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Abstract: Introduction Apolipoprotein E 4 (APOE4)-related genetic risk for sporadic Alzheimer's disease is associated with an early impairment of cognitive brain networks. The current study determines relationships between APOE4 carrier status, cortical iron, and cortical network-functionality. Methods Sixty-nine cognitively healthy old-aged individuals (mean age [SD] 66.1 [\pm 7.2] years; Mini-Mental State Exam [MMSE] 29.3 \pm 1.1) were genotyped for APOE4 carrier-status and received 3 Tesla magnetic resonance imaging (MRI) for blood oxygen level-dependent functional magnetic resonance imaging (MRI) at rest, three-dimensional (3D)-gradient echo (six echoes) for cortical gray-matter, non-heme iron by quantitative susceptibility mapping, and 18F-flutemetamol positron emission tomography for amyloid- β . Results A spatial pattern consistent with the default mode network (DMN) could be identified by independent component analysis. DMN activity was enhanced in APOE4 carriers and related to cortical iron burden. APOE4 and cortical iron synergistically interacted with DMN activity. Secondary analysis revealed a positive, APOE4 associated, relationship between cortical iron and DMN connectivity. Discussion Our findings suggest that APOE4 moderates effects of iron on brain functionality prior to manifestation of cognitive impairment.

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NEUROIMAGING

APOE4 moderates effects of cortical iron on synchronized default mode network activity in cognitively healthy old-aged adults

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Abstract

Introduction: Apolipoprotein E ϵ 4 (APOE4)-related genetic risk for sporadic Alzheimer's disease is associated with an early impairment of cognitive brain networks. The current study determines relationships between APOE4 carrier status, cortical iron, and cortical network-functionality.

Methods: Sixty-nine cognitively healthy old-aged individuals (mean age [SD] 66.1 [\pm 7.2] years; Mini-Mental State Exam [MMSE] 29.3 \pm 1.1) were genotyped for APOE4 carrier-status and received 3 Tesla magnetic resonance imaging (MRI) for blood oxygen level-dependent functional magnetic resonance imaging (MRI) at rest, three-dimensional (3D)-gradient echo (six echoes) for cortical gray-matter, non-heme iron by quantitative susceptibility mapping, and 18F-flutemetamol positron emission tomography for amyloid- β

Results: A spatial pattern consistent with the default mode network (DMN) could be identified by independent component analysis. DMN activity was enhanced in APOE4 carriers and related to cortical iron burden. APOE4 and cortical iron synergistically interacted with DMN activity. Secondary analysis revealed a positive, APOE4 associated, relationship between cortical iron and DMN connectivity.

Discussion: Our findings suggest that APOE4 moderates effects of iron on brain functionality prior to manifestation of cognitive impairment.

KEYWORDS

APOE4, DMN, flutemetamol, fMRI, gradient echo, ICA, iron, MRI, PET, QSM, preclinical Alzheimer's disease

1 | INTRODUCTION

Apolipoprotein E (apoE) is an essential mediator for fat metabolism and facilitates cholesterol handling in the central nervous system (CNS).¹

The Apolipoprotein E ϵ 4 allele (APOE4) is the strongest known genetic risk factor for sporadic Alzheimer's disease (AD).²⁻⁴ In addition, several studies have demonstrated associations between APOE4 with established clinical phenotypes and pathological hallmarks of increased risk

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for AD. These include mild cognitive impairment,^{5,6} increased burden of brain amyloid- β ($A\beta$),⁷⁻¹¹ CNS proteinopathy,^{12,13} metabolic disorder,^{14,15} and vascular disease.^{16,17}

Cognitive dysfunction in AD is preceded by progressive changes of intrinsic brain network activity.¹⁸⁻²¹ Here, cognitive networks such as the default mode network (DMN) are primarily affected.^{19,22-24} The DMN is constituted by a distinct connectivity pattern of synchronous cortical neuronal activity in spatially distant brain regions that include the medial prefrontal cortex (MPFC), lateral parietal cortices, posterior cingulate, and hippocampus, which are activated at rest.²⁵⁻²⁷ There is a consistent link between APOE4 carrier status and increased $A\beta$ burden.^{8,9,28} Interestingly, the effect of APOE4 on cognitive brain networks is present prior to accumulation of $A\beta$, which may reflect risk for AD prior to manifestation of characteristic AD pathology.²⁹

Another pathological feature of AD is an increased burden of cerebral iron deposition.^{30,31} Although iron continuously accumulates in the human brain during aging,³²⁻³⁵ recently published postmortem data suggest an association between the amount of brain iron deposition and the progression and severity of cognitive decline in AD.³⁶⁻³⁸ Moreover, associations between APOE4 carrier status, clinical phenotypes of increased risk for AD, and increased iron burden in the CNS have been demonstrated.^{36,39,40} Paramagnetic susceptibility, as measured by quantitative susceptibility mapping (QSM) magnetic resonance imaging (MRI), has been demonstrated to closely relate to non-heme iron content of deep brain gray matter.⁴¹⁻⁴⁴ Although myelin content is a major determinant of MR phase differences between cortical gray and white matter,⁴⁵ recently published histological data suggest that iron in cortical gray matter regions is a major contributor to susceptibility measured by QSM.⁴⁶ This approach is considered to provide an indirect estimate of non-heme iron in the brain, and associated risk for oxidative damage.^{38,47} By applying suitable post-processing techniques that balance susceptibility measures with reference regions and correct for heme iron and other non-tissue iron sources, strong linear correlations resulted between postmortem gray matter tissue iron concentration and QSM measurements.^{43,48} Consistently, several QSM-MRI studies demonstrated an association between increased tissue magnetic susceptibility, AD, and AD-risk phenotypes.^{33,40,49,50} Considering possible damaging impacts of brain iron burden on brain tissue by increased oxidative stress and redox activity,⁵¹⁻⁵⁶ as well as programmed cell death conferred by ferroptosis,^{57,58} APOE might be a moderator for iron-related oxidative brain damage in individuals at risk for AD.^{36,59} In this context, cortical iron burden might indicate early cortical neurodegenerative alterations, as suggested by earlier published data of ours on brain iron in mild cognitive impairment.⁴⁰

Considering these prior studies, and allowing for the published evidence on earliest APOE4-moderated DMN alterations in cognitively unimpaired adults,^{29,60} we hypothesized that APOE4 may moderate the effects of increased cortical iron on cortical functionality, as reflected by DMN connectivity. Moreover, we hypothesized that APOE4-related changes should (a) be reflected by altered DMN activity and (b) precede the manifestation of significant AD pathology and cognitive dysfunction. To test this hypothesis, a study sample

HIGHLIGHTS

- Three-dimensional (3D) gradient recalled echo magnetic resonance imaging for quantitative susceptibility mapping (QSM) and 18F-flutemetamol positron emission tomography for amyloid- β ($A\beta$) in healthy old-aged adults.
- Spatial definition and assessment of the default mode network (DMN) by group independent component analysis.
- Synergistic effects of iron (as measured by QSM) and APOE ϵ 4 allele (APOE4) on DMN connectivity.
- Interactive effects of APOE4 and QSM-iron may precede $A\beta$ pathology.
- APOE4 may accelerate brain iron accumulation associated DMN disintegrity.

RESEARCH IN CONTEXT

1. The apolipoprotein E ϵ 4 allele (APOE4) is the strongest known genetic risk factor for sporadic Alzheimer's disease (AD). APOE4-associated impairment of brain-network connectivity manifests prior to dementia. Considering recent reports on relationships between cerebral iron load, genetic risk, and progression of AD, our study aims at investigating the effects of APOE4 on cortical iron and cortical network connectivity in non-demented old-aged adults. Brain iron may be non-invasively inferred on by its paramagnetic properties using quantitative susceptibility mapping (QSM) magnetic resonance imaging. QSM is considered to provide an indirect estimate of non-heme iron.
2. Our findings suggest that APOE4 may moderate iron effects on brain functionality, as reflected by altered synchronized network activity, which is consistent with earlier reports on APOE4-related default mode network alterations. Additional studies are needed to clarify mechanisms implicated in the interaction between APOE4, cortical iron burden, and progression of cortical dysfunction in AD.

of cognitively healthy, old-aged study participants was recruited. All participants received genotyping for detection of APOE4 carrier status,² and 18F-flutemetamol positron emission tomography (PET) for assessing brain $A\beta$ burden.^{61,62} Blood oxygen level-dependent (BOLD) functional MRI (fMRI) at rest was performed for assessing intrinsic network connectivity, and the CONN toolbox⁶³ was used to identify the DMN by independent component analysis (ICA)⁶⁴ and statistical testing for interactive effects of APOE4 and cortical iron load, inferred from gray matter susceptibility.

2 | MATERIALS AND METHODS

2.1 | Study sample

The study sample included 69 cognitively healthy old-aged adults (32 female, 37 male; mean age [SD] 66.1 [7.25] years, range 51–80 years, mean education [SD] 15.99 [2.77] years, range 11–20) recruited in the cantone Zurich, Switzerland, from an ongoing study at our center.⁶⁵ Study procedures were in concordance with regulations issued by the local, cantonal ethics authority (Kantonale Ethikkommission Zürich, www.kek.zh.ch), good clinical practice, and with the Declaration of Helsinki.⁶⁶ Written informed consent was obtained from all participants before inclusion in the study. Inclusion criteria were age between 50 and 80 years, unimpaired overall cognitive status as indicated by Mini-Mental State Examination (MMSE) $\geq 27/30$, neuropsychological testing, and comprehensive psychiatric examination. Exclusion criteria were presence of any condition possibly affecting cognition, any current medication or substance abuse with prompt effects on cognition, serious medical or psychiatric illness, and evidence of infarction or inflammation on cranial MRI. Furthermore, subjects with contraindications to MRI or PET, clinically relevant changes in red blood count, or significant exposure to radiation were excluded. After inclusion, all participants received standardized cognitive testing and a clinical workup, including medical history, blood sampling, and genotyping of APOE as described earlier.⁶⁵ The study population was dichotomized based on presence of the APOE4 genotype (“APOE4 carriers” vs “non-carriers”), for stratification by genetic risk for sporadic, late-onset AD.⁴

2.2 | Cognitive assessment of participants

Screening for cognitive impairment was performed by applying the MMSE⁶⁷ and the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) neuropsychological battery.⁶⁸ Moreover, the delayed recall Verbal Learning and Memory Test (VLMT)⁶⁹ was used to assess episodic memory performance, the Boston Naming Test for confrontational word retrieval,⁷⁰ the Stroop interference test⁷¹ as a measure of executive function, and Trail Making Test, Section B divided by Section A (TMT B/A) for assessment of mental flexibility.⁷²

2.3 | Acquisition of MRI data and QSM

Imaging of all 69 participants was conducted using a 3T GE SIGNA PET-MR whole-body scanner (GE Medical Systems, Milwaukee, WI) equipped with an 8-channel head coil. For anatomical referencing and automated image segmentation, T1-weighted BRAVO images (TI = 450 ms, voxel size = $1 \times 1 \times 1$ mm³, flip-angle = 12°, ASSET factor = 2, scan time = 6:00 minutes) were acquired. MR phase measurements used for QSM calculation were collected using a multi-echo 3D gradient recalled echo (GRE) sequence with six echoes (TR/TE1/ Δ TE = 40/6/4 ms, voxel size = $1 \times 1 \times 1$ mm³, flip angle = 15°, bandwidth = ± 62.5 kHz, flow compensated, ASSET factor = 2, scan

time = 7:53 minutes). For investigating BOLD synchronicity at rest (open eyes), a T2*-weighted single-shot gradient echo-planar imaging sequence was used to record 200 functional volumes (repetition time (TR) = 2.547 s; echo time (TE) = 13.8 ms; matrix = 64×64 ; flip angle = 90°, total scan time = 8.49 minutes), with 46 slices per volume (voxel size = $3.75 \times 3.75 \times 3.6$ mm³) for whole-brain coverage.

For QSM reconstruction, phase data acquired with an echo time in the range of 18 to 26 ms was used. QSM images were calculated from the MR phase images using algorithms described previously by our group^{40,48,65} using an in-house script written in MATLAB (MATLAB 2016a, Version 9.0). Briefly, processing included Laplacian-based phase unwrapping,⁴¹ brain masking using GRE magnitude image acquired at TE = 14 ms with FSL's brain extraction tool (BET, FMRIB Oxford, UK), dividing the unwrapped phase images by $2\pi \cdot TE$ to obtain frequency shift images (Hz) for each echo. For eliminating background fields the vSHARP method^{42,73} was used (maximum spherical kernel size = 4 mm, regularization parameter = 0.05). Spatially confined vascular objects such as veins and microbleeds were excluded from estimation of regional susceptibility values. To increase signal-to-noise ratios (SNR), images resulting from the last three echoes were averaged.⁷⁴ Sparse linear equation and least-squares (LSQR)-based minimization was used for inverse dipole calculations of susceptibility maps,^{75,76} with deep frontal white matter as reference.⁶⁵

2.4 | Acquisition of flutemetamol-PET data

18F-flutemetamol-PET was used for determination of participants A β plaque burden,^{61,62} as described earlier by our group.⁶⁵ In brief, an individual dose of 140 MBq of flutemetamol was injected into the cubital vein. Late-frame (85–105 minute) PET images were reconstructed with state of the art time-of-flight algorithms allowing for attenuation correction maps, which were generated using standard procedures implemented by the manufacturer. This resulted in 3D volumes of 18F-flutemetamol retention (matrix = $256 \times 256 \times 89$, voxel size = $1.17 \times 1.17 \times 2.78$ mm³). Individual measures of cortical A β plaque load were calculated based on average 18F retention in a composite cortical volume of interest, which was normalized to cerebellar gray matter composite cortical volume of interest standard uptake value ratio (COM-SUVR).⁶² Consistent with earlier studies on AD and healthy controls, extent of amyloid pathology was assessed in regard to a COM-SUVR threshold of 1.56.⁶²

2.5 | Analysis of functional MRI data

2.5.1 | Data preprocessing

BOLD fMRI data were processed for statistical analysis using “CONN functional connectivity toolbox” (ver.17.d; www.nitrc.org/projects/conn),⁶³ “statistical parametric mapping” (SPM12, Version 6906), MATLAB (Version 9.0, MathWorks Inc., Natick, MA, USA), and the “statistics and machine learning toolbox” (Version 10.2). After importing NIFTI functional and anatomical images into the CONN toolbox, individual

TABLE 1 Overview of sample demographics, neuropsychological test performance as well as cortical A β and cortical magnetic susceptibility measures as mean (\pm SD)

	Whole sample	APOE4	no-APOE4	t test (P)
N	69	18	51	-
Females/males	32/37	8/10	24/27	-
Age	66.1 (7.25)	66.28 (5.29)	66.04 (7.87)	0.89
Years of education	15.99 (2.77)	16.56 (2.48)	15.78 (2.87)	0.28
MMSE	29.32 (1.12)	29.12 (1.58)	29.4 (0.89)	0.49
Boston Naming Test	14.64 (0.7)	14.65 (1)	14.64 (0.57)	0.97
CERAD word fluency	24.05 (5.34)	22.47 (5.36)	24.62 (5.27)	0.62
TMT B/A	2.24 (0.76)	2.33 (0.88)	2.21 (0.72)	0.64
Stroop	28 (7.12)	28.59 (6.87)	27.79 (7.26)	0.17
Episodic memory (VLMT)	9.34 (3.9)	9 (3.22)	9.47 (4.14)	0.69
Cortical Abeta (18F-flutemetamol SUVR)	1.19 (0.19)	1.28 (0.33)	1.16 (0.11)	0.15
Abeta positives ($n > 1.56$)	2	2	0	-
Cortical magnetic susceptibility (QSM)	1.85 (0.81)	1.81 (0.65)	1.87 (0.86)	0.78

T test was performed to investigate differences on a group-level between APOE4 carriers (APOE4) and non-carriers (no-APOE4).

Abbreviations: APOE4: Apolipoprotein E ϵ 4 allele, MMSE: Mini-Mental State Exam, CERAD: Consortium to Establish a Registry for Alzheimer's Disease, TMT A/B: Trail Making Test, Section A divided by Section B, VLMT: Verbal Learning and Memory Test, SUVR: standard uptake value ratio, QSM: quantitative susceptibility mapping.

fMRI volumes were spatially realigned, and anatomical scans were coregistered to the mean functional image, corrected for timing differences between slices, spatially normalized to montreal neurological institute (MNI) template space, and smoothed using an 8 mm full width at half-maximum Gaussian kernel. The Artifact Detection Toolbox (ART, <https://web.mit.edu/swg/software.htm>) was used for detection and analysis of sources of artifacts in the investigated timeseries of 200 functional MR-volumes.⁶³

2.5.2 | Identification of resting-state networks

To obtain functional connectivity networks of all 69 participants, we performed group spatial ICA⁷⁷ as implemented in the CONN toolbox for data-driven blind source separation of fMRI data and identification of functional networks, represented by spatially distant patterns of BOLD synchronicity.^{64,78–80} The DMN, as a representation of intrinsic network activity, was identified based on characteristic spatial patterns that included signature regions of MPFC, posterior cingulate cortex (PCC), and left and right lateral parietal lobes (LPLs).²⁶

2.6 | Statistical analysis

The statistical analysis of fMRI data, as well as investigation of APOE4 and paramagnetic tissue iron effects on DMN connectivity, was performed using algorithms implemented in the CONN-toolbox (V17d).⁶³ As such, DMN activity was operationalized by level of BOLD synchronicity (β weights of connectivity) within the spatial confinement of the correspondent independent component.⁷⁷ Reliability of the ICA for detection of the DMN was estimated by probability of each voxel identified by ICA to belong into the DMN by one sample t test. Moreover,

CONN was used for investigating group differences in DMN activity as a function of APOE4 carrier status and cortical magnetic susceptibility by linear regression and one-way analysis of covariate interaction tests in order to study the effects of high cortical magnetic susceptibility and APOE4 carrier status on DMN activity. False discovery rate was used to adjust raw P-values for multiple testing (P-FDR).⁸¹

Descriptives of the sample data are presented using mean \pm SD. Tests for differences in demographic, clinical, neuropsychological, or imaging-based (SUVR, QSM) parameters between subjects in the ApoE4- and no-ApoE4 group were performed with independent samples t tests. A median split of the study sample was used for separation of participants based on average cortical magnetic susceptibility: QSM levels above or equal to the group median were categorized as “high cortical magnetic susceptibility” and QSM levels below the group median as “low cortical magnetic susceptibility.” The resulting dichotomous variable was used as a categorical operator of cortical iron burden.

3 | RESULTS

3.1 | Characteristics of the study population

Within the study population of 69 participants, 18 carriers of the APOE4 allele could be identified (allelic frequencies were: APOE ϵ 2/ ϵ 4 = 1, APOE ϵ 3/ ϵ 4 = 16, APOE ϵ 4/ ϵ 4 = 1). When comparing APOE4 carriers with non-carriers, there were no significant differences in demographic characteristics between groups. Moreover, neuropsychological screening by MMSE as well as domain-specific testing using the CERAD neuropsychological battery consistently indicated high levels of cognitive performance in all study participants,

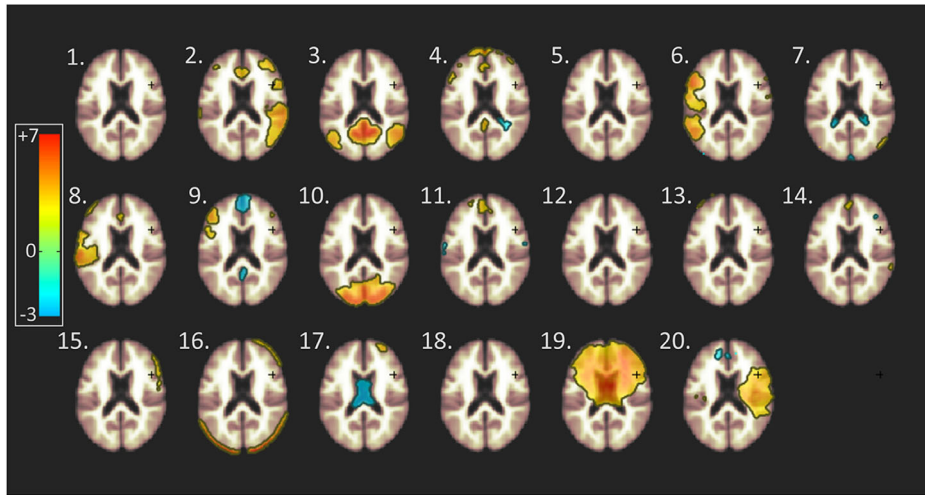


FIGURE 1 Identification of resting-state networks by independent component analysis (ICA) of blood oxygen level-dependent (BOLD) time course synchronicity at rest. Using ICA, 20 independent components could be identified, representing distinct BOLD time course synchronicity patterns at rest. Heat maps represent factor loading by voxel for each spatial component, estimated by group ICA (color bar: lowest, blue = -3; maximum, red = +7, horizontal lines in the green area = 0)

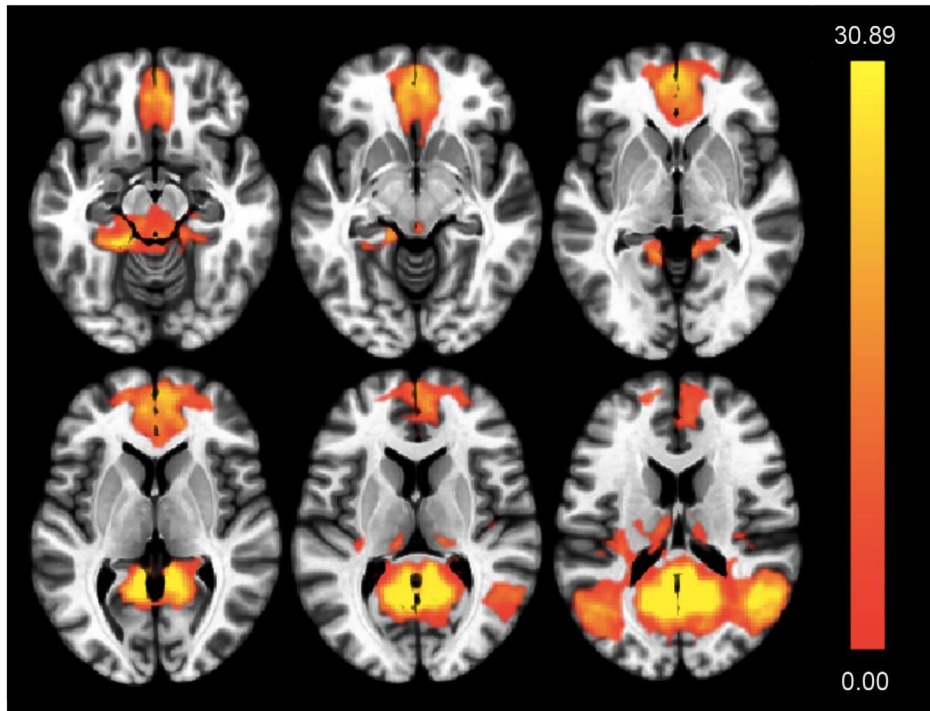


FIGURE 2 Spatial definition of the default mode network (DMN) based on blood oxygen level-dependent (BOLD) time course synchronicity within independent component 3. Indicated are axial slices indicating brain regions included by component 3. Significance levels of voxel-level BOLD synchronicity are color coded (T -map, highest values are yellow)

without significant differences between APOE4 carriers and non-carriers (Table 1). Only in two APOE4 carriers was increased brain $A\beta$ load observable. For the rest of the study population, no substantial brain amyloid pathology was present, as indicated by average COM-SUVr (mean (\pm SD)) of 1.19(0.19) without significant differences on a group level (APOE4 carriers: 1.28(0.33), non-carriers: 1.16(0.1)) (Table 1). Overall cortical magnetic susceptibility was 1.85 (0.81) ppb,

without significant differences between APOE4 carriers (1.81(0.65) ppb) and non-carriers (1.87(0.65) ppb) (Table 1).

3.2 | Identification and spatial definition of the DMN

By performing group ICA for blind source decomposition of 3D resting-state BOLD data, 20 spatial components of resting-state

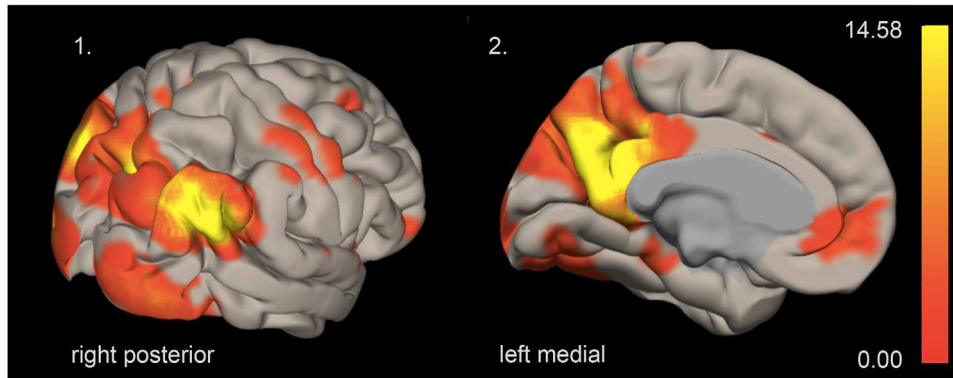


FIGURE 3 Effect of APOE4 carrier status on default mode network (DMN) activity. DMN activity, as indicated by synchronicity of blood oxygen level–dependent (BOLD) time courses, is associated with APOE4 carrier status (F -map, highest values are yellow)

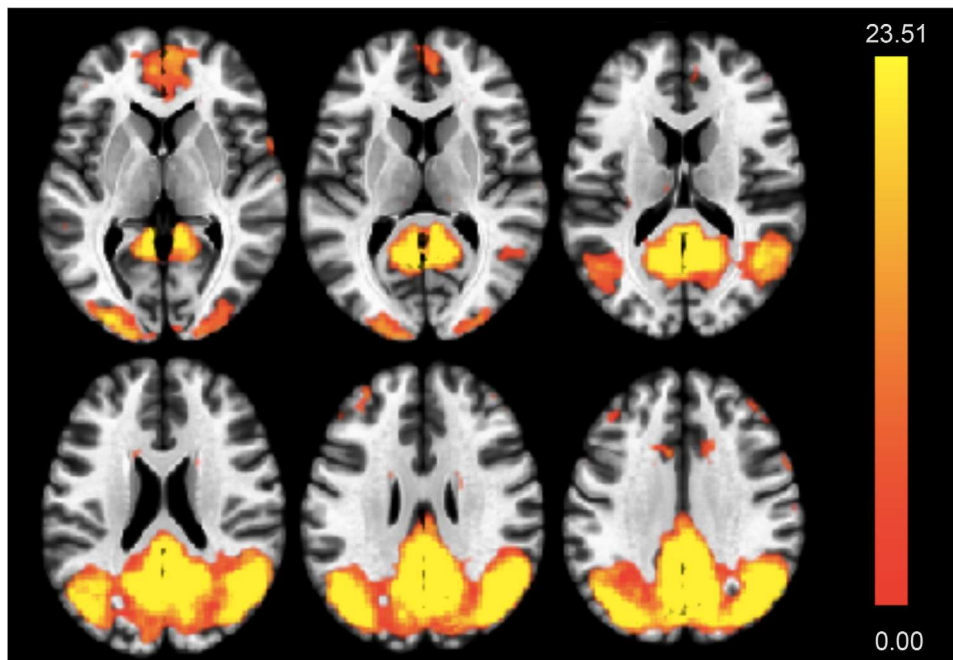


FIGURE 4 Effect of cortical iron burden on default mode network (DMN) activity. DMN activity is increased in study participants with high cortical iron (estimated by QSM) (F -map, highest values are yellow)

network activity could be identified based on factor loading by voxel (Figure 1). These represented established functional brain networks, including one consistent with the DMN (component #3: $T(68) = 4.07$, $P(\text{FDR}) = 0.0024$, $k_{\text{min}} = 24$), as defined by a spatial pattern of MPFC, hippocampus, parahippocampal formation, PCC, precuneus, and lateral parietal cortex (Figure 2).

3.3 | Synergistic interaction of APOE4 and cortical iron on default mode network activity

By analysis of covariance, a significant effect of APOE4 carrier status on increased DMN activity could be observed: $F(2,66) = 17.95$, $P(\text{FDR}) < 0.001$. The strongest effects were observed in the

posterior cingulate cortex, precuneus, and lateral parietal cortex (Figure 3).

Moreover, ANCOVA also indicated significant effects of cortical iron burden on connectivity within the DMN component ($F(2,66) = 14.24$, $P(\text{FDR}) < 0.001$) (Figure 4). Finally, to compare regression effects attributable to APOE4 and cortical iron, respectively, a one-way ANCOVA interaction was performed by using second-level analysis algorithms included in CONN.⁶³ Additive synergism of APOE4 and iron effects was indicated by a positive relationship between cortical iron and DMN connectivity that was associated with APOE4 carrier status ($T(65) = 3.22$, $P(\text{FDR}) < 0.001$, Figure 5A). Here, significant iron effects within the subgroup of APOE4 carriers were observed ($F(2,16) = 10.97$, $P(\text{FDR}) = 0.0026$, Figure 5B). The strongest local effects consistently resulted for voxels localized in the posterior cingulate cortex, the precuneus, and lateral parietal cortex (Figures 5A, B).

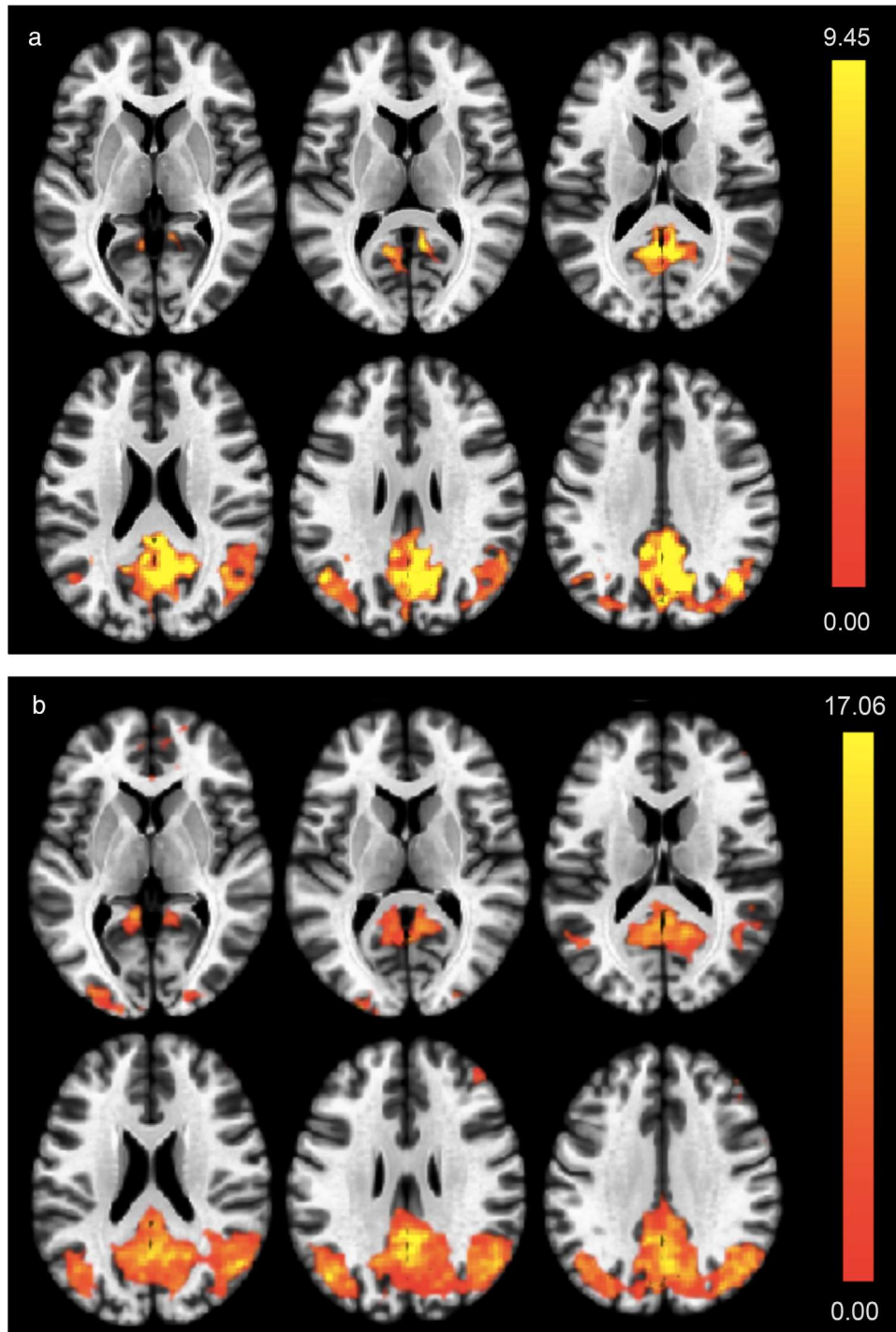


FIGURE 5 (A) Synergistic interactions between APOE4 carrier status and cortical iron on default mode network (DMN) activity. Colors indicate local effect sizes, as generated by second level, one-way analysis of covariance (ANCOVA) interaction analysis (T-map, highest values are yellow). (B) DMN activity in APOE4 carriers relates to cortical iron burden. DMN activity is increased in APOE4 carriers with high cortical iron, as indicated by regression (F-map, highest values are yellow)

4 | DISCUSSION

We identified a synergistic interaction of cortical gray matter susceptibility (QSM) and BOLD synchronicity at rest, suggesting a moderator effect of APOE4 on the relationship between cortical non-heme

iron and DMN activity. This effect was observable in a population of old-aged, cognitively healthy adults. Because for the majority of the investigated participants no significant increase of A β burden could be observed, our findings might reflect preclinical brain alterations associated with APOE4-related increased risk for AD.

The current study used established neuropsychological testing for assessment of cognitive domains typically affected in AD.⁸² Moreover, all study participants were genotyped for presence of the APOE4 allele, allowing for additional stratification of the study population by individual risk for AD.^{1,4} Each participant also was investigated by 18F-flutemetamol PET to assess brain A β burden. Flutemetamol has been used previously for assessing patients with AD,^{61,62} as well as cognitively unimpaired old-aged adults.^{83,84} Although there was no significant difference between APOE4 carriers and non-carriers regarding A β pathology, longitudinal follow-up of our sample might reveal possible faster accumulation of brain A β in the APOE4 group, as suggested by earlier reports.^{10,85} Considering the synchronicity of BOLD contrast variation in spatially distinct brain regions a proxy of neuronal functionality,^{25,26,86} ICA was performed for detection and statistical group-inferences.^{63,77} Blind source decomposition is capable of identifying many different brain networks from fMRI data recorded at rest.^{26,64} In the current study we focused on the DMN, considering earlier studies that provide evidence that DMN connectivity is an indicator of cognitive functionality,^{25,27,87} which is affected at early, preclinical stages of AD.^{18,19,21,22,84} Although the chosen focus on the DMN may be a limitation regarding general inferences on global intrinsic network activity, the unambiguous spatial characteristics of the DMN component may have facilitated differentiation from components representing noise. Iron burden was assessed using QSM,^{44,47,88} which is a potential pathology in AD and AD-risk populations.^{40,49,50,89} A close association between paramagnetic susceptibility in gray matter brain regions (with low myelin content) and high tissue iron content is supported by post mortem analysis of deep brain gray matter.^{43,90} Recently published histological data on cortical and deep gray matter regions furthermore support a positive relationship between QSM and iron, and an inverse relationship between QSM and myelin.⁴⁶ Although paramagnetic susceptibility in human gray matter is regarded as a valid measure of non-heme iron,^{38,91} particularly cortical susceptibility measures should be interpreted carefully. When inferring on local non-heme iron, myelin may have a confounding impact due to its phase shifting capacity.⁴⁵ Moreover, there may be a relationship between susceptibility measured by locally increased BOLD contrast and QSM. Although the current study investigated BOLD synchronicity over time rather than local increases of BOLD contrast, the fact that BOLD and QSM may not be completely independent might nevertheless represent another limitation of our experimental approach. In addition, QSM at higher spatial resolution might reduce the risk of confounding heme iron by vascular objects that may have been missed due to SNR limitations of the 3 Tesla GRE sequence applied here. Further studies implying an additional, independent measure of blood flow might provide insight on the interdependence of both measures.

Although our data are consistent with earlier reports on an association between APOE4 and altered DMN properties in preclinical stages of AD,²⁹ our findings of an association between APOE4 and increased DMN connectivity may be consistent with earlier considerations that increased DMN connectivity might represent characteristic phenomena in populations at risk for AD.⁸⁴ Moreover, the finding of a genotype effect may corroborate biological relevance of our observation. Our

main finding was that APOE4 effects synergistically interacted with effects associated with increased brain iron (as estimated by QSM). To our knowledge no interactive effects between magnetic susceptibility, as a reflection of iron, and APOE4 on DMN activity have been reported so far. However, our findings support earlier considerations that detrimental effects associated with increased brain iron may be promoted by the APOE4 genotype.^{36,59,89}

Although the APOE4 genotype alone is not associated with increased iron,³⁷ increased prevalence of APOE4 in cognitively impaired individuals with higher levels of brain iron might reflect accelerated cognitive deterioration.^{39,40} Moreover, recently published data suggest that alterations of cortical networks such as the DMN may also indicate progression of tau pathology.^{92,93} Here, additional longitudinal studies are needed to carefully investigate potential interactions between pathological tau and iron in neurodegenerative disease and healthy aging.⁹⁴ Our current findings of a possibly synergistic impact of paramagnetic susceptibility and APOE4 on BOLD synchronicity within the DMN might support recently suggested therapeutic interventions aimed at brain iron load^{54,95} but also restoring physiological network architecture within the neocortex.⁹⁶ Recent studies suggest potentially deleterious effects of unbound iron by oxidative stress^{52,97} or programmed cell death such as ferroptosis,⁵⁸ an association of brain iron burden with life style,⁹⁸ and a possible role of iron for maintained cognitive function at old age.⁶⁵ Thus, longitudinal cohort studies are needed to better understand the interplay between altered MR measures of cortical susceptibility, its relationship to dysbalanced brain iron homeostasis, and APOE4-related risk for AD.

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