

Quantitative Susceptibility Mapping of Brain Iron and β -Amyloid in MRI and PET Relating to Cognitive Performance in Cognitively Normal Older Adults

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Conflicts of interest are listed at the end of this article.

See also the editorial by Chiang in this issue.

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Background: For individuals with mild cognitive impairment (MCI) or dementia, elevated brain iron together with β -amyloid is associated with lower cognitive functioning. But this needs further investigation among cognitively normal older adults.

Purpose: To investigate via quantitative susceptibility mapping (QSM) in MRI and PET how cerebral iron together with β -amyloid affects cognition among cognitively normal older adults.

Materials and Methods: In this secondary analysis of a prospective study, cognitively normal older adults underwent QSM MRI to measure brain iron. A majority underwent PET to measure cerebral β -amyloid within 30 days of MRI. Multiple linear regression analyses were performed for 12 cortical and subcortical gray matter regions to assess the effect of brain iron on cognitive functions. Voxel-based analyses investigated the associations between tissue iron and β -amyloid load and their relationship to cognitive performance.

Results: Evaluated were 150 cognitively normal older adults (mean age, 69 years \pm 8 [standard deviation]; 93 women). Of 150, 97 underwent PET; 22 of the 97 (mean age, 71 years \pm 6; 13 women) were positive for β -amyloid. In all participants, brain iron content in the hippocampus negatively correlated with global cognitive composite score (standardized β = -0.24 ; 95% CI: -0.40 , -0.07 ; P = .005). In the PET subgroup, brain iron in the hippocampus negatively correlated with episodic memory (β = -0.24 ; 95% CI: -0.40 , -0.08 ; P = .004) and visuospatial score (β = -0.34 ; 95% CI: -0.56 , -0.12 ; P = .003) independent of β -amyloid burden. Both negative and positive correlations between brain iron and β -amyloid were observed in the PET subgroup, revealing clusters where brain iron content negatively correlated with β -amyloid and global cognitive scores (eg, in the frontal cortex: β = -0.13 ; 95% CI: -0.23 , -0.02 ; P = .02). No clusters showed associations between β -amyloid and global cognition.

Conclusion: Among cognitively normal older adults, quantitative susceptibility mapping in MRI and PET indicated that elevated cerebral iron load was related to lower cognitive performance independent of β -amyloid.

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It is clear that Alzheimer disease (AD) pathology, including the extracellular accumulation of β -amyloid plaques, occurs many years before the emergence of clinical symptoms of AD and can be detected noninvasively at PET imaging (1,2). However, large variability in the rate of cognitive decline among individuals with amyloid pathology (3,4) and ineffectiveness of antiamyloid therapeutics to slow cognitive decline (5,6) suggest that other factors, such as brain iron (7), may alter risk of cognitive decline (8,9).

As an essential transition metal, iron has many important roles in the brain biologic functions, including neurotransmitter synthesis, generation of myelin sheets, and metabolism (10). But animal models suggest elevated brain iron content can cause oxidative stress, promote β -amyloid toxicity and tau-protein dysfunction, enhance neuronal cell death, and lead to neurodegeneration and cognitive dysfunction (11). Although local brain iron increases during

normal aging (12,13), a greater-than-normal iron accumulation among older adults is associated with neurodegeneration and amyloid plaque in AD (10,14).

Advances in quantitative susceptibility mapping (QSM) MRI techniques have made it possible to noninvasively measure tissue iron content with high spatial resolution and high sensitivity (15). Several studies that used QSM MRI reported elevated cerebral iron in subcortical nuclei and cortical regions among individuals with mild cognitive impairment (MCI) and AD dementia compared with cognitively normal individuals (16–18). Also, interactions between iron and amyloid among individuals with MCI or AD dementia (19,20) showed greater cognitive decline among those with both high iron and high amyloid load compared with those with only one of these pathologic conditions (20). A recent PET/MRI study among participants without dementia further suggested that in frontal and temporal regions, where

Abbreviations

AD = Alzheimer disease, APOE $\varepsilon 4$ = apolipoprotein E $\varepsilon 4$, MCI = mild cognitive impairment, QSM = quantitative susceptibility mapping, ROI = region of interest

Summary

Quantitative susceptibility mapping in MRI and PET indicated that elevated brain iron was related to lower cognitive performance independent of β -amyloid in cognitively normal older adults.

Key Results

- Brain iron content in the hippocampus negatively correlated with episodic memory ($\beta = -0.24$; $P = .004$) and visuospatial scores ($\beta = -0.34$; $P = .003$), whereas β -amyloid showed no associations with cognition in cognitively normal older adults.
- Voxel-based analyses also revealed clusters (eg, in the frontal cortex) where brain iron content negatively correlated with β -amyloid and global cognitive scores ($\beta = -0.13$; $P = .02$) with no associations between β -amyloid and cognition.

iron and amyloid- β levels are locally correlated, higher regional amyloid is associated with lower global cognition. But the study did not show direct associations between iron and cognition (21).

In our study, the effects of elevated cerebral iron load on cognitive functions and its possible interaction with amyloid- β burden were examined with relevant clinical, genetic, and neurodegeneration factors, including age, sex, education, apolipoprotein E $\varepsilon 4$ (APOE $\varepsilon 4$) genotype, and brain atrophy in cognitively normal older adults. Elevated local cerebral iron load was hypothesized to relate to lower cognitive performance. To our knowledge, no study has examined such effects in a well-characterized sample of cognitively normal older adults. Unlike previous studies, participants with MCI were excluded.

Materials and Methods

This secondary analysis of a prospective study was approved by the Johns Hopkins University institutional review board. Written informed consent were obtained with compliance with the Health Insurance Portability and Accountability Act.

Study Participants

Participants were part of an ongoing, longitudinal study, known as Biomarkers for Older Controls at Risk for Dementia, or BIOCARD (22). The analyses herein are on the basis of cognitively normal older adults who underwent QSM MRI between January 2015 and September 2018. Exclusion criteria included corrupted image reconstruction or severe artifacts or diagnosis of MCI. The majority of participants underwent a PET examination using ^{11}C -labeled Pittsburgh compound B tracer within 30 days (mean, 1 day \pm 3 [standard deviation]) of their MRI to assess amyloid- β levels, and these participants are referred to as the PET group (see Appendix E1 [online] for details about participant recruitment and diagnostic procedures).

APOE Genotyping and Clinical and Cognitive Assessments

APOE $\varepsilon 4$ carrier status was coded by a dichotomous indicator variable: $\varepsilon 4$ carriers (ie, individuals with at least one APOE $\varepsilon 4$ allele) were coded as 1, and noncarriers were coded as 0.

Each participant included in the present analyses received a consensus diagnosis by the staff of the Johns Hopkins University BIOCARD Clinical Core as (a) cognitively normal or (b) impaired not MCI (Appendix E1 [online]).

All participants underwent a comprehensive battery of neuropsychologic tests at the same visit as their MRI (22). Four domain-specific composite scores, reflecting verbal episodic memory, executive function, visuospatial processing, and language ability were calculated on the basis of 12 neuropsychologic test scores, as described previously (23) and summarized in Appendix E2 (online). In addition, global cognitive performance was measured by using a global cognitive composite score (24) (Appendix E2 [online]). For both the global and the domain-specific cognitive composite scores, higher scores mean better cognitive performance.

MRI Acquisition and Processing

MRI was conducted by using a 3.0-T scanner (Achieva; Philips Healthcare, Best, the Netherlands). A three-dimensional multi-echo gradient-recalled echo sequence ($1 \times 1 \times 1 \text{ mm}^3$ resolution) was used for QSM, whereas a three-dimensional T1-weighted magnetization prepared rapid gradient-echo sequence ($1 \times 1 \times 1.2 \text{ mm}^3$ resolution) was used for anatomic referencing and automated image segmentation (Appendix E3 [online]).

For assessing brain iron content, QSM images were reconstructed by using the Johns Hopkins University/Kennedy Krieger Institute QSM Toolbox (version 3.0; <http://godzilla.kennedykrieger.org/QSM/>) (25). The phase preprocessing and QSM dipole inversion procedures are detailed in Appendices E4 and E5 (online).

For brain segmentation, T1-weighted magnetization prepared rapid gradient-echo images were coregistered to the QSM space. Twelve regions of interest (ROIs) including the superior and middle frontal cortex, inferior and orbital frontal cortex, parietal cortex, temporal cortex, occipital cortex, entorhinal cortex, cingulate cortex, amygdala, hippocampus, caudate, putamen, and globus pallidus were automatically segmented on the basis of human brain atlases for quantifying tissue magnetic susceptibility (iron measure) and structure volume in each region (Appendix E6 [online]).

PET Image Acquisition and Processing

The dynamic ^{11}C -labeled Pittsburgh compound B tracer PET scans were performed on an Advance PET scanner (GE Healthcare, Milwaukee, Wis), and distribution volume ratio images were calculated in the native space of each PET image (26,27). Mean distribution volume ratio in each selected ROI was quantified and a global index of cortical distribution volume ratio value greater than a threshold of 1.06 was considered positive for Pittsburgh compound B (or positive for amyloid- β ; Appendix E7 [online]).

Statistical Analysis

Multiple linear regression models were tested to assess the association between local cerebral iron load and the global cognitive composite score in each selected ROI. First, for all participants (model 1), the continuous global cognitive

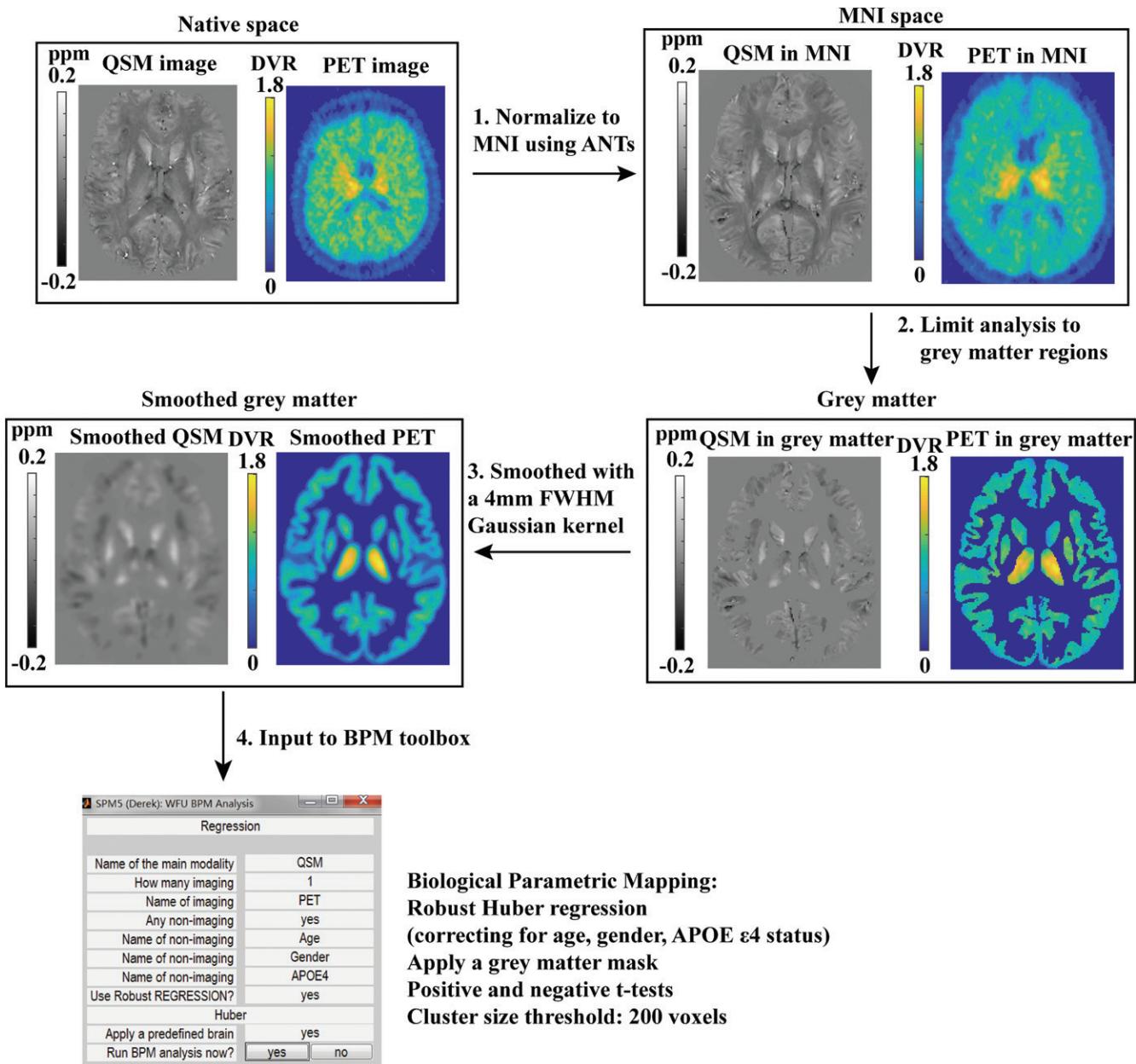


Figure 1: Diagram of the processing steps for performing voxel-based analysis on the local correlation between brain iron and amyloid- β load by using the corresponding quantitative susceptibility mapping (QSM) and PET distribution volume ratio (DVR) images with the Biologic Parametric Mapping toolbox. ANTs = Advanced Normalization Tools, APOE $\epsilon 4$ = apolipoprotein E $\epsilon 4$, FWHM = full width at half maximum, MNI = Montreal Neurologic Institute.

composite score was regressed on age, sex, APOE $\epsilon 4$ status, years of education, structure volume, and tissue magnetic susceptibility, with sex and APOE $\epsilon 4$ status as dichotomous variables. To test whether this association was independent of β -amyloid deposition, all models were rerun on the PET group (model 2) with local distribution volume ratio value as an extra predictor (see Appendix E8 [online] for details about sensitivity analyses). In addition, we reran the regression models in participants negative for amyloid- β to further investigate the association between brain iron and cognition in participants presumably at even lower risk of cognitive decline. Benjamini-Hochberg corrections for multiple comparisons were performed (12 tests for 12 ROIs; false discovery rate, 0.25). All regression analyses were then

repeated by using the four domain-specific continuous cognitive composite scores as the outcome.

The Biologic Parametric Mapping toolbox (28) was used to estimate the voxel-based correlations between brain iron and β -amyloid in gray matter regions in the PET group (Appendix E9 [online], Fig 1). The associations of iron load and β -amyloid burden with cognition was further examined in clusters with significant positive or negative correlations between iron and β -amyloid. Ridge regressions were then conducted with the global or domain-specific cognitive score as an outcome. Benjamini-Hochberg correction for multiple comparisons was applied (number of tests determined by the number of clusters identified by Biologic Parametric Mapping; false discovery rate, 0.25).

Table 1: Demographic Information and Neuropsychologic Testing Results

Parameter	All Participants with Normal Cognition	Total	PET Group		Participants without PET	P Value [*]	P Value [†]
			Amyloid- β Negative	Amyloid- β Positive			
No. of participants	150	97	75	22	53		
No. of women	93	62	49	13	31		
No. of men	57	35	26	9	22		
Age (y)	69 ± 8	69 ± 9	68 ± 10	71 ± 6	.24	71 ± 7	.40
Education (y)	17 ± 2	17 ± 2	17 ± 2	18 ± 2	.14	18 ± 2	.81
No. of APOE ϵ 4 carriers [‡]	49 (33)	30 (31)	15 (20)	15 (68)	<.001	19 (36)	.54
Global cognitive composite score	0.31 ± 0.57 (-1.66 to 1.45) [0.36]	0.33 ± 0.54	0.35 ± 0.52	0.27 ± 0.62	.78	0.23 ± 0.61	.39
Episodic memory score	0.88 ± 1.38 (-3.44 to 3.93) [0.92]	1.01 ± 1.37	1.08 ± 1.30	0.75 ± 1.62	.34	0.62 ± 1.37	.06
Executive score	0.61 ± 1.28 (-5.41 to 3.48) [0.74]	0.64 ± 1.34	0.63 ± 1.40	0.67 ± 1.14	.71	0.55 ± 1.16	.25
Visuospatial score	0.54 ± 1.49 (-3.98 to 3.42) [0.84]	0.59 ± 1.47	0.59 ± 1.55	0.58 ± 1.18	.71	0.47 ± 1.57	.65
Language score	0.66 ± 1.09 (-3.38 to 3.39) [0.62]	0.77 ± 1.13	0.70 ± 1.10	1.00 ± 1.24	.23	0.41 ± 1.05	.07

Note.—Data are means ± standard deviation unless otherwise indicated. Data in parentheses are range unless otherwise indicated; data in brackets are median. All cognitive scores have *z*-score per unit. All *P* values are from Mann-Whitney *U* tests. APOE ϵ 4 = apolipoprotein E ϵ 4.

* *P* values are from tests comparing the amyloid- β -negative group and the amyloid- β -positive group.

† *P* values are from tests comparing the total PET group (*n* = 97) and participants without PET.

[‡] Data in parentheses are percentages.

All regression analyses were performed by using software (SPSS version 25.0; SPSS, Chicago, Ill).

Results

Participant Characteristics

We initially identified 174 participants of the BIOCARD study. Two participants were excluded for severe imaging artifacts and 22 participants were excluded for diagnosis of MCI. We evaluated 150 cognitively normal older adults (mean age, 69 years ± 8 [standard deviation]; 93 women); 121 participants were cognitively normal and 29 participants were impaired not MCI (see Table E1 [online] for demographic information and see Appendix E8 [online] for information regarding how these participants were treated in the analysis). Of those 150 participants, 97 underwent a Pittsburgh compound B PET examination within 30 days of MRI to assess amyloid- β levels. Of the 97 participants with PET examinations, 22 were positive for β -amyloid (mean age, 71 years ± 6; 13 women); 75 participants were negative for amyloid- β .

The demographic information and neuropsychologic measurements for all participants and, separately, for the PET subgroup are summarized in Table 1. A higher percentage of APOE ϵ 4 carriers was observed in β -amyloid positive versus the β -amyloid negative group (*P* < .001). Typical susceptibility maps with the macroscopic vein masked out are shown in Figure 2, *A* (see Fig E1 [online] for more slices), and the selected cortical and deep gray matter ROIs are shown in Figure 2, *B*.

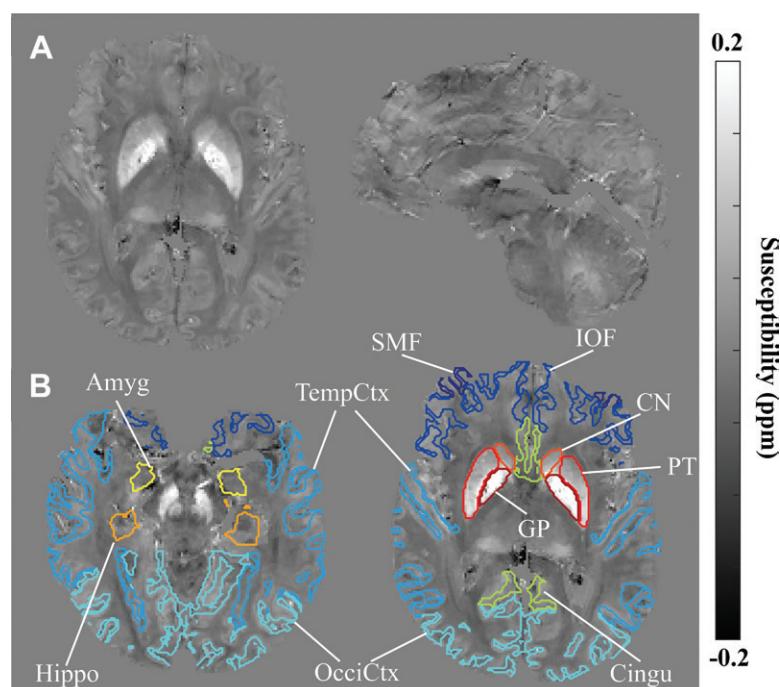


Figure 2: *A*, Example quantitative susceptibility maps in a 72-year-old man with normal cognition in axial (left) and sagittal (right) view. Regions that contained macroscopic veins were masked out to better quantify the nonheme tissue iron content. *B*, The selected regions of interest in the cortex and basal ganglia are indicated on the susceptibility maps. Amyg = amygdala, Cingu = cingulate cortex, CN = caudate nuclei, GP = globus pallidus, Hippo = hippocampus, IOF = inferior and orbital frontal cortex, OcciCtx = occipital cortex, PT = putamen, SMF = superior and middle frontal cortex, TempCtx = temporal cortex.

Relationship between Brain Iron, Amyloid, and Global Cognitive Performance

Associations between the global cognitive composite score and regional brain iron in all participants and in the PET group

Table 2: Results from Multiple Linear Regression of Global and Domain-specific Cognitive Composite Scores on Brain Iron Levels

Parameter	Model 1 (<i>n</i> = 150)		Model 2 (<i>n</i> = 97)		β-Amyloid Negative (<i>n</i> = 75)	
	β Coefficient	P Value	β Coefficient	P Value	β Coefficient	P Value
Global cognition						
SMF cortex	-0.11 (-0.27, 0.05)	.19	-0.22 (-0.43, -0.11)	.04	-0.34 (-0.59, -0.10)	.007
Temporal cortex	-0.08 (-0.23, 0.08)	.32	-0.20 (-0.41, 0.01)	.06	-0.27 (-0.52, -0.02)	.03
Entorhinal cortex	-0.12 (-0.28, 0.04)	.15	-0.15 (-0.37, 0.08)	.19	-0.33 (-0.60, -0.06)	.02
Hippocampus	-0.24 (-0.40, -0.07)	.005	-0.22 (-0.45, 0.02)	.07	-0.43 (-0.71, -0.16)	.003
Putamen	-0.15 (-0.32, 0.02)	.09	-0.17 (-0.39, 0.06)	.14	-0.28 (-0.55, -0.00)	.047
Globus pallidus	-0.10 (-0.26, 0.07)	.25	-0.26 (-0.48, -0.05)	.02	-0.30 (-0.55, -0.06)	.02
Episodic memory						
SMF cortex	-0.10 (-0.26, 0.07)	.24	-0.16 (-0.38, 0.06)	.14	-0.27 (-0.52, -0.02)	.04
Temporal cortex	-0.10 (-0.26, 0.06)	.21	-0.18 (-0.39, 0.03)	.10	-0.27 (-0.52, -0.01)	.04
Entorhinal cortex	-0.16 (-0.33, 0.00)	.06	-0.15 (-0.38, 0.08)	.19	-0.32 (-0.59, -0.05)	.02
Hippocampus	-0.22 (-0.38, -0.05)	.01	-0.24 (-0.40, -0.08)	.004	-0.44 (-0.72, -0.16)	.002
Putamen	-0.14 (-0.31, 0.04)	.13	-0.11 (-0.35, 0.12)	.33	-0.16 (-0.44, 0.13)	.27
Globus pallidus	-0.06 (-0.23, 0.11)	.48	-0.12 (-0.34, 0.01)	.27	-0.15 (-0.40, 0.10)	.24
Executive score						
SMF cortex	-0.09 (-0.24, 0.07)	.29	-0.16 (-0.37, 0.05)	.12	-0.23 (-0.48, 0.01)	.06
Temporal cortex	-0.04 (-0.20, 0.11)	.60	-0.11 (-0.31, 0.10)	.30	-0.13 (-0.37, 0.12)	.31
Entorhinal cortex	-0.09 (-0.25, 0.08)	.30	-0.13 (-0.35, 0.09)	.24	-0.22 (-0.49, 0.05)	.10
Hippocampus	-0.17 (-0.34, -0.01)	.04	-0.19 (-0.41, 0.04)	.11	-0.31 (-0.59, -0.04)	.03
Putamen	-0.18 (-0.35, -0.01)	.04	-0.24 (-0.46, -0.03)	.03	-0.34 (-0.60, -0.08)	.01
Globus pallidus	-0.23 (-0.39, -0.08)	.004	-0.39 (-0.58, -0.19)	<.001	-0.44 (-0.66, -0.21)	<.001
Visuospatial score						
SMF cortex	-0.04 (-0.20, 0.12)	.61	-0.15 (-0.36, 0.06)	.15	-0.16 (-0.41, 0.09)	.20
Temporal cortex	-0.05 (-0.21, 0.11)	.53	-0.16 (-0.37, 0.04)	.12	-0.16 (-0.41, 0.08)	.19
Entorhinal cortex	-0.10 (-0.27, 0.06)	.23	-0.14 (-0.36, 0.08)	.20	-0.23 (-0.50, 0.03)	.08
Hippocampus	-0.25 (-0.41, -0.09)	.002	-0.34 (-0.56, -0.12)	.003	-0.45 (-0.71, -0.20)	.001
Putamen	-0.08 (-0.25, 0.09)	.36	-0.16 (-0.37, 0.06)	.16	-0.21 (-0.47, 0.06)	.12
Globus pallidus	-0.08 (-0.24, 0.09)	.36	-0.25 (-0.46, -0.05)	.02	-0.29 (-0.52, -0.06)	.01
Language score						
SMF cortex	-0.10 (-0.26, 0.07)	.24	-0.11 (-0.32, 0.11)	.33	-0.19 (-0.45, 0.06)	.14
Temporal cortex	-0.05 (-0.21, 0.12)	.57	-0.10 (-0.31, 0.12)	.38	-0.14 (-0.40, 0.12)	.28
Entorhinal cortex	-0.10 (-0.26, 0.08)	.28	-0.22 (-0.44, -0.00)	.05	-0.39 (-0.65, -0.12)	.005
Hippocampus	-0.13 (-0.30, 0.04)	.13	-0.02 (-0.26, 0.21)	.85	-0.12 (-0.41, 0.18)	.43
Putamen	-0.18 (-0.35, -0.00)	.05	-0.18 (-0.40, 0.05)	.12	-0.28 (-0.56, -0.01)	.04
Globus pallidus	-0.14 (-0.30, 0.03)	.10	-0.27 (-0.48, -0.07)	.01	-0.32 (-0.56, -0.07)	.01

Note.—Data in parentheses are 95% CIs. *P* values less than .05 indicate statistical significance. Model 1 represents all participants, and model 2 represents participants in the PET group. The units of all variables were *z* score per unit. Results for inferior and orbital frontal cortex, parietal cortex, occipital cortex, cingulate cortex, amygdala, and caudate are not shown because of less or no significant associations between brain iron and cognition found in these regions. Associations between β-amyloid plaque load (PET distribution volume ratio) and cognitive test scores were not significant in any listed region with all *P* values of .08 or greater. Complete list of model coefficients can be found in Tables E2 and E3 (online) for global cognition, E5 and E6 (online) for episodic memory, E8 and E9 (online) for executive function score, E11 and E12 (online) for visuospatial score, and E14 and E15 (online) for language score. SMF = superior and middle frontal.

are summarized in Table 2 and Table E2 (online). Older age, less education, and male sex were associated with lower global cognitive scores. A negative correlation between brain iron and global cognitive performance was found in the hippocampus (model 1: standardized $\beta = -0.24$; 95% CI: $-0.40, -0.07$; $P = .005$) (Fig 3, *A*). Similar analyses in the PET group (model 2) confirmed such negative associations in superior and middle frontal cortex ($\beta = -0.22$; 95% CI: $-0.43, -0.11$; $P = .04$) and globus pallidus ($\beta = -0.26$; 95% CI: $-0.48, -0.05$; $P = .02$), but not in hippocampus ($\beta = -0.22$; 95% CI: $-0.45,$

0.02 ; $P = .07$) (Fig 3, *B*), whereas β-amyloid showed no association with global cognition in all selected ROIs (eg, in the hippocampus [$\beta = 0.05$; 95% CI: $-0.17, 0.27$; $P = .67$]) (Table E2 [online]).

In participants negative for amyloid-β, similar negative correlations between brain iron and global cognition were found in more ROIs including superior and middle frontal cortex ($\beta = -0.34$; 95% CI: $-0.59, -0.10$; $P = .007$), temporal cortex ($\beta = -0.27$; 95% CI: $-0.52, -0.02$; $P = .03$), entorhinal cortex ($\beta = -0.33$; 95% CI: $-0.60, -0.06$; $P = .02$), hippocampus

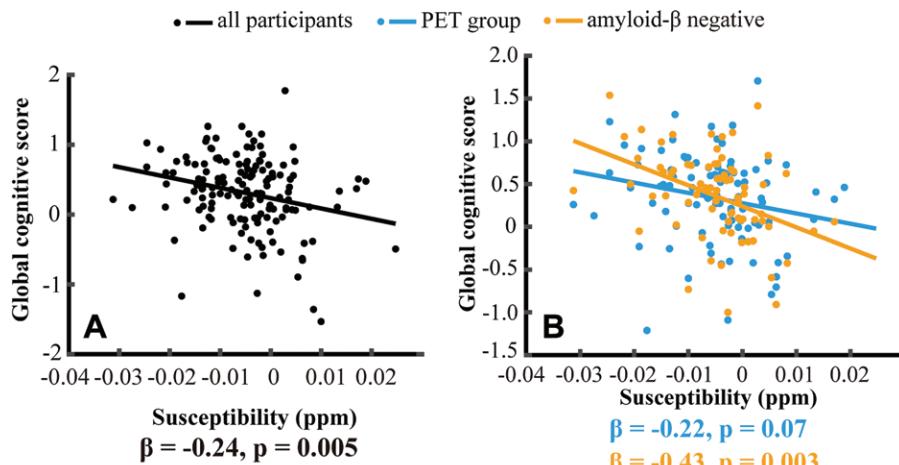


Figure 3: Scatterplot of the adjusted response data and adjusted response function of the multiple linear regression model between global cognitive composite score (continuous variable with z-score per unit) and tissue susceptibility values in the hippocampus of, A, all participants with normal cognition ($n = 150$), B, and in the PET subgroup ($n = 97$, blue) and in participants negative for amyloid- β ($n = 75$, orange). The reported β values are standardized coefficients.

($\beta = -0.43$; 95% CI: $-0.71, -0.16$; $P = .003$) (Fig 3, B), globus pallidus ($\beta = -0.30$; 95% CI: $-0.55, -0.06$; $P = .02$) and others (Table 2, Table E3 [online]). Sensitivity analyses excluding the impaired-not-MCI group are shown in Table E4 (online).

Relationship between Brain Iron, Amyloid, and Domain-specific Cognitive Performance

Regarding episodic memory, older age and male sex were associated with lower scores (Table E5 [online]). Brain iron levels in the hippocampus were negatively associated with episodic memory score in all participants ($\beta = -0.22$; 95% CI: $-0.38, -0.05$; $P = .01$) and in the PET group ($\beta = -0.24$; 95% CI: $-0.40, -0.08$; $P = .004$) (Fig 4, A, B; Table 2), independent of β -amyloid levels ($\beta = -0.03$; 95% CI: $-0.26, 0.19$; $P = .78$) (Table E5 [online]). For participants negative for amyloid- β , negative correlations between iron and episodic memory score were found in hippocampus ($\beta = -0.44$; 95% CI: $-0.72, -0.16$; $P = .002$; Fig 4, B) and other ROIs including superior and middle frontal cortex ($\beta = -0.27$; 95% CI: $-0.52, -0.02$; $P = .04$), temporal cortex ($\beta = -0.27$; 95% CI: $-0.52, -0.01$; $P = .04$), and entorhinal cortex ($\beta = -0.32$; 95% CI: $-0.59, -0.05$; $P = .02$) (Table 2, Table E6 [online]). Sensitivity analyses excluding the impaired-not-MCI group are shown in Table E7 (online).

For executive function, younger age and higher education was related to better performance (Table E8 [online]). Brain iron levels in hippocampus negatively correlated with executive function scores in all participants ($\beta = -0.17$; 95% CI: $-0.34, -0.01$; $P = .04$; Fig 4, C). Negative correlations between iron and executive function scores were also observed in globus pallidus ($\beta = -0.23$; 95% CI: $-0.39, -0.08$; $P = .004$) in all participants and in the PET group ($\beta = -0.39$; 95% CI: $-0.58, -0.19$; $P < .001$) (Table 2, Table E8 [online]). For participants negative for amyloid- β , similar negative correlations were observed in hippocampus ($\beta = -0.31$; 95%

CI: $-0.59, -0.04$; $P = .03$; Fig 4, D) and globus pallidus ($\beta = -0.44$; 95% CI: $-0.66, -0.21$; $P < .001$; Table 2, Table E9 [online]). Sensitivity analyses excluding the impaired-not-MCI group are shown in Table E10 (online).

For visuospatial function, older age and female sex were associated with lower performance (Table E11 [online]). A negative correlation was observed between the visuospatial score and brain iron load in hippocampus in all participants ($\beta = -0.25$; 95% CI: $-0.41, -0.09$; $P = .002$) and in the PET group ($\beta = -0.34$; 95% CI: $-0.56, -0.12$; $P = .003$; Fig 4, E, F), independent of β -amyloid levels ($\beta = -0.03$; 95% CI: $-0.24, 0.17$; $P = .75$) (Table E11 [online]). For participants negative for amyloid- β , negative

correlations were found in hippocampus ($\beta = -0.45$; 95% CI: $-0.71, -0.20$; $P = .001$; Fig 4, F) and globus pallidus ($\beta = -0.29$; 95% CI: $-0.52, -0.06$; $P = .01$) (Table 2, Table E12 [online]). Sensitivity analyses excluding the impaired-not-MCI group are shown in Table E13 (online).

For language function, higher education was related to higher scores (Table E14 [online]). Negative associations between the language scores and brain iron in globus pallidus ($\beta = -0.27$; 95% CI: $-0.48, -0.07$; $P = .01$) were observed in the PET group (Table 2, Table E14 [online]). For participants negative for amyloid- β , negative correlations were found in more ROIs including entorhinal cortex ($\beta = -0.39$; 95% CI: $-0.65, -0.12$; $P = .005$) and globus pallidus ($\beta = -0.32$; 95% CI: $-0.56, -0.07$; $P = .01$; Table E15 [online]). Sensitivity analyses excluding the impaired-not-MCI group are shown in Table E16 (online).

Voxel-level Associations between Brain Iron and Amyloid and Their Correlations with Cognition

There were nine clusters (brain regions) of negative correlation and three clusters of positive correlation between iron and amyloid levels (Table 3, Fig 5, A). Two clusters among the nine clusters with negative amyloid-iron correlations demonstrated negative correlations between iron level and the global cognitive composite score: one in the frontal cortex ($\beta = -0.13$; 95% CI: $-0.23, -0.02$; $P = .02$) and one in the cingulate cortex area ($\beta = -0.11$; 95% CI: $-0.21, -0$; $P = .047$; Fig 5, B). The frontal cortex cluster also showed negative associations between iron levels and episodic memory scores ($\beta = -0.12$; 95% CI: $-0.22, -0.01$; $P = .04$; Fig 5, C) and language scores ($\beta = -0.13$; 95% CI: $-0.23, -0.02$; $P = .02$; Fig 5, D). No association was observed between β -amyloid and global cognitive composite score in any of these 12 clusters. Sensitivity analyses excluding the impaired-not-MCI group are shown in Table E17 (online) and Figure E2 (online).

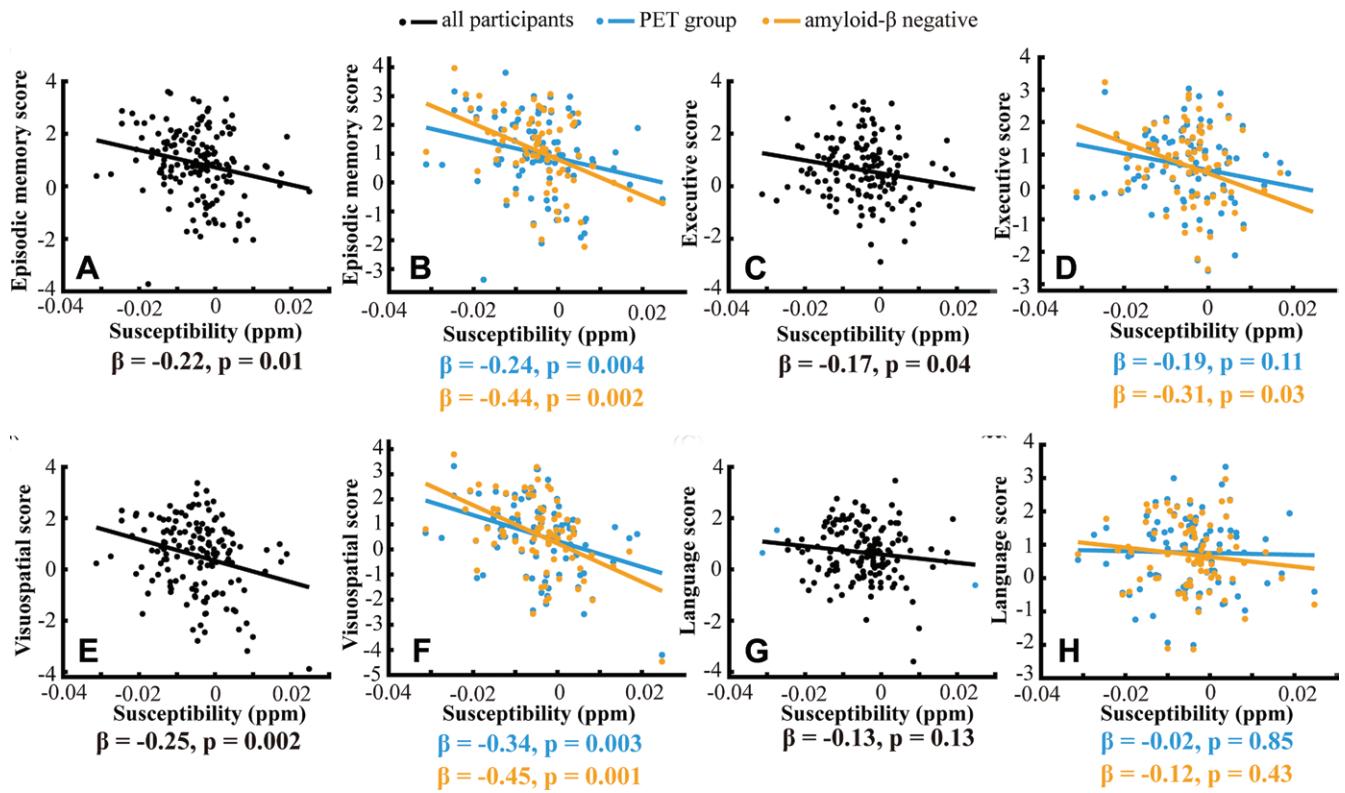


Figure 4: Scatterplot of the adjusted response data and adjusted response function of the multiple linear regression model between, A, B, episodic memory score, C, D, executive function score, E, F, visuospatial score, G, H, language score, and tissue susceptibility values in the hippocampus of all participants with normal cognition ($n = 150$), in the PET group ($n = 97$), and of participants negative for amyloid- β in this group ($n = 75$). All cognitive scores are continuous variables and have z-score per unit. The reported β values are standardized coefficients.

Table 3: Voxel-based Analysis Results of the PET Group Testing Associations between Iron Load and β -Amyloid Load

Cluster Number	Negative Correlation		Positive Correlation	
	Brain Region	No. of Significant Voxels	Brain Region	No. of Significant Voxels
1	Hippocampus head	1542	Caudate nucleus	787
2	Insula	1029	Hippocampus tail	372
3	Frontal cortex	925	Thalamus	321
4	Cingulate cortex	819		
5	Occipital cortex	743		
6	Amygdala	458		
7	Temporal cortex	425		
8	Parietal cortex	284		
9	Basal forebrain	222		

Note.—Listed are clusters showing correlations between brain iron load (quantitative susceptibility mapping) and β -amyloid plaque load (PET distribution volume ratio) ($n = 97$). Brain regions are sorted by the number of significant voxels. Clusters in the frontal cortex and cingulate cortex showed negative correlations between the global cognitive composite score and tissue susceptibility (see Fig 5).

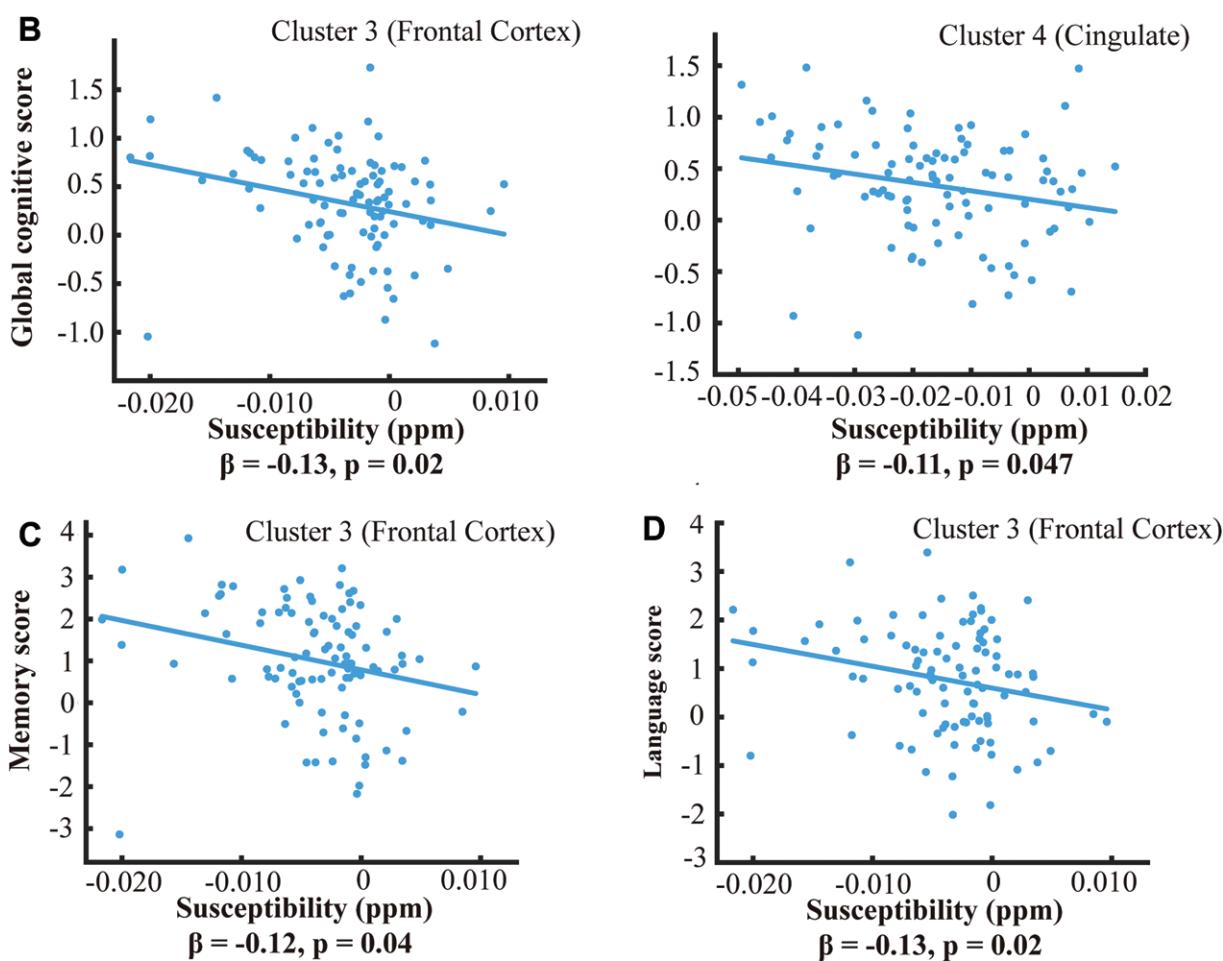
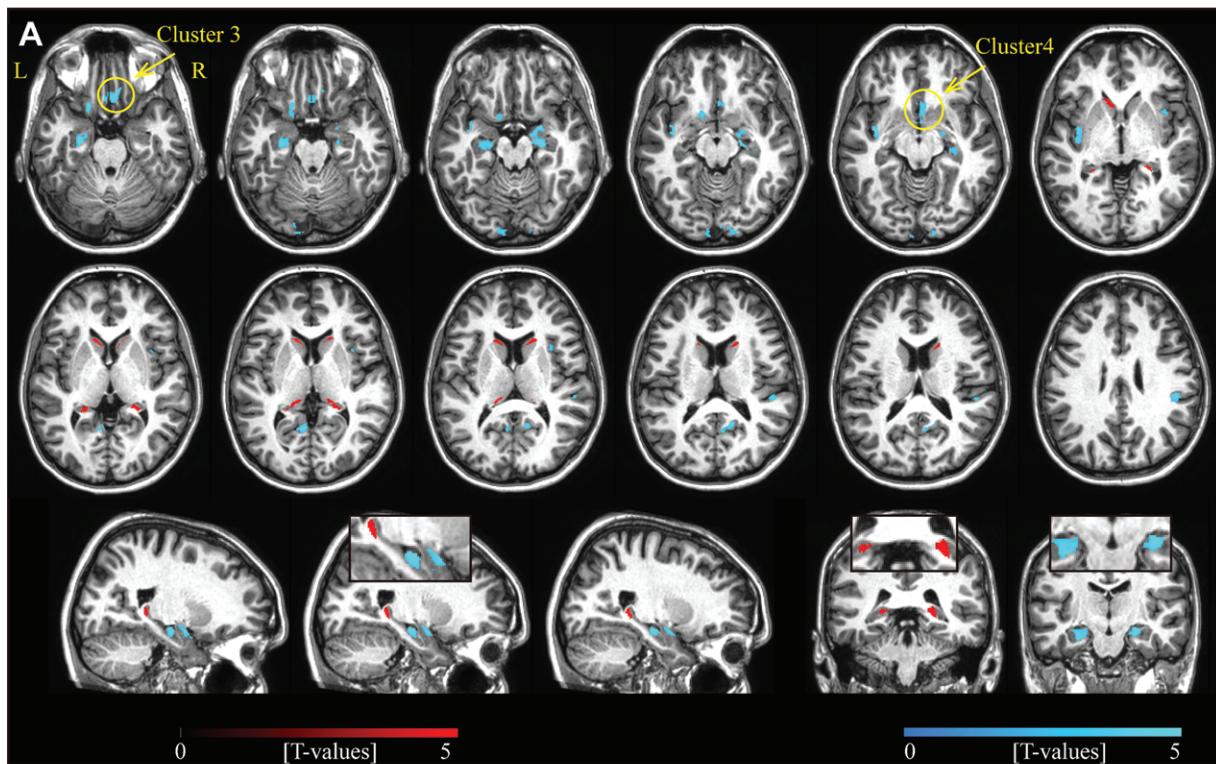
Associations between Structure Volume and QSM or Distribution Volume Ratio

Further analyses confirmed no significant correlations between structure volume and iron (QSM) or β -amyloid (distribution volume ratio) in all selected ROIs (Appendix E10 [online]).

Discussion

Previous studies suggested that elevated brain iron in the presence of β -amyloid is associated with lower cognitive performance among individuals with mild cognitive impairment or dementia, but it is unclear whether this is also the case for cognitively normal older adults. In our study, by using quantitative susceptibility mapping in MRI and PET imaging of β -amyloid in cognitively normal older adults, we demonstrated that elevated brain iron content especially in the hippocampus is associated with lower performance on tests of global cognition ($\beta = -0.24; P = .005$), episodic memory ($\beta = -0.24; P = .004$), and visuospatial function ($\beta = -0.34; P = .003$), independent of β -amyloid burden.

Possible interactions between brain iron and the characteristic AD pathology of β -amyloid and τ have been suggested in postmortem histologic studies that have found amyloid plaques



and neurofibrillary tangles to be enriched with iron (14,29). Versus the interaction with β -amyloid, our results suggest that among older individuals with normal cognition, iron appears to be more consistently related to cognitive performance than β -amyloid. Brain iron level in the hippocampus better predicted global cognitive composite scores than volumetric measures, whereas β -amyloid measures were unrelated to cognitive performance. Our results are consistent with a previous meta-analysis (30) that reported that increased amyloid burden is only weakly associated with episodic memory and measures of global cognition among individuals who are cognitively normal. In addition, our results demonstrated that elevated brain iron correlated with lower cognitive performance with larger coefficients and smaller P values in participants negative for β -amyloid. This may be partly because of the increased detrimental effect on cognition from other disease processes including β -amyloid, τ , and neurodegeneration within the participants positive for β -amyloid.

APOE $\epsilon 4$ is the major known genetic risk factor for late-onset AD (22). However, studies examining cross-sectional associations between APOE $\epsilon 4$ status and cognition among cognitively normal older adults have been mixed (31). This likely reflects cohort differences in the age of participants, cognitive measures used, and levels of preclinical AD pathology. Consistent with present results, studies that carefully screened participants for cognitive deficits at enrollment or subsequently excluded participants with incipient cognitive impairment tended to find no differences in cognitive test performance between $\epsilon 4$ carriers and noncarriers (32). Possible interactions from APOE to brain iron and β -amyloid have been suggested in MCI (19) but may not be evident in the preclinical phase of AD (20,21).

Concerns regarding whether lower scores on cognitive measures predict subsequent cognitive decline were addressed by demonstrated associations between baseline global cognitive scores and the rate of cognitive decline in our participants, which indicated that lower baseline cognitive scores are indeed associated with a higher risk of progression to MCI and dementia (Appendix E12 [online]).

We speculate that the damaging effect of iron on cognitive function among cognitively normal older adults reflects β -amyloid-independent mechanisms, such as iron-related oxidative stress and iron-dependent cell death (ie, ferroptosis) (33). Such mechanisms may also include possible interactions between iron and τ tangles (11), which usually start to accumulate in the hippocampus (34). The associations we found between elevated hippocampal iron levels and episodic memory and visuospatial processing are consistent with the

role of the hippocampus in both cognitive domains. The association between hippocampal iron levels and the global cognitive score is likely because the global score contains two episodic memory tests. Other brain regions including the frontal cortex, temporal cortex, and entorhinal cortex may have similar relevance to the memory and global cognition. In addition, elevated iron content in the putamen and globus pallidus have been observed during aging and many neurodegenerative diseases likely suggesting an altered systematic brain iron homeostasis. Our results also suggest that elevated brain iron content in these regions are related to lower performance in global cognition, executive, visuospatial, and language functions.

The limitations of our study included its cross-sectional design and the separate acquisition of PET and MRI images. The false discovery rate of 0.25 used in the Benjamini-Hochberg correction was set to increase statistical power. Therefore, several reported correlations with marginal significance (eg, $P = .04$) may lose significance if stricter multiple comparison corrections are used. Spatial variations and different statistical powers may explain some observed discrepancy between the ROI-based and voxel-based analyses (eg, the lack of associations of brain iron in the hippocampus clusters with cognition in the whole group) (Fig 5) versus the ROI-based analysis.

In conclusion, our results suggest that cerebral iron elevation is related to lower cognitive function among older adults with normal cognition, independent of β -amyloid load. Further investigations regarding the interaction between iron and τ are warranted, along with longitudinal studies to determine whether brain iron levels predict variability in cognitive trajectories among individuals at different stages along the Alzheimer disease (AD) continuum. With no current effective therapies or preventive strategies for AD, understanding the role of brain iron in AD is vital because it could be a new target of treatment (35).

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Figure 5: A, Overlay of regions with positive (red) and negative (cyan) correlations between iron load (ie, quantitative susceptibility mapping) and β -amyloid plaque load (PET distribution volume ratio) in all participants of the PET group ($n = 97$) with family-wise error—corrected cluster level significance of P value less than .05 (combined with an uncorrected voxel-level significance $P < .001$) and a cluster size threshold of 200 voxels on example axial slices of anatomic MRI (top two rows) and sagittal and coronal slices showing the hippocampus (bottom row). B, Scatterplot of the adjusted response data and adjusted response function of the multiple linear regression model between global cognitive composite score and susceptibility values in two clusters (marked by arrows in A), in which the cognitive composite score negatively correlates with tissue susceptibility values. Similarly, negative associations were observed between tissue susceptibility values in the frontal cortex cluster and, C, episodic memory scores and, D, language scores. All cognitive scores are continuous variables and have z-score per unit. The reported β values are standardized coefficients.

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