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A comprehensive analysis of *APOE* genotype effects on human brain structure in the UK Biobank

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Alzheimer's disease (AD) risk is increased in carriers of the apolipoprotein E (*APOE*) $\epsilon 4$ allele and decreased in $\epsilon 2$ allele carriers compared with the $\epsilon 3\epsilon 3$ genotype. The aim of this study was to determine whether: the *APOE* genotype affects brain grey (GM) or white matter (WM) structure; and if differences exist, the age when they become apparent and whether there are differential effects by sex. We used cross-sectional magnetic resonance imaging data from ~43,000 (28,494 after pre-processing) white British cognitively healthy participants (7,446 *APOE* $\epsilon 4$ carriers) aged 45–80 years from the UK Biobank cohort and investigated image-derived phenotypes (IDPs). We observed no statistically significant effects of *APOE* genotype on GM structure volumes or median T2* in subcortical structures, a measure related to iron content. The volume of white matter hyperintensities differed significantly between *APOE* genotype groups with higher volumes in *APOE* $\epsilon 4\epsilon 4$ (effect size 0.14 standard deviations [SD]) and $\epsilon 3\epsilon 4$ carriers (effect size 0.04 SD) but no differences in $\epsilon 2$ carriers compared with $\epsilon 3\epsilon 3$ carriers. WM integrity measures in the dorsal (mean diffusivity [MD]) and ventral cingulum (MD and intracellular volume fraction), posterior thalamic radiation (MD and isotropic volume fraction) and sagittal stratum (MD) indicated lower integrity in *APOE* $\epsilon 4\epsilon 4$ carriers (effect sizes around 0.2–0.3 SD) and $\epsilon 3\epsilon 4$ (effect sizes around 0.05 SD) carriers but no differences in $\epsilon 2$ carriers compared with the *APOE* $\epsilon 3\epsilon 3$ genotype. Effects did not differ between men and women. *APOE* $\epsilon 4$ homozygotes had lower WM integrity specifically at older ages with a steeper decline of WM integrity from the age of 60 that corresponds to around 5 years greater “brain age”. *APOE* genotype affects various white matter measures, which might be indicative of preclinical AD processes. This hypothesis can be assessed in future when clinical outcomes become available.

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INTRODUCTION

Carriers of the apolipoprotein E gene (*APOE*) $\epsilon 4$ allele (~ 25% of white Europeans) have a higher risk of Alzheimer's disease (AD) (odds ratio of ~3 for heterozygotes and ~15 for homozygotes) while carriers of the rarer $\epsilon 2$ allele have a lower AD risk (odds ratio of 0.6) compared with carriers of the most common *APOE* $\epsilon 3\epsilon 3$ genotype [1, 2]. There may be an interaction between *APOE* genotype and sex because only female but not male $\epsilon 4$ heterozygotes showed increased AD risk [2], however, this view has recently been challenged by a much larger meta-analysis [3].

Magnetic resonance imaging (MRI) can be used to identify changes in brain structure in symptomatic AD patients [4]. In studies of familial AD cases and controls, differences in cortical thickness between participants carrying autosomal dominant AD genes and non-carriers can be observed years before onset of symptoms with some regions such as the precuneus and parahippocampus showing thinning ~10 years before disease onset [5]. Therefore, changes in brain structure in healthy adults carrying *APOE* $\epsilon 4$ might be MRI biomarkers of pre-symptomatic AD that could identify potential participants for clinical trials of AD prevention or treatment before the onset of clinical symptoms, at which point interventions might be more effective [6].

Although small cross-sectional studies reported significant effects of *APOE* genotype on grey matter (GM) ([7], $N = 65$ $\epsilon 4$ carriers) and white matter (WM) structures ([8], $N = 275$ $\epsilon 4$ carriers), most studies of *APOE* genotype effects did not report significant differences ([9–15], $N = 40$ –1070 $\epsilon 4$ carriers). In adolescents *APOE* genotype was not associated with differences in brain structure in some ([16, 17], $N = 120$ –340 $\epsilon 4$ carriers), although not all studies ([18, 19], $N = 60$ –310 $\epsilon 4$ carriers). While some studies only reported effects of *APOE* genotype on brain regions that are affected by early AD pathology (e.g., refs. [7, 8]), such as the hippocampus and posterior cingulate cortex and their interconnecting tracts, there are also studies that show effects on regions across the whole brain (e.g., refs. [18, 19]). However, all of these studies were likely underpowered because they were small, or aggregated data from different MRI scanners with different data acquisition protocols and data analysis methods.

To more reliably estimate differences by *APOE* genotype, studies of a larger number of participants scanned with identical MRI hardware and data acquisition protocols are needed. In this study, we assessed whether, in about 43,000 (28,494 after pre-processing) UK Biobank participants with MRI brain scanning with a common protocol, *APOE* genotype affects brain grey or white matter structure

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across the whole brain and if differences exist, at what age they become apparent and whether there are differential effects by sex.

MATERIALS AND METHODS

Study design and participants

The UK Biobank (UKB) cohort is a large prospective epidemiological study of around 0.5 million participants [20]. Between 2006 and 2010 men and women aged 40 to 69 years were recruited and data collected on their lifestyle, environment, medical history and physical measures, along with biological samples. In 2014, UKB started to collect brain, heart and body imaging data from 100,000 UKB participants [21]. Data from 43,796 UK Biobank participants with pre-processed neuroimaging data released in February 2021 were included here.

Ethical approval

UKB has ethics approval from the North West Multi-centre Research Ethics Committee (MREC), which covers the UK (for more information, see: <http://www.ukbiobank.ac.uk/ethics/>). Informed consent was obtained from all subjects. This project was approved by UK Biobank (8835).

Genetic data

Procedures for genotyping in the UK Biobank are described in more detail elsewhere [22] and online on <http://www.ukbiobank.ac.uk/scientists-3/genetic-data>. For this project, *APOE* genotype information was derived from the combined allelic information of two SNPs, rs423958 and rs7412. Imputed and directly genotyped SNPs were identical where both were present but more participants had complete imputed genetic information available (99.8% vs ~85% for direct genotyping), so imputed genotypes were used in all analyses.

Neuroimaging data

The neuroimaging data acquisition and pre-processing pipelines have been described previously [23, 24]. In brief, data used in this project were acquired at three imaging centres using identical MRI scanners (3 T Siemens Skyra, software VD13) and the standard Siemens 32-channel receive head coil. The scanning protocol consisted of structural T1-weighted MRI (T1), resting-state functional MRI (rsfMRI), task fMRI (tfMRI), T2-weighted fluid-attenuated inversion recovery (FLAIR) imaging, diffusion MRI (dMRI) and susceptibility-weighted MRI (swMRI). These different modalities provide information on GM structure (T1), functional connectivity (rsfMRI), brain activation in response to a specific task (tfMRI), volumes of white matter hyperintensities (FLAIR), WM structure/ connectivity (dMRI) and vascular lesions/ iron content (swMRI). Automated pre-processing pipelines were developed for the UK Biobank imaging data to create image-derived phenotypes (IDPs). For T1-weighted data, the IDPs analysed in this study were: total GM, WM and cerebrospinal fluid (CSF) volumes and GM volumes for cortical regions of interest (ROIs), not including the cerebellum, derived using the FAST algorithm as well as subcortical GM volumes derived using the algorithm FIRST. For FLAIR imaging, total volume of white matter hyperintensities (WMHs) was provided as an IDP. All volumetric IDPs (GM and WMH volumes) were corrected for head size using the volumetric scaling factor provided in the UK Biobank data before running statistical tests. The IDPs used from diffusion MRI were fractional anisotropy (FA), mean diffusivity (MD) and three neurite orientation dispersion and density imaging (NODDI) measures (intra-cellular volume fraction [ICVF], isotropic/free water volume fraction [ISOVF] and orientation dispersion [OD]) averaged across skeletonised white matter tracts, excluding tracts in the cerebellum. The white matter tracts were defined by Alfaro-Almagro et al. [24] based on 48 standard-space tract masks developed by the group of Susumu Mori at Johns Hopkins University [25, 26]. Note that the two IDPs indicating different parts of the cingulum were renamed in this study to use more "standard" notation: the cingulum cingulate gyrus corresponds to the dorsal cingulum in standard notation and the cingulum hippocampus to the ventral cingulum [27]. For swMRI IDPs that denoted median T2* across subcortical structures were investigated. Functional data were not investigated in this study, only 1 IDP that indicated motion during the resting-state fMRI scan was used as a confounder in later analyses.

Medical history and physical measures

Self-reported medical history was used to define history of neurological or cerebrovascular disease or neurological cancers. Additionally, cardiovascular

disease (CVD: angina, myocardial infarction, heart or cardiac problem, peripheral vascular disease, leg claudication/ intermittent claudication, arterial embolism, aortic aneurysm, aortic aneurysm rupture), CVD risk factors (hypertension, high cholesterol or diabetes, no distinction between type 1 and type 2), treated hypertension, hypocholesteraemia, or diabetes as well as maternal and paternal AD/ dementia family history were defined by participant report. Participants were classified as hypertensive if the average of two blood pressure measurements at the imaging visit were ≥ 140 mmHg for systolic blood pressure and/ or ≥ 90 mmHg for diastolic blood pressure.

Measurements of standing height and weight at the imaging visit were used to calculate body-mass index (BMI) for participants. If standing height was not measured at the imaging visit, measurements from previous visits were used but if weight was not measured at the imaging visit BMI was not calculated and treated as missing.

Other phenotype data

Townsend deprivation index was used as a measure of socioeconomic status [28]. Education was categorised into: no qualification, O-levels or equivalent, A levels or equivalent and University degree or equivalent (includes vocational and professional qualifications).

Exclusion criteria

Participants were excluded: (i) if they reported medical history of conditions that might lead to structural brain abnormalities or cognitive impairment, i.e. any central or peripheral nervous system or nerve tumour, any chronic neurological illness or nervous system trauma, any cerebrovascular disease or intracranial haemorrhage, any infection of nervous system, cranial nerve palsy, spinal cord disorder, epilepsy, or cerebral palsy, (ii) if their UK Biobank genetic quality control flag indicated unusually high heterozygosity or $>5\%$ missing genotype rate, or if information on kinship indicated that participants were related to individuals within the sample (3rd degree or closer), or if participants did not have a Caucasian genetic ethnic background, or if there was a mismatch between self-reported sex and genetic sex, or if they carried *APOE* genotypes other than $\epsilon 2/\epsilon 2$, $\epsilon 2/\epsilon 3$, $\epsilon 3/\epsilon 3$, $\epsilon 3/\epsilon 4$ or $\epsilon 4/\epsilon 4$, (iii) if they did not have complete MRI datasets with data from all structural modalities and the rsfMRI scan, were outliers (3 standard deviations (SD) above the mean) on head motion during the rsfMRI acquisition or on the number of outlier slices detected during pre-processing of the dMRI data. For the computation of cut-offs, all available MRI data were used irrespective of scan site.

Statistical analyses

Data were analysed with SAS version 9.4 and figures were prepared using R version 3.6.2 (<https://cran.r-project.org/>). *APOE* genotype groups were classified as $\epsilon 2$ carriers (genotypes $\epsilon 2\epsilon 2$ and $\epsilon 2\epsilon 3$), $\epsilon 3$ homozygotes ($\epsilon 3\epsilon 3$), $\epsilon 4$ heterozygotes ($\epsilon 3\epsilon 4$) or $\epsilon 4$ homozygotes ($\epsilon 4\epsilon 4$).

Baseline characteristics were compared between *APOE* genotype groups with analysis of variance (ANOVA) for continuous variables (age, Townsend deprivation index, BMI) or Pearson's χ^2 test for categorical variables (sex, education, CVD, hypertension, high cholesterol, diabetes, dementia family history).

To analyse effects of *APOE* genotype on brain structure, multiple linear regression was run for every IDP of interest to determine whether standardised means differed significantly between *APOE* genotype groups. The IDP was used as the dependent variable and *APOE* genotype group and confounders as independent variables. One IDP (volume of WMHs) was log-transformed before statistical analyses because the distribution was positively skewed. Before analyses all IDPs were standardised by dividing by their standard deviation. Results were displayed in 'Manhattan' style plots of p values for the heterogeneity of each IDP with *APOE* genotype categories.

Confounders. Models included age and age², sex, age*sex, age²*sex, educational attainment and twenty principal components of genetic ancestry provided by UK Biobank. Models included neuroimaging confounders, as described previously [29, 30]: the volumetric scaling factor to correct for head size (only used in analyses of non-volumetric IDPs, volumetric IDPs were corrected for head size before statistical analyses), head motion and head motion² from the rsfMRI analysis and head position (x, y, z) in the MRI scanner as linear and quadratic terms. Since z position and table position in the MRI scanner are highly anticorrelated ($r = -0.91$), table position was not used as confounder. Month of scan (calculated from the date of first scan) was also added as a

Table 1. Participant characteristics at the imaging visit by *APOE* genotype.

	ε2 carriers (n = 3842)	ε3ε3 (n = 17,206)	ε3ε4 (n = 6794)	ε4ε4 (n = 652)	P value
Age (years)					
Mean (SD)	64.1 (7.7)	64.1 (7.6)	63.6 (7.6)	63.2 (7.3)	3.0 × 10 ⁻⁶
Median [Min, Max]	65.0 [46.0,81.0]	65.0 [45.0,82.0]	64.0 [46.0,82.0]	64.0 [47.0,79.0]	
Sex					
Female (%)	2057 (53.5)	9099 (52.9)	3659 (53.9)	372 (57.1)	0.12
Townsend deprivation index					
Mean (SD)	-2.07 (2.66)	-2.07 (2.61)	-2.01 (2.64)	-2.16 (2.47)	0.38
Median [Min, Max]	-2.77 [-6.26,9.23]	-2.73 [-6.26,9.74]	-2.68 [-6.26,8.91]	-2.84 [-6.26,7.18]	
Education					
No qualifications (%)	208 (5.4)	922 (5.4)	339 (5.0)	29 (4.4)	0.90
O-levels/CSE/equivalent (%)	443 (11.5)	1976 (11.5)	773 (11.4)	80 (12.3)	
A-levels (%)	183 (4.8)	840 (4.9)	335 (4.9)	27 (4.1)	
Degree/professional (%)	3007 (78.3)	13467 (78.3)	5347 (78.7)	516 (79.1)	
Body mass index (kg/m²)					
Mean (SD)	26.4 (4.2)	26.3 (4.2)	26.3 (4.3)	25.7 (3.9)	1.0 × 10 ⁻³
Median [Min,Max]	25.8 [15.7,46.9]	25.8 [13.4,57.2]	25.7 [14.1,53.4]	25.3 [16.0,42.3]	
AD family history					
Mother (%)	550 (14.3)	2768 (16.1)	1538 (22.6)	189 (29.0)	6.2 × 10 ⁻⁵⁰
Father (%)	305 (7.9)	1450 (8.4)	860 (12.7)	109 (16.7)	1.4 × 10 ⁻³²
Medical conditions					
Hypertension (%)	1958 (51.0)	8793 (51.1)	3432 (50.5)	318 (48.8)	0.60
Diabetes (%)	106 (2.8)	443 (2.6)	154 (2.3)	8 (1.2)	0.063
Hypercholesterolemia (%)	672 (17.5)	3978 (23.1)	1786 (26.3)	200 (30.7)	2.8 × 10 ⁻²⁷
CVD (%)	30 (0.8)	157 (0.9)	80 (1.2)	2 (0.3)	0.045

P values refer to comparisons using χ^2 tests for categorical variables and ANOVAs for continuous variables.

categorical confounder to account for temporal drifts in the data. These confounders roughly correspond to the “simple” set of confounders in [30]. Analyses where data from different scan sites were combined (e.g. the replication dataset) were additionally adjusted for the scanner site and the interaction between the site and each imaging confounder.

Correcting for multiple comparisons. Bonferroni correction was applied to correct for multiple comparisons as follows. The alpha level of 0.05 was divided by the total number of modalities investigated, i.e. 4 (T1, dMRI, swMRI and FLAIR), to derive one alpha level per modality, i.e. 0.0125. Within modality, this alpha level was then divided by the number of brain regions investigated: 113 for T1, 200 for dMRI (40 regions*5 different diffusion measures), 14 for swMRI and 1 for FLAIR. This resulted in the following Bonferroni correction thresholds for the different modalities: $p < 1 \times 10^{-4}$ for T1, $p < 6 \times 10^{-5}$ for dMRI, $p < 9 \times 10^{-4}$ for swMRI and 0.0125 for FLAIR.

Discovery and replication dataset. The data were split based on the MRI scanner used for data acquisition. Cheadle was the discovery dataset ($n = 17239$) and Newcastle ($n = 7465$) and Reading ($n = 3790$) were combined as the replication dataset to determine whether IDPs passing the level of significance in the discovery analysis (‘hits’) could be replicated. The p value threshold for significance at replication per modality was 0.0125 divided by the number of significant hits in that modality in the discovery cohort.

Post-hoc analyses of hits in the discovery cohort. For the replicating hits within each modality likelihood ratio tests were used to assess whether hits in left and right hemisphere IDPs remained statistically significant after adjustment for their average level, and if they did not then the average level was used in further analyses.

Effects (in units of standard deviation of the IDP) of each *APOE* genotype in comparison to the $\epsilon 3\epsilon 3$ genotype were estimated. Analyses were first

run separately for the discovery and replication dataset and then data from all scan sites were combined in one analysis, adjusted for site.

Sensitivity analyses were conducted altering the level of adjustment for age and neuroimaging confounders: finer adjustment for age using individual years of age as a single year categorical variable, no adjustment for neuroimaging confounders, rank inverse-normal transformation of all IDPs, age and neuroimaging confounders except for imaging centre. All sensitivity analyses were adjusted for twenty principal components of genetic ancestry.

Subgroup analyses. Subgroup analyses were conducted in a combined analysis of all datasets for hits that achieved replication. Differences in the strength of associations by sex and age, separately, were investigated in *APOE* $\epsilon 3\epsilon 4$ and $\epsilon 4\epsilon 4$ carriers compared with the *APOE* $\epsilon 3\epsilon 3$ genotype group. To determine more finely at what age *APOE* genotype differences start to become apparent a further analysis divided participants into five age groups (<55, 55-59, 60-64, 65-69, ≥ 70 years) and the strength of associations were compared between all *APOE* genotype groups in each of these age bins. Adjusted rate of cross-sectional change with age at imaging was calculated for all *APOE* genotype groups from linear regression of IDPs on age at imaging, principal components of genetic ancestry and the other imaging confounders.

RESULTS

After data cleaning steps and applying exclusion criteria, these analyses include 28,494 participants (Supplementary Figure 1).

Participant characteristics at the imaging visit

With absence of an *APOE* $\epsilon 2$ allele or a greater number of $\epsilon 4$ alleles, people had a greater prevalence of hypercholesterolemia and family history of AD (Table 1). *APOE* $\epsilon 4$ carriers were a median

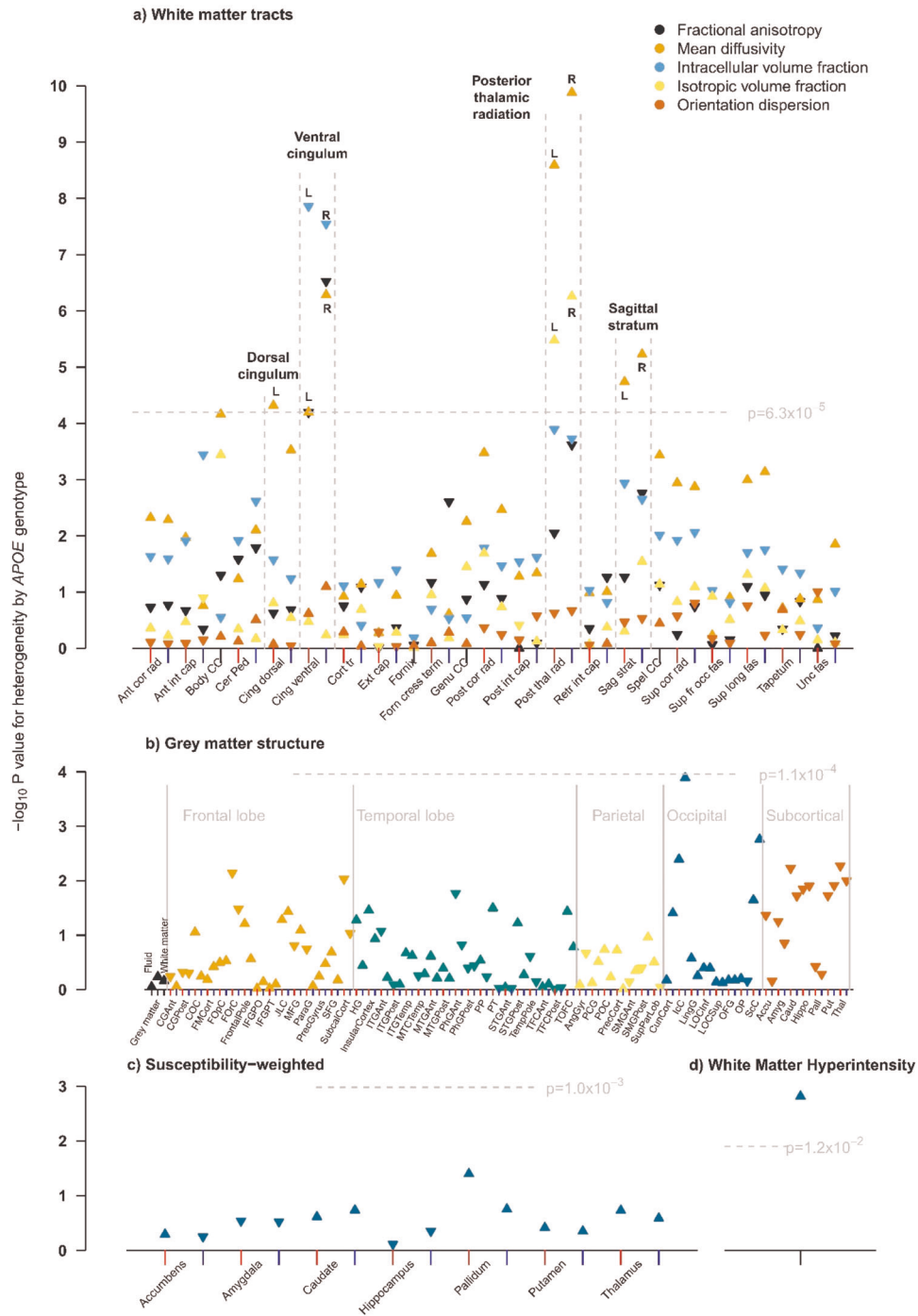


Fig. 1 Manhattan plots showing p values for heterogeneity across APOE genotypes for different measures of brain structure in the discovery cohort. Measures shown are: **a** white matter, **b** grey matter, **c** swMRI and **d** volume of white matter hyperintensities. For IDPs that have left and right components, the x positions of left and right components are indicated with red and blue tick marks respectively. L and R is used to distinguish left from right. For grey matter structure, the markers have been ordered by brain region: whole brain, frontal lobe, temporal lobe parietal, occipital, and subcortical. An upwards pointing triangle indicates that the IDP is higher in participants with the APOE ε4ε4 genotype compared with the ε3ε3 genotype, a downwards pointing triangle indicates it is lower.

1 year younger and a had a lower BMI compared with other genotypes. Other characteristics were similar by genotype.

Discovery analyses

In the discovery cohort, WM integrity of four tracts showed statistically significant differences between APOE genotype groups: dorsal cingulum, ventral cingulum, posterior thalamic

radiation, and sagittal stratum (Fig. 1a). 3D-images of those brain structures can be found on <https://identifiers.org/neurovault.collection:9357>.

The WM integrity measures that showed genotype differences were: MD (left only) for the dorsal cingulum; ICVF, MD (both left and right) and FA (right only) for the ventral cingulum; MD and ISOVF (both left and right) for the posterior thalamic radiation; and

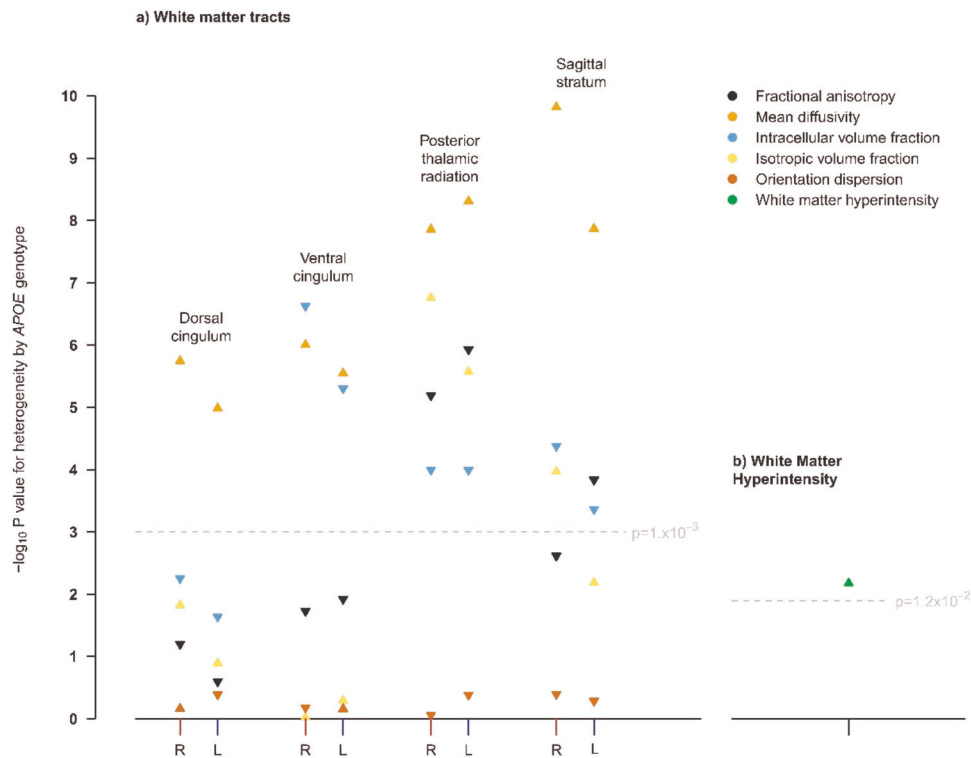


Fig. 2 Manhattan plots showing p values for heterogeneity across *APOE* genotypes in the replication cohort for IDPs that reached significance in the discovery cohort. The measures shown are: **a** white matter IDPs and **b** volume of white matter hyperintensities. L and R is used to distinguish left from right. An upwards pointing triangle indicates that the IDP is higher in participants with the *APOE* $\epsilon 4\epsilon 4$ genotype compared with the $\epsilon 3\epsilon 3$ genotype, a downwards pointing triangle indicates it is lower.

MD (left and right) for the sagittal stratum. In each tract, WM integrity was lower (increased MD and ISOVF, decreased FA and ICVF) in *APOE* $\epsilon 4\epsilon 4$ carriers compared with the $\epsilon 3\epsilon 3$ genotype. Additionally, total WMH volume differed statistically significantly between *APOE* genotype groups with higher volumes in carriers of *APOE* $\epsilon 4\epsilon 4$ genotype (Fig. 1d).

In contrast, there were no statistically significant differences in volumes of grey matter structures (Fig. 1b) or median T2* in subcortical structures (Fig. 1c) between *APOE* genotype groups.

Replication analyses

The significance threshold for the replication analysis with data from the other two imaging centres was $0.0125 / 12 \text{ hits} = 1.0 \times 10^{-3}$ for the analysis of white matter IDPs and 0.0125 for the volume of white matter hyperintensities.

The following WM integrity IDPs differed significantly by genotype (in the same direction) in both the discovery and replication cohort: MD in the left dorsal cingulum; ICVF and MD in the left and right ventral cingulum; MD and ISOVF in the left and right posterior thalamic radiation; and MD in the left and right sagittal stratum (Fig. 2a). The association of *APOE* genotype with WMH volume was also replicated (Fig. 2b). An analysis of all IDPs in the replication cohort provided visual confirmation that patterns in the Manhattan plots for the discovery cohort were replicated (Supplementary Figure 2).

Independence of left and right WM hits

In the discovery data, there was no evidence that *APOE* genotype associations with WM integrity differed by side of tract (left or right) (Supplementary Table 1), so an average of both tracts was used in subsequent analyses.

Sensitivity analyses

Sensitivity analyses were conducted for one white matter IDP (MD in the posterior thalamic radiation). They showed very similar

results for all analyses irrespective of the types of adjustments (Supplementary Figure 3).

Comparison of WM integrity and WMH volumes between *APOE* genotype groups

For the IDPs that achieved replication for statistically significant effects of *APOE* genotype, effects were investigated further to determine, which *APOE* genotype groups differed significantly from *APOE* $\epsilon 3\epsilon 3$ carriers. *APOE* $\epsilon 4\epsilon 4$ carriers showed the largest differences of 0.14–0.31 standard deviations (SDs) compared with $\epsilon 3\epsilon 3$ carriers, $\epsilon 3\epsilon 4$ carriers showed very small but statistically significant differences of around 0.05 SDs and $\epsilon 2$ carriers did not differ significantly from $\epsilon 3\epsilon 3$ carriers. Thus, the effect size for *APOE* $\epsilon 4$ homozygotes was much stronger than twice the effect for *APOE* $\epsilon 3\epsilon 4$ carriers (Fig. 3).

Subgroup analyses by sex and age

For the IDPs that achieved replication for heterogeneity across *APOE* genotype, there was no strong evidence for effect modification by sex given the number of comparisons when comparing *APOE* $\epsilon 3\epsilon 4$ or $\epsilon 4\epsilon 4$ carriers with the reference genotype *APOE* $\epsilon 3\epsilon 3$ (Fig. 4). The comparison of results of two age groups (< 65 years) and (≥ 65 years) indicated that effects differed markedly in *APOE* $\epsilon 4\epsilon 4$ carriers where the older age group showed double or more the effect sizes of the younger group, in particular for WM integrity in the posterior thalamic radiation and sagittal stratum; effects of the *APOE* $\epsilon 3\epsilon 4$ genotype were similar for both age groups (Fig. 4).

A more detailed analysis of 5-year age groups showed worsening WM integrity (increase in MD and decrease in ICVF) and an increase in WMH volume with age for all *APOE* genotype groups in this cross-sectional analysis. For *APOE* $\epsilon 2$ carriers, $\epsilon 3\epsilon 3$ and $\epsilon 3\epsilon 4$ genotypes the WM integrity worsened at a similar rate with age, whereas for the *APOE* $\epsilon 4\epsilon 4$ genotype there was a higher rate of change per year from the age group 55–59 years. The mean

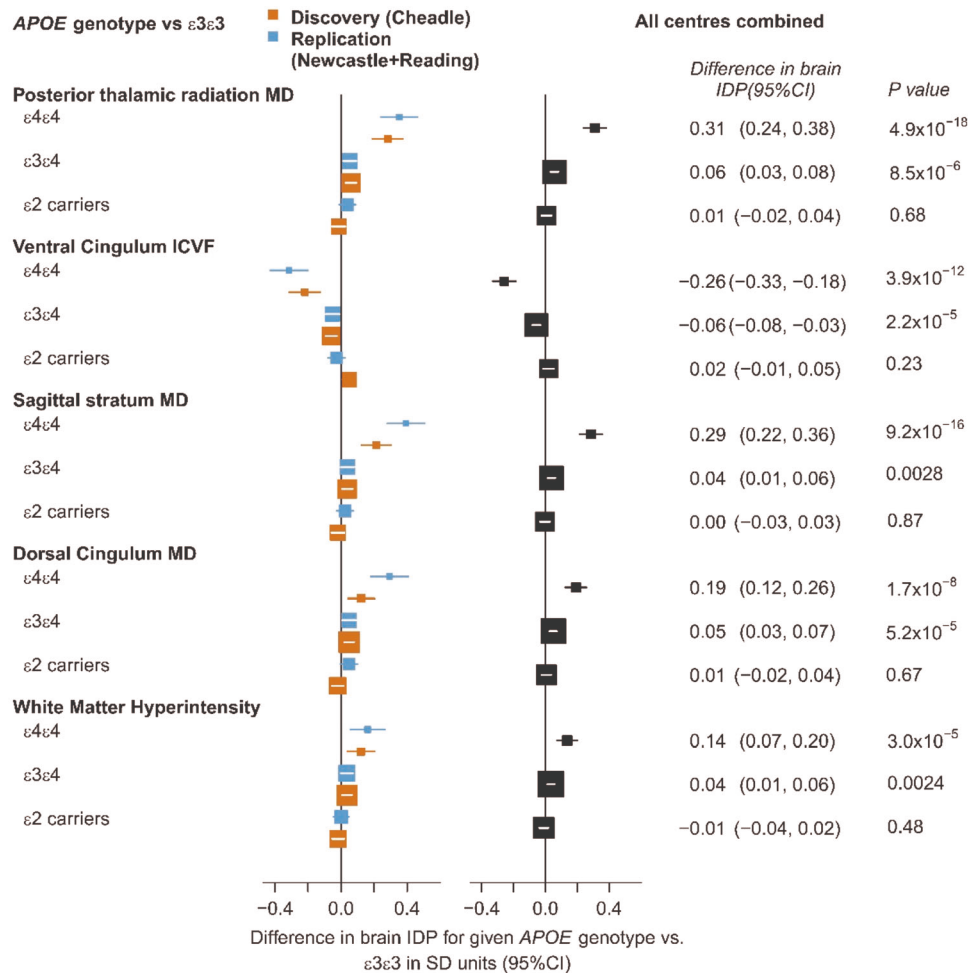


Fig. 3 Differences in WM integrity and WMH volume between *APOE* genotype groups for IDPs that achieved replication for heterogeneity with *APOE* genotype. The *APOE* ε3ε3 genotype is the reference group. On the left, there are results for separate analyses for the discovery (red) and replication (blue) dataset. On the right there are results for the combined analysis of all data.

difference in WM integrity between *APOE* ε4ε4 carriers and all other genotype groups from age group 65–69 years onwards corresponded to the mean IDP difference seen with around 5 years greater age cross-sectionally over the whole population (Fig. 5).

DISCUSSION

In this analysis of neuroimaging data from 28,494 UK Biobank participants we found statistically significant effects of *APOE* genotype on WM integrity and WMH volume but did not observe statistically significant effects on GM volumes or subcortical swMRI measures.

White matter structure

Effects of *APOE* genotype on several measures of WM integrity were observed: MD in the dorsal cingulum; ICVF and MD in the ventral cingulum; MD and ISOVF in the posterior thalamic radiation and MD in the sagittal stratum. WM integrity was lower (lower ICVF, higher MD) in those with highest AD risk. However, differences were greatest between *APOE* ε4 homozygote and ε3ε3 carriers, small but consistent between ε4 heterozygotes and ε3ε3 carriers but not statistically significant between ε2 carriers and ε3ε3 carriers. Therefore, *APOE* genotype differences in WM integrity did not clearly map to AD risk, which is lowest in ε2 carriers [2], but were based on negative effects of the *APOE* ε4 allele only.

There have been conflicting findings in the literature of possible *APOE* genotype effects on white matter structure in smaller studies, e.g. positive in [31] or no associations in [9, 32]. In their analysis of a smaller UK Biobank sample Lyall et al. [33] did not find significant effects of *APOE* genotype on a general factor of FA (gFA), which is a single factor capturing common variance across all white matter tracts. We show here that the differences between genotypes are very localised and mainly driven by the *APOE* ε4ε4 genotype, which is why, in combination with the smaller study size, they might not have been picked up by Lyall et al. (2020).

The analyses of NODDI measures provide insight into the microstructural properties and biological mechanisms underlying the observed differences. ICVF is a measure of neurite density that is also related to myelination and ISOVF is thought to reflect CSF contamination [34]. Therefore, *APOE* genotype effects on WM integrity in the ventral cingulum could be driven by neuronal mechanisms and in particular reduced myelination, a process that might play a role in early AD development [35, 36]. The ventral cingulum connects the hippocampus, parahippocampal areas and entorhinal cortex with the posterior cingulate cortex and retrosplenial cortex [27], i.e. areas that show neuronal and synapse loss early in AD [37]. Reduced white matter integrity of this tract has been shown in patients with mild cognitive impairment and AD [27]. A study combining dMRI and histopathology showed that reduced FA and increased MD of the ventral cingulum were associated with higher Braak stages of neurofibrillary tangle

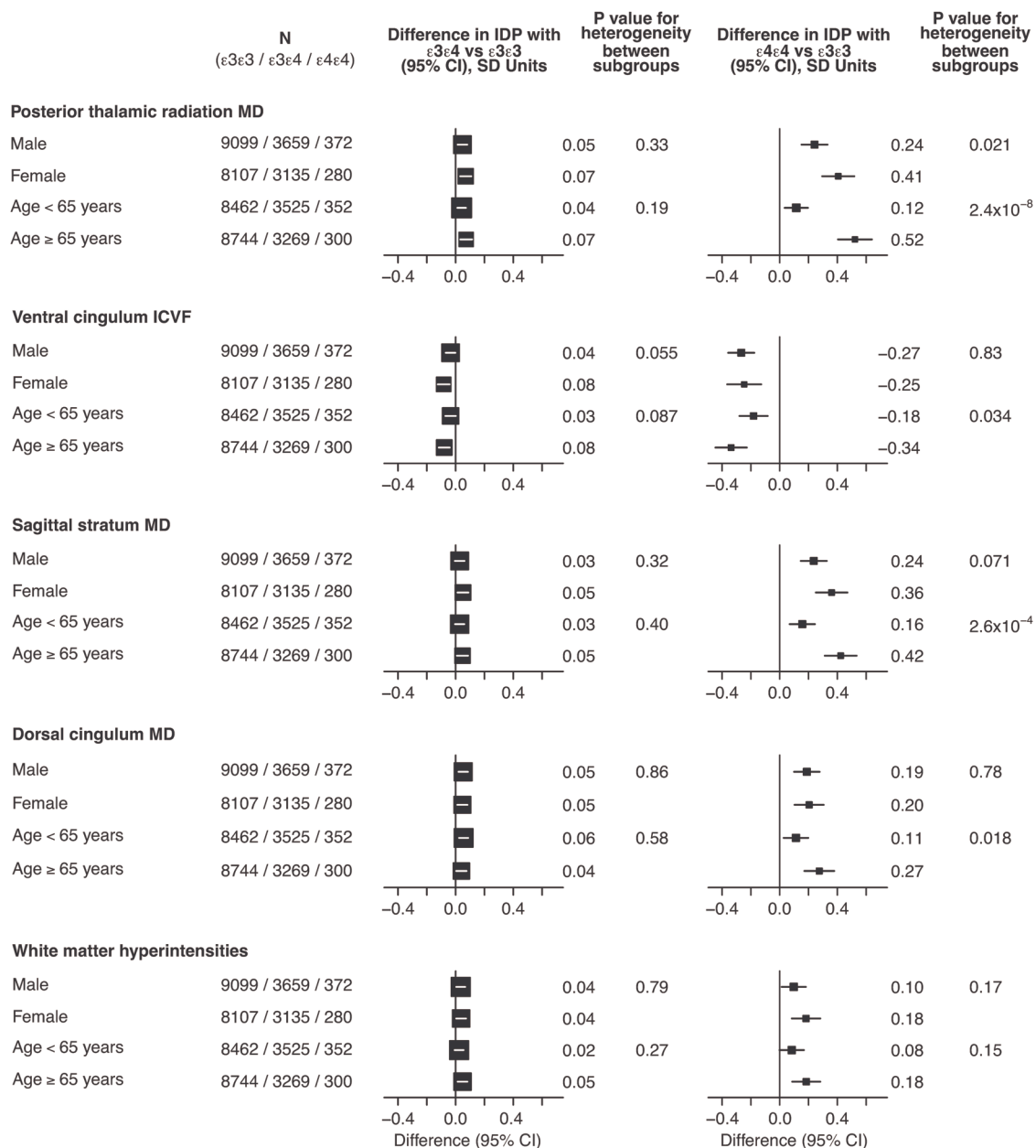


Fig. 4 Combined analysis of all centres with subgroup analyses by sex and age group. Comparison of *APOE* $\epsilon 3\epsilon 4$ (left) and *APOE* $\epsilon 4\epsilon 4$ carriers (right) with the reference group *APOE* $\epsilon 3\epsilon 3$ by sex (male vs female) and by age group (<65 vs \geq 65 years) for the IDPs that achieved replication for heterogeneity by *APOE* genotype.

pathology in AD patients [38]. Therefore, the findings of reduced integrity of the ventral cingulum here might be indicative of preclinical AD pathology in those at higher genetic AD risk.

In contrast, *APOE* genotype effects on MD and ISOVF of the posterior thalamic radiation could reflect localised expansion of ventricles, a well-known feature of AD pathology that correlates with cognitive decline [39]. *APOE* genotype differences in MD in the dorsal cingulum and sagittal stratum were not accompanied by differences in NODDI measures, so the biological mechanisms underlying differences in those tracts are unclear. However, there are previous reports that have shown reduced WM integrity of these tracts in cognitively impaired patients [40, 41].

Effects of age and sex on WM integrity

We conducted an exploratory analysis of WM integrity across the age range in this sample, which indicated that *APOE* $\epsilon 4\epsilon 4$ carriers

seem to deviate from the other *APOE* genotype groups particularly from the age group of 55–59 years. The differences in WM integrity measures in *APOE* $\epsilon 4\epsilon 4$ carriers compared with all other genotype groups correspond to WM integrity measure changes over 5 years in this cross-sectional study. Therefore, they might indicate faster ageing processes in older *APOE* $\epsilon 4\epsilon 4$ carriers. If confirmed in longitudinal studies, this would point to a role of *APOE* genotype on changes associated with preclinical AD processes rather than effects that exist across the lifespan. This would fit with some larger studies in children and adolescents that have reported no significant effects of *APOE* genotype on brain structure at younger ages [16, 17].

We did not find any evidence of differences between men and women in *APOE* genotype effects on brain structure. This would support a recent meta-analysis that reported no sex differences in *APOE* genotype effects on AD risk [3].

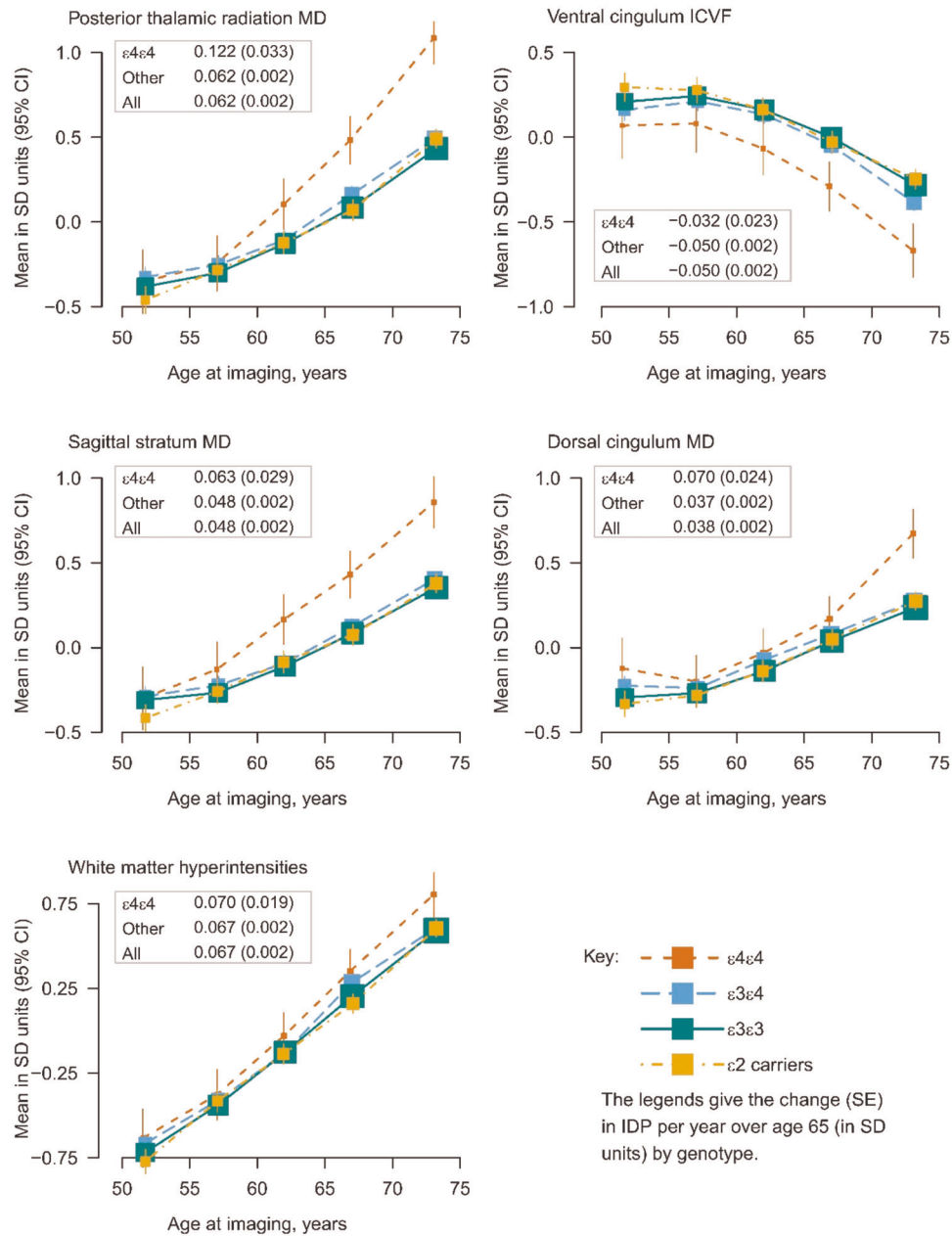


Fig. 5 Mean levels of IDPs that achieved replication by APOE genotype group averaged across 5-year age groups. IDPs are standardised to a mean of zero and a standard deviation of one. APOE $\epsilon 2$ carriers are shown in yellow, $\epsilon 3$ homozygotes in green, $\epsilon 4$ heterozygotes in blue and $\epsilon 4$ homozygotes in red.

White matter hyperintensities

There have been conflicting reports in the literature investigating possible APOE genotype effects on WMH volume, e.g. positive association in [42], no association in [43]. Significant effects of APOE genotype on WMH volumes have been reported previously in a smaller sample of UK Biobank data [33] and our results are consistent with that report. WMHs are one marker of cerebral small vessel disease [44]. Therefore, APOE genotype might affect AD risk via effects on the vasculature in the brain.

Effect sizes

While we reliably observed effects of APOE genotype on WM structure and WMH volumes, the effect sizes for differences between groups were generally modest with 0.3 SDs or less. On the one hand, this has implications for follow-up MRI studies of APOE genotype effects on brain structure, which need to have large enough sample

sizes to be able to detect small effects. On the other hand, it is unclear whether these modest changes can be translated into MRI biomarkers that would be sensitive enough to detect preclinical changes associated with AD development on an individual basis.

Grey matter structure

In this study, we did not observe any significant effects of APOE genotype on volumes of GM structures. This is not necessarily surprising since most studies with larger sample sizes do not report effects on GM structure in cognitively healthy participants, with many focusing specifically on the hippocampus [9, 12–14, 45]. This is the largest and most homogenous study that investigated effects on GM structure and did not find any effects. However, it is clear from histopathological studies that GM structures, particularly in the medial temporal lobe, are affected early by neuronal loss in AD development [37]. These changes might be too localised to translate

into volumetric differences that can be detected with the IDP MRI measures used in this study. Additionally, histopathological studies have shown that synaptic loss can exceed and predate neuronal loss [37]. It is possible that WM integrity MRI measures investigated here are sensitive enough so that these preclinical changes in neuronal connectivity can be detected here.

Susceptibility-weighted imaging

Similarly, we did not find any significant effects on median T2* in subcortical structures. Increased iron levels that affect median T2* [46], particularly in subcortical structures, have been reported in AD patients [47]. However, there is no study to date that investigated APOE genotype effects on median T2* levels. SWI is sensitive to iron accumulation and therefore bleeding events. A number of meta-analyses reported significant APOE genotype effects on volumes of cerebral microbleeds [42, 48]. Therefore, it might be more appropriate to use the T2* signal to identify localised microbleeds and investigate the volumes of these bleeds, similar to the analysis of WMHs in this paper, rather than averaging the signal across structures.

Limitations

We only included UK Biobank participants with Caucasian genetic ethnic background in this study, i.e. of white British ancestry, because APOE genotype effects on AD risk differ by ethnicity [49] and in turn effects on brain structure might differ as well, and the numbers of participants from other ancestries were too small for separate analysis. Therefore, the results might not be generalisable to other genetic and ethnic backgrounds.

In this large-scale investigation of UK Biobank data we decided to focus on IDPs and did not analyse the native imaging data directly. It is currently unclear whether the use of IDPs, which are obtained by averaging measures such as T2* or FA/MD across structures or tracts, is the best way to identify changes that might happen on a smaller scale and only affect certain parts of a structure. This would become visible in analyses conducted on a voxel-by-voxel basis but not necessarily in the analysis of IDPs averaged across the whole structure.

Additionally, it is important to note that even where we did find significant differences, the effect sizes were modest. This is a cross-sectional study and we do not yet know which participants will go on to develop AD in the coming decades. Therefore, we can only speculate whether the differences between APOE genotypes in white matter integrity might be caused by preclinical AD processes. Participants in the UK Biobank have also been shown to be healthier than the general population [50] and we only included cognitively healthy participants in this study. Therefore, one possible reason why we mostly report null effects could be that a large number of participants will either not develop AD or develop AD relatively late in their life. This would affect our ability to capture the effects of preclinical AD using APOE genotype as a substitute measure for AD risk.

However, evidence from cross-sectional histopathological and PET studies suggests that APOE genotype does affect processes that are relevant at preclinical AD, e.g. the accumulation of amyloid plaques [14, 51]. Our results suggest that these changes might only translate into modest structural changes visible using MRI modalities.

CONCLUSION

In this comprehensive analysis of APOE genotype effects on brain structure in 28,494 white British UK Biobank participants we showed consistent significant effects on WMH volume and WM integrity of the dorsal and ventral cingulum, posterior thalamic radiation and sagittal stratum in men and women. We did not observe significant differences between APOE genotype groups in volumes of GM structures or median T2* in subcortical structures. APOE genotype effects on WM integrity in the ventral cingulum may be driven by neuronal mechanisms and reduced myelination.

We saw that APOE $\epsilon 4$ homozygotes appear to have lower WM integrity at older ages with a potentially steeper decline of WM integrity from the age of 60 that corresponds to around 5 years greater “brain age”. Therefore, APOE genotype effects might be associated with preclinical AD. Whether this is the case may become clear in future, when these participants have been followed up for clinical outcomes and AD detected.

Pre-registration

The pre-registered study protocol is available on doi: <https://doi.org/10.17605/OSF.IO/BRN3H>. Changes from the pre-registered protocol are described in the supplementary methods.

Open data

UK Biobank is an open access resource and researchers can apply to use the data following procedures outlined on their website: <https://www.ukbiobank.ac.uk/enable-your-research>.

CODE AVAILABILITY

Computer code used to generate the results and figures is available from the corresponding author upon reasonable request.

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AUTHOR CONTRIBUTIONS

Verena Heise: conceptualisation, data curation, formal analysis, methodology, project administration, software, validation, visualisation, and writing – original draft preparation; Alison Offer: data curation, formal analysis, software, validation, visualisation, and writing – review and editing; William Whiteley: conceptualisation and writing – review and editing; Clare Mackay: conceptualisation, supervision, and writing – review and editing; Jane Armitage: conceptualisation, supervision, and writing – review and editing; Sarah Parish: conceptualisation, funding acquisition, methodology, resources, supervision, and writing – review and editing

CONFLICT OF INTEREST

The authors declare no competing interests.

ADDITIONAL INFORMATION

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