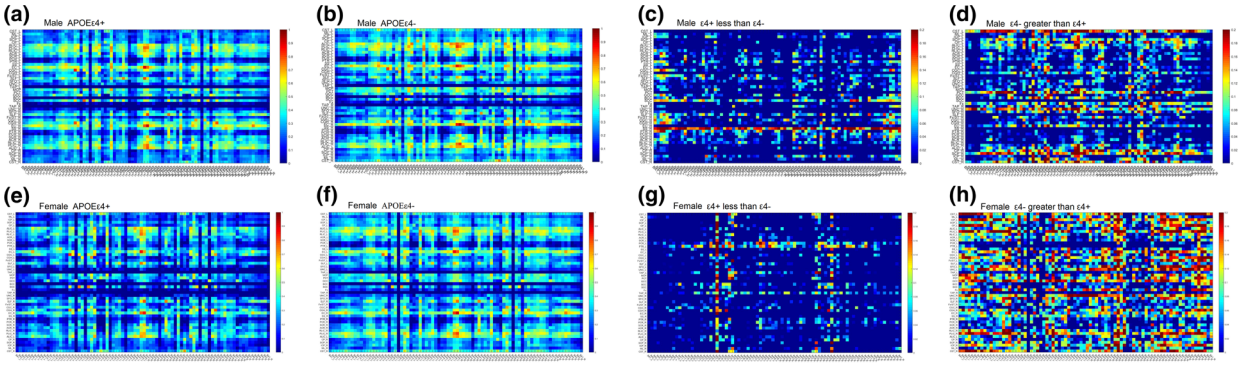

Figure 1 Sex modifies the apolipoprotein E (APOE)ε4 effect on the temporal correlations in blood-oxygen-level-dependent signals between gray matter regions and white matter bundles. Average maps (a, b, e and f) and divergence maps (c, d, g and h) are shown with hot colors (red and yellow) denoting positive differences.

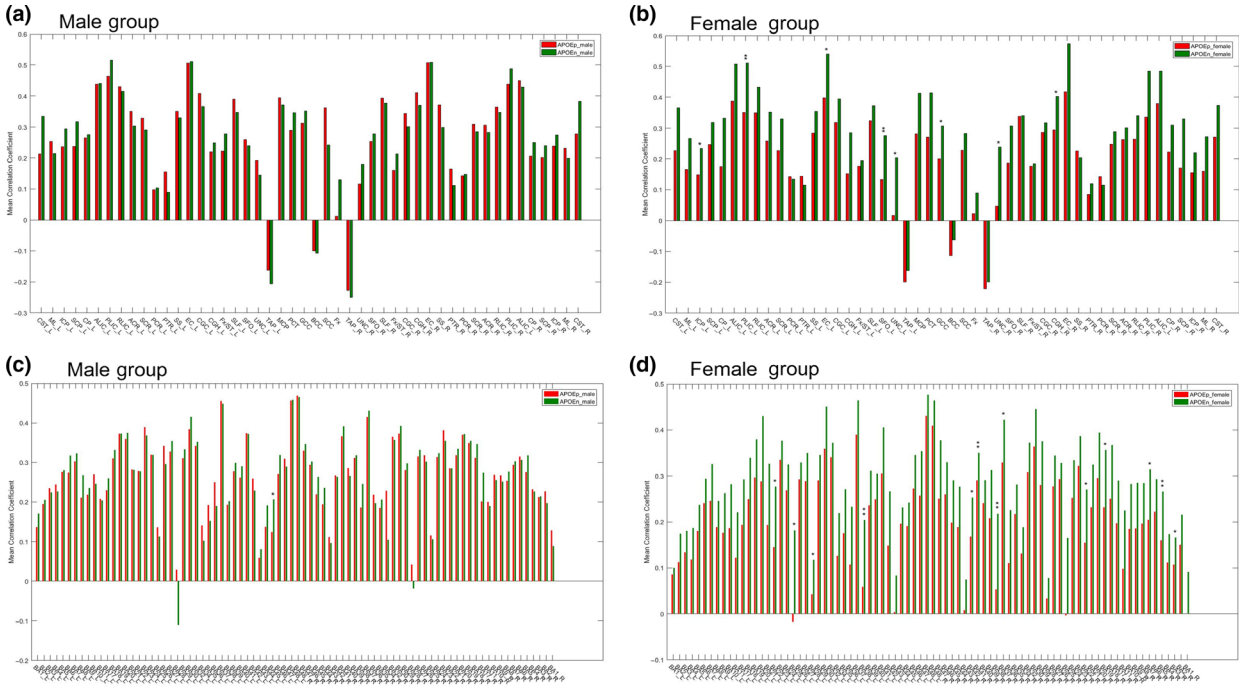

Figure 2 Sex differences between apolipoprotein E (APOE)ε4 carriers (red) and non-carriers (green) with amnestic mild cognitive impairment (aMCI) in the averaged correlations of (a and b) white matter bundle and (c and d) gray matter region. *$P < 0.05$. **Significant differences with correction.

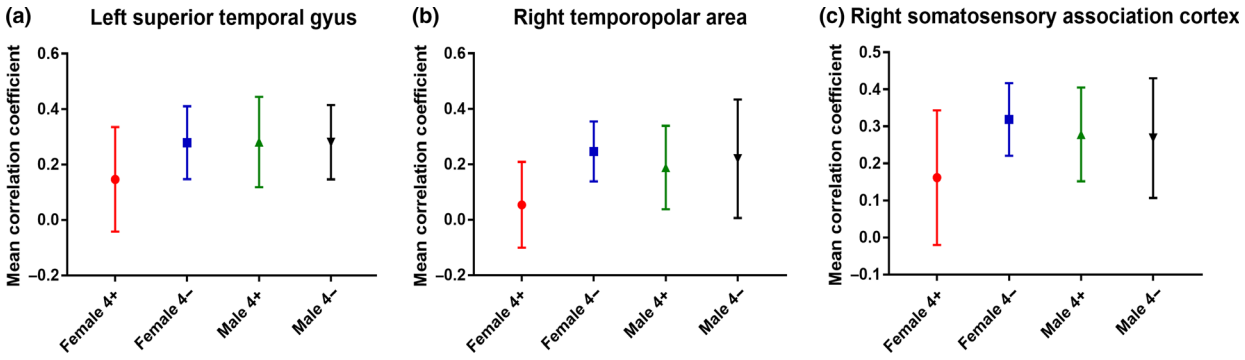

Figure 3 *Post hoc* comparison results in gray matter regions.

factors of sex and APOEε4 and their additive influences on brain functional degeneration in patients with MCI, which may accelerate the progression to dementia. Here, we observed significant functional connectivity reduction in the left posterior limb of internal capsule and left superior fronto-occipital fasciculus in female APOEε4 carriers compared with non-carriers using a stratified analysis. Moreover, a significant interaction effect of sex and APOEε4 on functional connectivity in the right somatosensory association cortex, left temporopolar area and left superior temporal gyrus was found. Interestingly, the connectivity of the above regions and fibers was also reduced in APOEε4 carriers with aMCI compared with non-carriers in our previous study, indicating that these regions were susceptible to genetic factors and potentially modified by sex. In line with our results, the multicenter European diffusion tensor imaging study on dementia has suggested that elderly APOE ε4 carriers had lower fractional anisotropy values in the inferior fronto-occipital, corpus callosum and internal and external capsule compared with APOE ε4 non-carriers. Sex differences have not been established in the study [28].

Finally, we found a significant negative correlation between MoCA scores and the right somatosensory association cortex only in men. In men, greater effects of APOEε4 were shown in longitudinal decline in memory and executive function, whereas in women, greater APOEε4 effects were shown in longitudinal decline in attention [29]. Male ε4 carriers are at a behavioral advantage in midlife on a sensitive task of short-term memory [30]. Thus, the negative association between global cognitive performance and the somatosensory association cortex in men compared with women may be due to compensation and highlights the interaction of gender on the influence of APOE in cognition.

The present study presents some limitations. Firstly, this study used cross-sectional data. The ongoing multicenter longitudinal study, SILCODE, plays an important role in verifying the current assumptions. Second, with respect to interpretation of the results, the limited sample size should be taken into consideration. Third, in this study we only used Mini Mental State Examination and MoCA scores to evaluate the association with brain function alterations. It would be important to include wider psychological tests to capture the neuropsychiatric performance in a more comprehensive manner.

The current study focused on the regulation of APOEε4 risk allele on resting-state brain function in male and female patients with aMCI. APOE-related vulnerability in women with aMCI may be associated with decreased activity in both GM and WM compared with men. This APOE by sex interaction may contribute to refining the most at-risk population and should be considered in clinical trials where APOEε4 status is a selection criterion.

Disclosure of conflicts of interest

The authors declare no financial or other conflicts of interest.

Acknowledgements

This work was supported by the National Key Research and Development Program of China (2016YFC1306300, 2018YFC1312001) and National Natural Science Foundation (grants 61633018, 81871438, 81771909).

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Supplementary methods.

References

1. Vermunt L, Sikkes SAM, van den Hout A, *et al*. Duration of preclinical, prodromal, and dementia stages of Alzheimer's disease in relation to age, sex, and APOE genotype. *Alzheimers Dement* 2019; **15:** 888–898.
2. Altmann A, Tian L, Henderson VW, Greicius MD. Alzheimer's disease neuroimaging initiative. I. Sex modifies the APOE-related risk of developing Alzheimer disease. *Ann Neurol* 2014; **75:** 563–573.
3. Liu M, Paranjpe MD, Zhou X, *et al*. Sex modulates the ApoE epsilon4 effect on brain tau deposition measured by (18)F-AV-1451 PET in individuals with mild cognitive impairment. *Theranostics* 2019; **9:** 4959–4970.
4. Woitowich NC, Woodruff TK. Opinion: Research community needs to better appreciate the value of sex-based research. *Proc Natl Acad Sci USA* 2019; **116:** 7154–7156.
5. Veitch DP, Weiner MW, Aisen PS, *et al*. Understanding disease progression and improving Alzheimer's disease clinical trials: Recent highlights from the Alzheimer's Disease Neuroimaging Initiative. *Alzheimers Dement* 2019; **15:** 106–152.
6. Spampinato MV, Langdon BR, Patrick KE, *et al*. Gender, apolipoprotein E genotype, and mesial temporal atrophy: 2-year follow-up in patients with stable mild cognitive impairment and with progression from mild cognitive impairment to Alzheimer's disease. *Neuroradiology* 2016; **58:** 1143–1151.
7. Cacciottolo M, Christensen A, Moser A, *et al*. The APOE4 allele shows opposite sex bias in microbleeds and Alzheimer's disease of humans and mice. *Neurobiol Aging* 2016; **37:** 47–57.
8. Cavedo E, Chiesa PA, Houot M, *et al*. Sex differences in functional and molecular neuroimaging biomarkers of Alzheimer's disease in cognitively normal older adults with subjective memory complaints. *Alzheimers Dement* 2018; **14:** 1204–1215.
9. Gawryluk JR, Mazerolle EL, D'Arcy RC. Does functional MRI detect activation in white matter? A review of emerging evidence, issues, and future directions. *Front Neurosci* 2014; **8:** 239.
10. Huang Y, Bailey SK, Wang P, Cutting LE, Gore JC, Ding Z. Voxel-wise detection of functional networks in white matter. *NeuroImage* 2018; **183:** 544–552.
11. Li M, Newton AT, Anderson AW, Ding Z, Gore JC. Characterization of the hemodynamic response function in white matter tracts for event-related fMRI. *Nat Commun* 2019; **10:** 1140.
12. Ding Z, Huang Y, Bailey SK, *et al*. Detection of synchronous brain activity in white matter tracts at rest and under functional loading. *Proc Natl Acad Sci* 2018; **115:** 595–600.
13. Gore JC, Li M, Gao Y, *et al*. Functional MRI and resting state connectivity in white matter – a mini-review. *Magn Reson Imaging* 2019; **63:** 1–11.
14. Wang J, Yang Z, Zhang M, *et al*. Disrupted functional connectivity and activity in the white matter of the sensorimotor system in patients with pontine strokes. *J Magn Reson Imaging* 2019; **49:** 478–486.
15. Wang P, Meng C, Yuan R, *et al*. The organization of the human corpus callosum estimated by intrinsic functional connectivity with white-matter functional networks. *Cereb Cortex* 2020; pii: bhz311. https://doi.org/10.1093/cercor/bhz311. [Epub ahead of print].

16. Farago P, Toth E, Kocsis K, *et al*. Altered resting state functional activity and microstructure of the white matter in migraine with aura. *Front Neurol* 2019; **10:** 1039.
17. Petersen RC. Mild cognitive impairment as a diagnostic entity. *J Intern Med* 2004; **256:** 183–194.
18. Albert MS, DeKosky ST, Dickson D, *et al*. The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement* 2011; **7:** 270–279.
19. Sun Y, Wang X, Wang Y, *et al*. Anxiety correlates with cortical surface area in subjective cognitive decline: APOE epsilon4 carriers versus APOE epsilon4 non-carriers. *Alzheimers Res Ther* 2019; **11:** 50.
20. Mori S, Oishi K, Jiang H, *et al*. Stereotaxic white matter atlas based on diffusion tensor imaging in an ICBM template. *NeuroImage* 2008; **40:** 570–582.
21. Lynall ME, Bassett DS, Kerwin R, *et al*. Functional connectivity and brain networks in schizophrenia. *J Neurosci* 2010; **30:** 9477–9487.
22. Plassman BL, Langa KM, McCammon RJ, *et al*. Incidence of dementia and cognitive impairment, not dementia in the United States. *Ann Neurol* 2011; **70:** 418–426.
23. Oveisgharan S, Arvanitakis Z, Yu L, Farfel J, Schneider JA, Bennett DA. Sex differences in Alzheimer's disease and common neuropathologies of aging. *Acta Neuropathol* 2018; **136:** 887–900.
24. Farrer LA, Cupples LA, Haines JL, *et al*. Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis. APOE and Alzheimer Disease Meta Analysis Consortium. *JAMA* 1997; **278:** 1349–1356.
25. Corder EH, Ghebremedhin E, Taylor MG, Thal DR, Ohm TG, Braak H. The biphasic relationship between regional brain senile plaque and neurofibrillary tangle distributions: modification by age, sex, and APOE polymorphism. *Ann N Y Acad Sci* 2004; **1019:** 24–28.
26. Ghebremedhin E, Schultz C, Thal DR, *et al*. Gender and age modify the association between APOE and AD-related neuropathology. *Neurology* 2001; **56:** 1696–1701.
27. Liu CC, Liu CC, Kanekiyo T, Xu H, Bu G. Apolipoprotein E and Alzheimer disease: risk, mechanisms and therapy. *Nat Rev Neurol* 2013; **9:** 106–118.
28. Cavedo E, Lista S, Rojkova K, *et al*. Disrupted white matter structural networks in healthy older adult APOE epsilon4 carriers – An international multicenter DTI study. *Neuroscience* 2017; **357:** 119–133.
29. Williams OA, An Y, Armstrong NM, *et al*. Apolipoprotein E epsilon4 allele effects on longitudinal cognitive trajectories are sex and age dependent. *Alzheimers Dement* 2019; **15:** 1558–1567.
30. Zokaei N, Giehl K, Sillence A, *et al*. Sex and APOE: A memory advantage in male APOE epsilon4 carriers in midlife. *Cortex* 2017; **88:** 98–105.