

Intracranial Internal Carotid Artery Calcification Is Not Predictive of Future Cognitive Decline

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Keywords: Internal carotid artery, Calcification, Clinical Dementia Rating, white matter hyperintensities, Mini-Mental State Exam, 11C-Pittsburgh compound B, PiB, Centiloid

Posted Date: September 22nd, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-910476/v1>

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Version of Record: A version of this preprint was published at Alzheimer's Research & Therapy on February 11th, 2022. See the published version at <https://doi.org/10.1186/s13195-022-00972-2>.

Abstract

Background

Intracranial internal carotid artery (ICA) calcification is a common incidental finding in non-contrast head CT. We evaluated the predictive value of ICA calcification for future risk of dementia and compared the results with conventional imaging biomarkers of dementia.

Methods

In a retrospective observational cohort, we included 230 participants with a PET-CT scan within 18 months of a baseline clinical assessment and longitudinal imaging assessments. Intracranial ICA calcification was quantified on baseline CT scans using the Agatson calcium score. The ability of baseline ICA calcification to discriminate between a control group (participants who maintained a Clinical Dementia Rating (CDR™) score of zero over all follow-up visits) and a converter group (participants who had a baseline CDR of zero but received a persistent CDR>0 at any follow-up visit) was evaluated along with the predictive value of baseline ICA calcification for longitudinal clinical and imaging biomarkers.

Results

Baseline ICA calcium score could not distinguish participants who converted to CDR>0. ICA calcium score was also unable to predict longitudinal changes in cognitive scores, imaging biomarkers of small vessel disease such as white matter hyperintensities (WMH) volume, or AD such as hippocampal volume, AD cortical signature thickness, and amyloid burden. Severity of intracranial ICA calcification increased with age, male sex, and higher WMH volumes at baseline visit. Higher WMH volume and amyloid burden as well as lower hippocampal volume and AD cortical signature thickness at baseline predicted lower Mini-Mental State Exam scores at longitudinal follow-up. Baseline ICA calcification was indirectly associated with longitudinal cognitive decline, fully mediated through WMH volume.

Conclusions

In elderly and preclinical AD populations, atherosclerosis of large intracranial vessels as demonstrated through ICA calcification is not directly associated with a future risk of dementia, cognitive impairment, or progression of imaging biomarkers of AD or small vessel disease.

1. Introduction

Mineralization of the intimal layer of the vessel wall is an integral part of the atherosclerotic process (1). Calcification of the cervical internal carotid artery (ICA) is a well-studied example that is associated with the presence of cardiovascular risk factors and risk of stroke (2).

Intracranial ICA calcification is an expression of intracranial atherosclerosis and a common incidental finding on non-contrast computed tomography of the head (CT) (3). Its prevalence ranges from 46%-82%

in the general adult population to almost 100% in individuals older than 90 years (4). The most common sites of calcification are the cavernous carotid and the carotid siphon where the severity of calcification is associated with the presence of small vessel disease and white matter lesions (5–8), both of which have been shown to adversely affect cognition in older adults (9).

Studies have demonstrated an inverse relationship between the severity of intracranial ICA calcification and cognitive performance in terms of memory, executive function, global cognition and processing speed in healthy adults (10, 11), as well as a relationship between extracranial ICA calcification and the risk of dementia (12). Disrupted cerebral blood flow autoregulation, blood-brain barrier dysfunction, and increased amyloid deposition following increased ICA stiffness are among the suggested underlying mechanisms (13). Little is known about the association of intracranial ICA calcification and Alzheimer disease (AD), in particular its association with imaging biomarkers of AD, including β -amyloid deposition and cortical and hippocampal atrophy.

We conducted a retrospective cohort study on 230 participants to assess the relationship between intracranial ICA calcification and imaging biomarkers of AD and small vessel disease as well as cognitive outcomes. The objectives of the study were to investigate: 1) whether there is a relationship between the presence and the severity of baseline ICA calcification and conversion from normal to impaired cognition, 2) whether there is a relationship between ICA calcification and imaging biomarkers of AD and small vessel disease, including amyloid burden, white matter lesions, cortical and hippocampal atrophy, and 3) how ICA calcification performs relative to imaging biomarkers of AD and small vessel disease in predicting future cognitive outcomes.

2. Methods

2.1 Participants

Participants were selected from a cohort of individuals enrolled and recruited from February 2009 through March 2018 in the ongoing longitudinal studies of memory and aging at the Charles F. and Joanne Knight Alzheimer Disease Research Center (Knight ADRC) at the Washington University School of Medicine in St. Louis. Inclusion criteria for this study were: 1) having a PET-CT scan within 18 months of a clinical assessment, which was considered the baseline visit, and 2) having at least one follow-up clinical and psychometric assessment. Figure 1 provides a summary of inclusion and exclusion criteria and how the sample size was arrived at. A total number of 230 participants (age range 52–90 years) met these inclusion criteria and were divided into three groups based on their Clinical Dementia Rating™ scale CDR™ (14): (1) *controls* (n = 106) who had a CDR = 0 in baseline and remained cognitively normal (CDR = 0) at their follow-up assessment, (2) *converters* (n = 52) who converted from CDR = 0 at baseline to CDR > 0 at any follow-up assessment, and (3) *impaired* (n = 72) who had a CDR > 0 at their baseline visit. Participants with a CDR = 0.5 who reverted back to a CDR = 0 at any later visit were excluded from both the controls and converters. Controls and converters were 2:1 matched by age and sex (Fig. 1).

2.2 Clinical and psychiatric assessments

All participants underwent annual cognitive assessments which included a global CDR score evaluated by experienced clinicians utilizing a semi-structured participant interview and information from collateral sources. For longitudinal assessments of cognition, CDR Sum of Boxes score (CDR-SB), a summation of scores for each domain measured, was utilized (15). Also included in the longitudinal cognitive assessment was the mini-mental state examination (MMSE) score that was extracted from participants' annual clinical visits. Finally, we derived the Preclinical Alzheimer Cognitive Composite (PACC) score for each individual as a measure of overall cognitive performance from a subset of the Knight ADRC cognitive battery as described before (16).

2.3 Imaging assessments

2.3.1 CT scan calcium scoring

Non-contrast head CT scans were obtained using a Siemens Biograph 40 PET CT scanner and transferred to a Vitrea 2 workstation (Vital Images Inc, Plymouth, MN). A semi-automated coronary calcium scoring software (VScore) was used to calculate the Agatston calcium score and volume (17).

Training cohort was defined by randomly choosing 15% of the 230 participants. Once trained to the satisfaction of the board-certified neuroradiologist rater (H.O.), two raters (M.N. and F.R.) scored the entire set blindly and independently by drawing ROIs around areas of ICA calcification starting from the distal petrous apex to the ICA terminus (cavernous, clinoid, ophthalmic and communicating segments). The scores were submitted to non-raters (C.D.C, N.S.M., and A.D.) to be evaluated for consistency between the two independent raters. For this purpose, Agatston calcium scores were divided into four categories based on the cut off values used to classify coronary artery calcification in clinical practice (18). The raters achieved a two-way agreement intraclass correlation coefficient (ICC) = 0.845 on raw calcium scores, and an ICC = 0.889 on calcium score categories. CT scans that received scores that differed between the two raters to the extent of receiving scores that belonged to different categories were blindly scored a second time, and any remaining discordant scans received a final resolution score by the board-certified experienced rater (H.O.). As both ICA calcium score and volume had a non-normal distribution, the natural log (Ln) -transformed values of both measures were used after adding one unit to the non-transformed values to deal with calcium scores of zero. Averaged scores between raters were used for analyses.

2.3.2 Amyloid PET acquisition and processing

Participants underwent amyloid PET imaging using either 11C-Pittsburgh compound B (PiB) or 18F-AV45 (florbetapir) radioligands. Methods for PiB and AV45 PET acquisition have been described previously (19, 20). Centiloid values were calculated to standardize the PiB and AV45 tracers (21). Amyloid positivity was defined as Centiloid values of > 16.4 (21).

2.3.3 MRI acquisition and processing

White matter hyperintensities (WMH) volume, total hippocampal volume and AD cortical signature thickness measurements were calculated for each MRI assessment. T1- and T2-weighted images were acquired using a magnetization-prepared rapid gradient-echo sequence on the Siemens 3T TIM Trio or Biograph mMR scanners. T1-weighted images were acquired with a 1 × 1 × 1 mm resolution, 2400 ms repetition time, 3.16 echo time, 8 degree flip angle, 176 frames, and a 256 × 256 field of view in sagittal orientation and T2-weighted images were acquired with a 1 x 1 x 1 mm resolution, 3200 ms repetition time, echo time of 455, 120 degree flip angle, a 256 x 256 field of view.

a) **White matter hyperintensity volume**

WMH volumes were calculated from a T2-weighted fluid-attenuated inversion recovery (FLAIR) and a T1 scan using the lesion segmentation toolbox (LST) implemented within SPM8 (22).

b) **Total hippocampal volume and AD cortical signature thickness**

Total hippocampal volumes were obtained on a T1-weighted image with the use of automated FreeSurfer segmentation as previously described (23). AD cortical signature thickness was also obtained from the T1-weighted image through an ROI cortical map representing the specific brain regions most susceptible to AD-related cortical atrophy as described previously (24).

2.4 Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics version 27 and R version 4.1.1. Baseline variables were evaluated for normality of distribution through Kolmogorov-Smirnov Goodness of Fit Test. Between-group comparisons were conducted using the Kruskal-Wallis and Mann-Whitney U test where applicable. The logistic and linear regression models were used to model the binary and continuous outcomes respectively. Univariable and multivariable analyses through stepwise selection were used to examine the relationship between these binary outcomes and baseline variables, where a significance level of 0.3 is required to allow a predictor into the multivariable model, and a significance level of 0.15 is required for a predictor to stay in the model. Only variables with p-value below 0.1 were reported in the final multivariable model. Variables were selected through a backward likelihood ratio (LR) method in the multivariable model.

For longitudinal analyses, the R packages “lmerTest” and “lme4” were used to run a linear mixed model to extract estimated annual rate of change in cognitive scores, WMH volume, and AD imaging biomarkers. Longitudinal measures of the variables of interest were considered dependent variables while time from baseline visit (*time*) and observations overtime for each subject (*time / subject*) were considered as fixed factors. Next, a simple linear model was devised to investigate the association between baseline Ln-transformed ICA calcium score and volume and estimated annual rates of change in the dependent variable with the significance level set to 0.05. The estimated marginal means of the target variables were then plotted against time through different levels of the fixed effect of interest. Survival analyses were performed using cox proportional hazards (P-H) model to investigate the effect of ICA calcification in

different levels of baseline amyloid status with conversion to CDR > 0 as outcome. The P-H assumption was tested through adding a time-dependent variable to the model.

The R package “mediation” was used to investigate a potential indirect effect of Ln-transformed ICA calcium score and volume on baseline and longitudinal MMSE scores, via baseline AD imaging biomarkers (25). The annual rate of change in MMSE score was estimated for each participant using the method described above. The significance was tested using 1000 bootstrapped samples.

3. Results

3.1 Cross-sectional analyses

3.1.1 No difference in ICA calcification, WMH volume, or AD imaging biomarkers between converters and controls

Figure 1 demonstrates a flow diagram showing the initial number of potentially eligible participants and those excluded for different reasons. A description of demographic, clinical, and cognitive risk factors of different groups in the baseline is presented in Table 1. There was no statistically significant difference between the control and converter groups in baseline calcium scores or volumes, WMH volume, or AD imaging biomarkers. The impaired group however had a higher WMH volume and Centiloid values as well as lower AD cortical signature thickness and total hippocampal volume compared to the control and converter group. Presence of ICA was not associated with any significant difference in cognitive scores or AD imaging biomarkers. Men had more ICA calcification compared to women (Table 2).

Table 1

Demographics, cognitive status, vascular risk factors, and AD imaging biomarkers of participant groups in the baseline

	Total (n = 230)	Control (n = 106)	Converter (n = 52)	Impaired (n = 72)	p-value
Age, years (mean ± SD)	73.7 ± 6.7	73.2 ± 6.6	73.1 ± 6.7	74.8 ± 6.9	0.418
Sex					
Men (n(%))	121(52.6)	54(50.9)	26(50)	41(56.9)	0.669
Women (n(%))	109(47.7)	52(49.1)	26(50)	31(43.1)	
Race					
Caucasian (n(%))	197(85.6)	93(87.8)	43(82.7)	61(84.7)	0.466
African American (n(%))	25(10.9)	11(10.4)	6(11.5)	8(11.2)	
Native American (n(%))	1(< 1)	0(0)	1(1.9)	0(0)	
Unknown (n(%))	7(3)	2(1.8)	2(3.9)	2(2.8)	
MMSE score (median (IQR))	29(2)	29 (1)	29 (2)###	28 (3)***	< 0.001
CDR-SB (median (min-max))	0(1)	0 (0-0.5)	0 (0-0.5)###	1.5 (0.5-6)***	< 0.001
PACC score (median (IQR))	-0.011 ± 0.47	0.13 ± 0.44	0.08 ± 0.38#	-0.23 ± 0.46***	< 0.001
WMH volume, mm ³ (mean ± SD)	16072(24128)	18024 ± 19131	21819 ± 18562	29603 ± 26163**	0.005
Ln ICA Ca Score (median (IQR))	3.2(4.8)	3.4(3.7)	2.9(3)	3.1(4.2)	0.349
Ln ICA Ca Volume (median (IQR))	3.7(4.9)	3.9(3.6)	3.6(3.4)	3.5(4.2)	0.520

*Control: participants with CDR = 0 throughout all visits; Converter: participants converting from CDR = 0 to CDR > 0 in any of the follow-up visits; Impaired: participants with CDR > 0 in the baseline visit; MMSE: Mini Mental-State Examination; CDR: Clinical Dementia Rating Scale; CDR-SB: Clinical Dementia Rating Scale Sum of Boxes; PACC: Preclinical Alzheimer Cognitive Composite; WMH volume: white matter hyperintensities volume; AA: African-American; C: Caucasian; Nat: Native American; U: unknown race; Ln ICA Ca score/volume: natural log transformed internal Carotid Artery Agatston calcium score/volume; Centiloid: measure of global amyloid disposition based on conversion of PIB or AV45 PET SUVRs to a standardized scale; AD cortical signature thickness: cortical thickness in signature regions affected in Alzheimer disease (see Dincer et al. 2020). Where variables did not have a normal distribution median ± interquartile range (IQR) or minimum and maximum values were reported. Annotations: * < 0.05 ** < 0.005 *** < 0.0001 significantly different from Controls. # < 0.05 ## < 0.005 ### < 0.0001 significantly different from Impaired.*

	Total (n = 230)	Control (n = 106)	Converter (n = 52)	Impaired (n = 72)	p-value
Total hippocampal volume, mm ³ (mean ± SD)	7004 ± 967.6	7397 ± 793	6991 ± 844 [#]	6397 ± 1008 ^{***}	< 0.001
AD cortical signature thickness, mm (mean ± SD)	2.5(0.15)	2.5 ± 0.12	2.5 ± 0.13 [#]	2.4 ± 0.17 ^{***}	< 0.001
Centiloid (mean ± SD)	6.9(69.9)	3.4 ± 35.3	66.5 ± 83.2	15.3 ± 75.1 ^{***}	< 0.001

*Control: participants with CDR = 0 throughout all visits; Converter: participants converting from CDR = 0 to CDR > 0 in any of the follow-up visits; Impaired: participants with CDR > 0 in the baseline visit; MMSE: Mini Mental-State Examination; CDR: Clinical Dementia Rating Scale; CDR-SB: Clinical Dementia Rating Scale Sum of Boxes; PACC: Preclinical Alzheimer Cognitive Composite; WMH volume: white matter hyperintensities volume; AA: African-American; C: Caucasian; Nat: Native American; U: unknown race; Ln ICA Ca score/volume: natural log transformed internal Carotid Artery Agatston calcium score/volume; Centiloid: measure of global amyloid disposition based on conversion of PIB or AV45 PET SUVRs to a standardized scale; AD cortical signature thickness: cortical thickness in signature regions affected in Alzheimer disease (see Dincer et al. 2020). Where variables did not have a normal distribution median ± interquartile range (IQR) or minimum and maximum values were reported. Annotations: * < 0.05 ** < 0.005 *** < 0.0001 significantly different from Controls. # < 0.05 ## < 0.005 ### < 0.0001 significantly different from Impaired.*

Table 2

Demographics, cognitive status, and AD imaging biomarkers of participants with and without internal carotid artery calcification in the baseline

	Calcification absent (n = 64)	Calcification present (n = 166)	p-value
Control/Converter/Impaired (n)	25/15/24	81/37/48	0.356
Age, years (mean±SD)	74.4 ± 6.8	74.9 ± 4.2	0.862
Sex			
Men (n(%))	22(34.3)	99(59.6)	0.001
Women (n(%))	42(65.7)	67(40.4)	
Race			
Caucasian (n(%))	50(78.1)	147(88.6)	0.110
African American (n(%))	12(18.7)	13(7.8)	
Native American (n(%))	0(0)	1(0.6)	
Unknown (n(%))	2(3.1)	5(3)	
MMSE (median (IQR))	29(3)	29(2)	0.061
CDR-SB (median (IQR))	0(0.5)	0(1)	0.38

*Control: participants with CDR = 0 throughout all visits; Converter: participants converting from CDR = 0 to CDR > 0 in any of the follow-up visits; Impaired: participants with CDR > 0 in the baseline visit; MMSE: Mini Mental-State Examination; CDR: Clinical Dementia Rating Scale; CDR-SB: Clinical Dementia Rating Scale Sum of Boxes; PACC: Preclinical Alzheimer Cognitive Composite; WMH volume: white matter hyperintensities volume; AA: African-American; C: Caucasian; Nat: Native American; U: unknown race; Centiloid: measure of global amyloid disposition based on conversion of PIB or AV45 PET SUVRs to a standardized scale; AD cortical signature thickness: cortical thickness in signature regions affected in Alzheimer disease (see Dincer et al. 2020). Where variables did not have a normal distribution median ± interquartile range (IQR) or minimum and maximum values were reported. Annotations: *<0.05 **<0.005 ***<0.0001 significantly different from Controls. #<0.05 ##<0.005 ###<0.0001 significantly different from Impaired.*

	Calcification absent (n = 64)	Calcification present (n = 166)	p-value
PACC score (mean±SD)	-0.047 ± 0.45	0.03 ± 0.48	0.334
WMH volume, mm ³ (median(IQR))	13134(27499)	17009(27167)	0.173
Total Hipp, mm ³ (mean±SD)	7052 ± 878	7203 ± 890	0.727
AD cortical signature thickness, mm (mean±SD)	2.5 ± 0.17	2.5 ± 0.11	0.771
Centiloid (median(IQR))	15.3(73.4)	5.3(75.9)	0.502

*Control: participants with CDR = 0 throughout all visits; Converter: participants converting from CDR = 0 to CDR > 0 in any of the follow-up visits; Impaired: participants with CDR > 0 in the baseline visit; MMSE: Mini Mental-State Examination; CDR: Clinical Dementia Rating Scale; CDR-SB: Clinical Dementia Rating Scale Sum of Boxes; PACC: Preclinical Alzheimer Cognitive Composite; WMH volume: white matter hyperintensities volume; AA: African-American; C: Caucasian; Nat: Native American; U: unknown race; Centiloid: measure of global amyloid disposition based on conversion of PIB or AV45 PET SUVRs to a standardized scale; AD cortical signature thickness: cortical thickness in signature regions affected in Alzheimer disease (see Dincer et al. 2020). Where variables did not have a normal distribution median ± interquartile range (IQR) or minimum and maximum values were reported. Annotations: *<0.05 **<0.005 ***<0.0001 significantly different from Controls. #<0.05 ##<0.005 ###<0.0001 significantly different from Impaired.*

3.1.2 Amyloid burden, but not ICA calcification, is associated with AD risk

Using univariable binary regression models, lower baseline MMSE scores and total hippocampal volume and higher Centiloid values were associated with higher odds of conversion to CDR above zero (Table 3). In the multivariable regression model, lower total hippocampal volume and higher Centiloid values were independently associated with higher odds of conversion to CDR above zero (Table 3) (overall model R-square:0.245, *p*-value < 0.001). Survival analyses revealed no association between ICA calcification in the baseline and risk of conversion (Hazard Ratio (HR) (95%CI): 0.866(0.474–1.579), *p*-value = 0.638). While amyloid positivity was associated with a risk of conversion to CDR > 0 (HR (95%CI):2.3(1.3–4.1), *p*-value = 0.04), there was no interaction between amyloid status and ICA calcification in predicting conversion risk (HR (95%CI): 0.826(0.242–2.817), *p*-value = 0.760).

Table 3

Binary logistic regression model to predict the odds of conversion to CDR above zero based on baseline biomarkers

	Odds of conversion from CDR = 0 to CDR > 0			
	Univariable		Multivariable	
	<i>p-value*</i>	<i>OR (95%CI)</i>	<i>p-value</i>	<i>OR**(95%CI)</i>
Age (year)	0.996	1(0.951–1.051)	-	-
MMSE score	0.023	0.737(0.566–0.958)	-	-
PACC score	0.07	0.349(0.112–1.088)	-	-
Ln ICA Calcium score	0.210	0.891(0.743–1.068)	-	-
Ln ICA Calcium volume	0.265	0.899(0.845–1.084)	-	-
WMH volume (1000 mm ³)	0.340	1.01(0.989–1.032)	-	-
Total hippocampal volume (100 mm ³)	0.006	0.940 (0.9-0.982)	0.071	0.958 (0.915–1.004)
AD cortical signature thickness (mm)	0.125	0.110(0.007–1.837)	-	-
Centiloid (5 unit)	0.003	1.08 (1.027–1.136)	0.012	1.070 (1.015–1.127)
* Odd ratios are demonstrated for 1000 mm ³ increment in WMH volume, 100 mm ³ increment in total hippocampal volume, and 5 unit increment in Centiloid scale				
** independent variables with a <i>p-value</i> below 0.3 and <i>OR(95%CI)</i> excluding the value 1 were selected from the univariable model (in Bold) and entered in a multivariable binary logistic model using the backward LR method. Significance level was set to 0.1 for the final model.				
*** beta coefficients adjusted for the effect of other variables in the model				
MMSE: Mini Mental-State Examination; CDR: Clinical Dementia Rating Scale; CDR-SB: Clinical Dementia Rating Scale Sum of Boxes; PACC: Preclinical Alzheimer Cognitive Composite; WMH volume: white matter hyperintensities volume; Centiloid: measure of global amyloid disposition based on conversion of PIB or AV45 PET SUVRs to a standardized scale; AD cortical signature thickness: cortical thickness in signature regions affected in Alzheimer disease (see Dincer et al. 2020); Ln ICA Calcium score/volume: natural log transformation of internal carotid artery Agatston calcium score/volume; <i>OR(95%CI)</i> : odds ratio and 95% confidence interval				

3.1.3 Severity of white matter disease and increased age predict ICA calcification at baseline

Increased WMH volume and age as well as decreased AD cortical signature thickness were associated with Ln-transformed ICA calcium score and volume using univariable linear regression models (Table 4). In the multivariable regression model, age and WMH volume were able to independently predict the Ln-transformed ICA calcium score and age was the only significant variable that predicted Ln-transformed ICA calcium volume (Table 4). Overall, the multivariable linear regression models were able to predict 16% and 18% of the variance in Ln-transformed ICA calcium score and Ln-transformed ICA calcium volume, respectively (Table 4).

Table 4

Linear regression model to explore baseline variables with statistically significant associations with baseline Ln-transformed ICA calcium score and volume

	Ln ICA calcium score				
	Univariable		Multivariable		
	<i>p</i> -value**	Coefficient(SE)	<i>p</i> -value	Coefficient(SE)***	
Age (year)	< 0.001	0.104(0.018)	0.003	0.078(0.026)	
MMSE	0.634	-0.033(0.07)	-	-	
CDR-SB	0.199	0.126(0.098)	-	-	
PACC	0.809	0.072(0.296)	-	-	
WMH volume (1000 mm ³)*	< 0.001	0.029(0.007)	0.039	0.017(0.008)	
Total hippocampal volume (100 mm ³)*	0.190	-0.018(0.014)	-	-	
AD cortical signature thickness (mm)	< 0.001	-3.12(0.871)	-	-	
Centiloid (5 units)*	0.365	-0.016(0.018)	-	-	
	Ln ICA calcium volume				
	Univariable		Multivariable		
	<i>p</i> -value**	Coefficient(SE)	<i>p</i> -value	Coefficient(SE)***	
Age (year)	< 0.001	0.108(0.017)	< 0.001	0.88(0.024)	
MMSE score	0.762	-0.02(0.067)	-	-	
CDR-SB	0.395	0.08(0.094)	-	-	
PACC score	0.557	0.164(0.279)	-	-	
WMH volume (1000 mm ³)*	< 0.001	0.027(0.007)	0.093	0.013(0.008)	
Total hippocampal volume (100 mm ³)*	0.190	-0.017(0.013)	-	-	
AD cortical signature thickness (mm)	< 0.001	-2.9(0.832)	-	-	

MMSE: Mini Mental-State Examination; CDR: Clinical Dementia Rating Scale; CDR-SB: Clinical Dementia Rating Scale Sum of Boxes; PACC: Preclinical Alzheimer Cognitive Composite; WMH volume: white matter hyperintensities volume; Centiloid: measure of global amyloid disposition based on conversion of PIB or AV45 PET SUVRs to a standardized scale; AD cortical signature thickness: cortical thickness in signature regions affected in Alzheimer disease (see Dincer et al. 2020). Ln ICA Calcium score/volume: natural log transformation of internal carotid artery Agatston calcium score/volume; SE: standard error of beta coefficient.

Ln ICA calcium score

Centiloid (5 units)*	0.398	-0.002(0.003)	-	-
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* coefficients are demonstrated for 1000 mm³ increment in WMH volume, 100 mm³ increment in total hippocampal volume, and 5 unit increment in Centiloid scale

** variables in the univariable model with p-value < 0.3 were selected for the multivariate model. Significance level was set to 0.1 for the final model.

*** beta coefficients adjusted for the effect of other variables in the model

MMSE: Mini Mental-State Examination; CDR: Clinical Dementia Rating Scale; CDR-SB: Clinical Dementia Rating Scale Sum of Boxes; PACC: Preclinical Alzheimer Cognitive Composite; WMH volume: white matter hyperintensities volume; Centiloid: measure of global amyloid disposition based on conversion of PIB or AV45 PET SUVRs to a standardized scale; AD cortical signature thickness: cortical thickness in signature regions affected in Alzheimer disease (see Dincer et al. 2020). Ln ICA Calcium score/volume: natural log transformation of internal carotid artery Agatston calcium score/volume; SE: standard error of beta coefficient.

3.2 Longitudinal Analyses

3.2.1 ICA calcification is not predictive of longitudinal changes in cognition, WMH volume, or AD imaging biomarkers

Table 5 summarizes the number of patients and sessions used in each set of longitudinal biomarkers analyses. Participants with and without ICA calcification at baseline were not different in their estimated annual rate of change in MMSE, CDR-SB, or PACC score, WMH volume, total hippocampal volume, AD cortical signature thickness, or Centiloid values (Fig. 2 and Table 6). On the other hand, higher WMH volume and Centiloid values as well as lower baseline hippocampal volume and AD cortical signature thickness at the baseline were associated with steeper annual decline in the MMSE score (Table 7).

Table 5
Participants and session characteristics of longitudinal follow up visits

Visit type	Variables involved	Total included visits (n)	Number of participants (n)	Between visit gap, month (mean \pm SD)	Number of visits (median(lower-upper quartile))
Clinical	CDR-SB/MMSE	1316/1257	231	14.5 \pm 6.7	3 (2–5)
Psychometric	PACC score	1008	229	14.4 \pm 6.6	3 (2–5)
MR session	AD cortical signature thickness/Total Hippocampal volume	341/341	118	25.5 \pm 18.3	2 (1–3)
WMH session	WMH volume	214	83	30.6 \pm 16.8	2 (1–2)
PIB/AV45 PET session	Centiloid	102	46	40.8 \pm 21.3	2 (1–2)
<p><i>MMSE: Mini Mental-State Examination; CDR: Clinical Dementia Rating Scale; CDR-SB: Clinical Dementia Rating Scale Sum of Boxes; PACC: Preclinical Alzheimer Cognitive Composite; WMH: white matter hyperintensities; PIB: Pittsburgh compound B; AV45: 18F-AV45 (florbetapir); Centiloid: measure of global amyloid disposition based on conversion of PIB or AV45 PET total cortical standardized uptake ratios to a standardized scale with arbitrary units ranging from 0 to 100; Total Hipp: total hippocampal volume; AD cortical signature thickness: cortical thickness in signature regions affected in Alzheimer disease; Total Hipp: total hippocampal volume</i></p>					

Table 6

Linear regression model to explore the association of estimated annual rate of change of cognitive and imaging biomarkers based on baseline ICA calcium score and volume

Ln ICA calcium score as independent variable		
<i>Estimated annual rate of change in:</i>	<i>p-value</i>	<i>Coefficient(SE)</i>
MMSE score	0.413	-0.016(0.019)
CDR-SB score	0.645	0.008(0.018)
PACC score	0.9	1.65e-4(0.001)
WMH volume (mm ³)	0.197	154.2(118.5)
Total hippocampal volume (mm ³)	0.957	0.161(2.9)
AD cortical signature thickness (mm)	0.154	-4.39e-4(3.41e-4)
Centiloid	0.927	0.01(0.106)
Ln ICA calcium volume as independent variable		
<i>Estimated annual rate of change in:</i>	<i>p-value</i>	<i>Coefficient(SE)</i>
MMSE score	0.485	-0.015(0.02)
CDR-SB score	0.715	0.007(0.018)
PACC score	0.755	4.16e-4(1.327e-3)
WMH volume (mm ³)	0.199	0.153(0.118)
Total hippocampal volume (mm ³)	0.745	0.962(2.94)
AD cortical signature thickness (mm)	0.200	-4.39e-4(3.41e-4)
Centiloid	0.845	0.004(0.021)
<p><i>MMSE: Mini Mental-State Examination; CDR: Clinical Dementia Rating Scale; CDR-SB: Clinical Dementia Rating Scale Sum of Boxes; PACC: Preclinical Alzheimer Cognitive Composite; WMH volume: white matter hyperintensities volume; Centiloid: measure of global amyloid disposition based on conversion of PIB or AV45 PET SUVRs to a standardized scale; AD cortical signature thickness: cortical thickness in signature regions affected in Alzheimer disease (see Dincer et al. 2020). Ln ICA Calcium score/volume: natural log transformation of internal carotid artery Agatston calcium score/volume; SE: standard error of beta coefficient.</i></p>		

Table 7

Linear regression model to explore the association of baseline imaging biomarkers on dementia with annual rates of change in cognitive scores

Estimated annual rate of change in MMSE score		
<i>Baseline imaging biomarkers of dementia</i>	<i>p-value</i>	<i>Coefficient(SE)</i>
WMH volume (1000 mm ³) *	< 0.001	-0.011(0.002)
Total hippocampal volume (100 mm ³) *	< 0.001	0.036(0.004)
AD cortical signature thickness (mm)	< 0.001	2.36(0.257)
Centiloid (5 unit) *	< 0.001	-0.040(0.004)
Estimated annual rate of change in PACC score		
<i>Baseline imaging biomarkers of dementia</i>	<i>p-value</i>	<i>Coefficient(SE)</i>
WMH volume (1000 mm ³) *	0.157	-2.66e-4(1.87e-4)
Total hippocampal volume (100 mm ³) *	< 0.001	0.002(0.0003)
AD cortical signature thickness (mm)	< 0.001	0.099(0.021)
Centiloid (5 unit) *	< 0.001	-1.969e-3(3.45e-4)
* coefficients are demonstrated for 1000 mm ³ increment in WMH volume, 100 mm ³ increment in total hippocampal volume, and 5 unit increment in Centiloid scale		
MMSE: Mini Mental-State Examination; CDR: Clinical Dementia Rating Scale; PACC: Preclinical Alzheimer Cognitive Composite; WMH volume: white matter hyperintensities volume; Centiloid: measure of global amyloid disposition based on conversion of PIB or AV45 PET SUVRs to a standardized scale; AD cortical signature thickness: cortical thickness in signature regions affected in Alzheimer disease (see Dincer et al. 2020); SE: standard error of beta coefficient.		

3.2.2 Effect of baseline ICA calcification on cognitive decline is fully mediated by white matter disease

Consistent with previous analyses, there was no significant main effect for baseline Ln-transformed ICA calcium score or volume on longitudinal MMSE scores. However, mediation analyses revealed an indirect effect of baseline Ln-transformed ICA calcium score or volume on longitudinal MMSE, mediated solely by baseline WMH volume and none of the other investigated imaging biomarkers. As Fig. 3 illustrates, the regression model between baseline Ln-transformed ICA calcium score and WMH volume as well as the regression model between baseline WMH volume and estimated annual rate of change in MMSE were significant. The indirect effect of one unit increment in Ln-transformed ICA calcium score on the estimated annual rate of change in MMSE was therefore estimated to be -0.035 (95%CI: [-0.06; -0.02], *p*-value < 0.001). Similarly, the indirect effect of one unit increase in Ln-transformed ICA calcium volume on

the estimated annual rate of change in MMSE was estimated as -0.034 (95%CI:[-0.05; -0.01], p -value < 0.001).

4. Discussion

We conducted a retrospective cohort study on a cohort of 230 adults from the Knight ADRC cohort to investigate the relationship between intracranial ICA calcification and cognitive outcomes, as well as imaging biomarkers of AD and small vessel disease. We demonstrated: 1) an independent association between increased age, white matter hyperintensities, and male sex with the odds and severity of ICA calcification, 2) no significant difference in the odds of conversion, longitudinal cognitive scores, WMH volume, hippocampal volume, AD cortical signature thickness, or amyloid burden between participants with and without ICA calcification in the baseline, 3) a significant indirect effect for baseline ICA calcification on longitudinal MMSE score which was purely mediated via baseline WMH volume, and 4) significant association between higher WMH volume and amyloid burden as well as lower hippocampal volume and AD cortical signature thickness in predicting lower MMSE scores in longitudinal follow-ups.

Results obtained through analysis of participants' baseline status were in line with previous literature indicating a direct relationship between age and severity of intracranial ICA calcification (2, 3). We also identified an association between the volume of WMH and ICA calcium scores and volume, independent from participants age, a result previously reported in the literature for cavernous carotid and carotid siphon (5, 6, 8, 26, 27). Interestingly, although ICA calcification was not associated with increases in WMH volume during follow-up visits, it mediated longitudinal cognition through its association with baseline WMH volume. As WMH are primarily an expression of small vessel disease and ICA calcification is a proxy of large vessel atherosclerosis (28), our results indicate that while both findings reflect atherosclerosis –hence correlated in severity when looked in a cross-sectional manner- they represent different clinical trajectories of atherosclerosis in different intracranial vascular beds.

We identified no direct association between the presence or severity of ICA calcification and baseline or longitudinal cognitive scores (MMSE, CDR-SB, and PACC) nor did we find any association with the risk of conversion to CDR above 0. This was in agreement with findings reported by Bos et al. from the Rotterdam study where intracranial ICA calcification was unable to predict the risk of AD in a large population-based cohort (12). Here we show in a more rigorous manner and using a longitudinally-followed cohort that intracranial ICA calcification was unable to predict the risk of AD even in the setting of preclinical AD. In contrast, two cross-sectional studies have identified a negative association between ICA calcification with scores in different cognitive domains (10, 11). Nonetheless, these studies did not include the presence of concomitant white matter lesions as a confounding factor that is related to both cognition and ICA calcification. Further longitudinal studies are needed to understand the potential effect of ICA calcification on individual cognitive domains rather than global cognitive measurements such as MMSE, CDR and PACC scores that were considered used in the current study.

In vivo modeling of ICA calcification can be achieved through direct application of calcium chloride to arterial intima in rodents (29). Rodents with ICA calcification demonstrate increased pulse pressure in distal, medium-sized arteries and a resulting impaired blood flow regulation in response to neuronal activity (13). This would lead to increased blood-brain barrier permeability, amyloid deposition, and oxidative stress in the hippocampus, followed by increased cortical and hippocampal gliosis, culminating in neurodegeneration and memory impairment in these animals (13, 29, 30). Similar to rodents with ICA calcification, Kang et al. identified an inverse relationship between the number of stenotic intracranial arteries and hippocampal volume, but no association with total cortical amyloid burden or AD cortical signature thickness (31). Similarly, post-mortem AD brain assessments have shown associations between amyloid pathology and reduced hippocampal volume yet no such association is demonstrated between *in vivo* measurement of atherosclerosis and amyloid burden in patients with AD (32, 33). When viewed in the context of animal model literature, our results present further evidence in human studies against the utility of ICA calcification in predicting in vivo AD imaging biomarkers in the setting preclinical AD.

LIMITATIONS

Calcium scoring in this project was performed using manual segmentation of ROI on axial head CT scans. However, the proximity of intracranial ICA to the petrous bone and bony skull base including the clinoid process limited our ability to delineate a ROI around calcifications without including bone. Nonetheless the raters achieved a high two-way agreement, and any discordant case was blindly scored a second time and, when necessary, was sent to a skilled neuroradiologist for adjudication.

Importantly, the sample size pool for our longitudinal data points was different among different biomarkers categories (Table 5), with the lowest number of data points and participants in the longitudinal Centiloid measures. We addressed this issue using linear mixed models to estimate the annual rates of longitudinal outcomes, a method known to be relatively unbiased in the presence of missing data (34).

Although ICA calcium score or volume did not show any predictive value for general cognitive outcomes in our longitudinal follow-up, cross-sectional data from the Rotterdam Study show evidence of an association between intracranial ICA calcification and worse performance in executive function, information processing speed, and motor speed domains (35). These are among domains commonly associated with WM disease in elderly population (36). It is therefore imperative for future longitudinal studies to investigate the association of ICA calcification with changes in AD-specific cognitive domains. Last but not least, despite ongoing efforts in the Knight ADRC to enroll a more diverse and representative cohort of older adults our study participants were primarily Caucasian.

Conclusions

ICA calcification is frequently identified as an incidental finding on head CT scan and physicians are often uncertain about its prognostic and diagnostic value of such finding. Together our results reveal that

intracranial ICA calcification is unable to predict the onset of dementia in cognitively normal adults, nor it is able to predict longitudinal changes in cognitive scores, imaging makers of small vessel disease such as WMH volume, or AD imaging biomarkers. However, an indirect effect of ICA calcification on longitudinal cognition was purely mediated by its effect on white matter disease at baseline. Therefore, WMH volume does impact longitudinal decline and may therefore be an important incidental finding to report.

Declarations

Ethics approval and consent to participate

The institutional ethics review board of Washington University School of Medicine in Saint Louis approved the protocol of this study. All participants consented to participate in the study and provided their informed consent to participate in the study. The study was in compliance with HIPAA and was performed in accordance with the Declaration of Helsinki.

Consent for publication

Not applicable

Availability of data and materials

De-identified participant data including clinical and cognitive assesment, CSF biomarkers and dMRI images are available upon request through the Knight ADRC Leadership Committee (<https://knightadrc.wustl.edu/research/resourcerequest.htm>).

Competing interests

Dr. Benzinger has investigator-initiated research funding from the NIH, the Alzheimer's Association, the Barnes-Jewish Hospital Foundation and Avid Radiopharmaceuticals (a wholly-owned subsidiary of Eli Lilly). Dr. Benzinger participates as a site investigator in clinical trials sponsored by Avid Radiopharmaceuticals, Eli Lilly, Biogen, Eisai, Jansen, and Roche, an unpaid consultant to Eisai and Siemens, and is on an Advisory Board and Speaker's Bureau for Biogen.

Funding

This research was supported by the following: ADRC Center Grant, NIH/NIA [P50AG005681](#), [The Barnes-Jewish Hospital Foundation \(BJHF\)](#), Barnes-Jewish Hospital Foundation (PIB imaging), NIH/NIA P01AG026276, Generous support from Fred Simmons and Olga Mohan, The McDonnell Center for Systems Neuroscience, 22 3922 26239N, NIH/NIA P01AG003991, Avid Radiopharmaceuticals, a wholly owned subsidiary of Eli Lilly (florbetapir and AV1451 imaging). For Florbetapir (AV45), Avid pharmaceuticals provided the doses and partial support for scanning through an investigator-initiated research grant awarded to Washington University (J.C. Morris, T.L.S. Benzinger). Additional support for

image acquisition was provided by CCIR/ICTS Human Imaging Unit (NIH/NCATS UL1TR000448) and support for imaging informatics (CNDA/XNAT) was provided in part by the Neuroimaging Informatics and Analysis Center (1P30NS098577) and R01 EB009352.

Authors' contributions

The study was conceived by FR, MN, and TLSB. HLPO, TLSB, and JCM were responsible for funding and data acquisition. Analysis design was conceived, conducted and interpreted by FR, CDC, NM, and AD with direct supervision from GC and JL. FR and MN contributed to the composition of the manuscript, and all authors contributed to the manuscript preparation and provided substantial revision. All authors have approved the submitted version and have agreed both to be personally accountable for the author's own contributions and to ensure that questions related to the accuracy or integrity of any part of the work are appropriately investigated, resolved, and the resolution documented in the literature.

Acknowledgements

Not applicable

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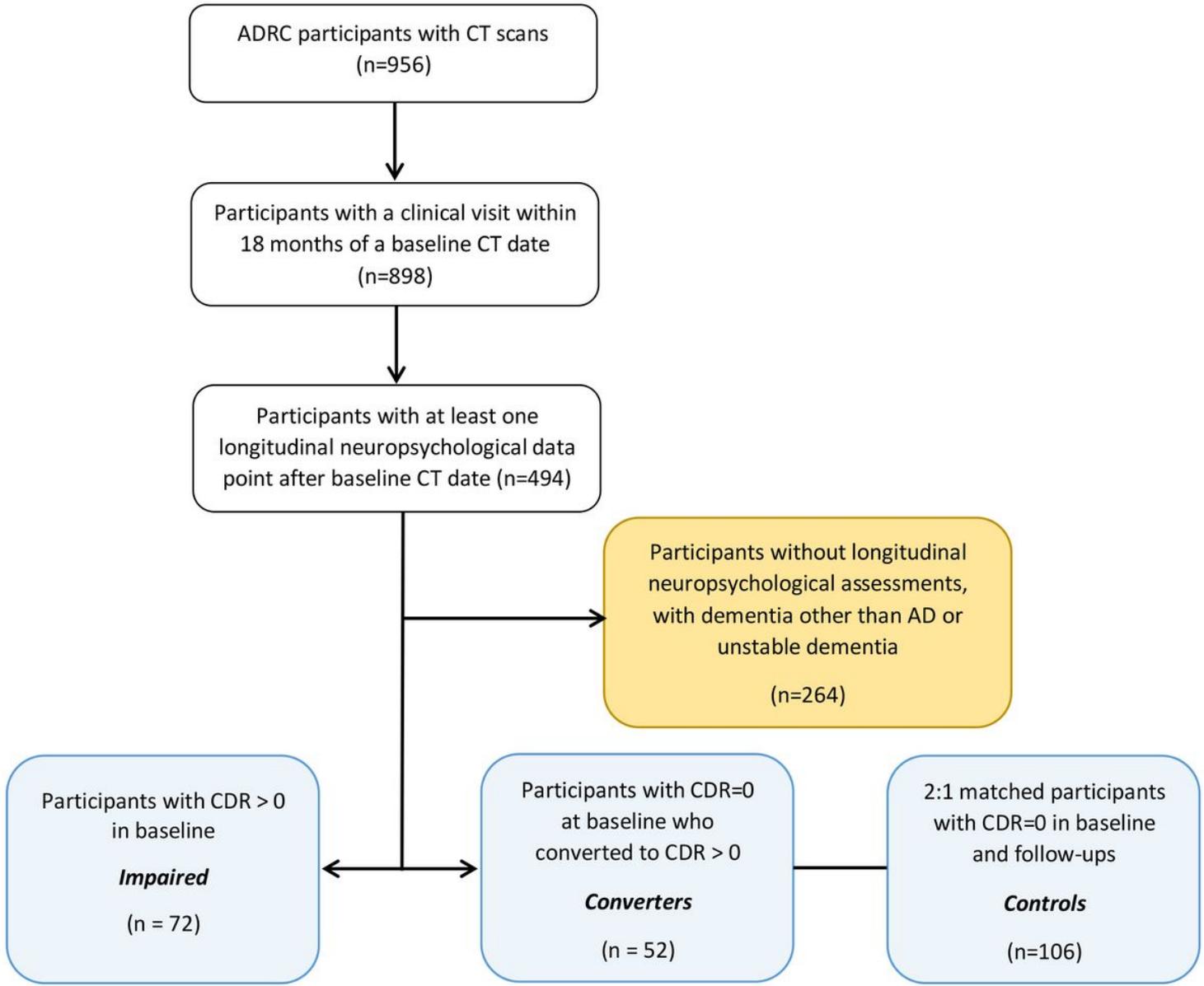
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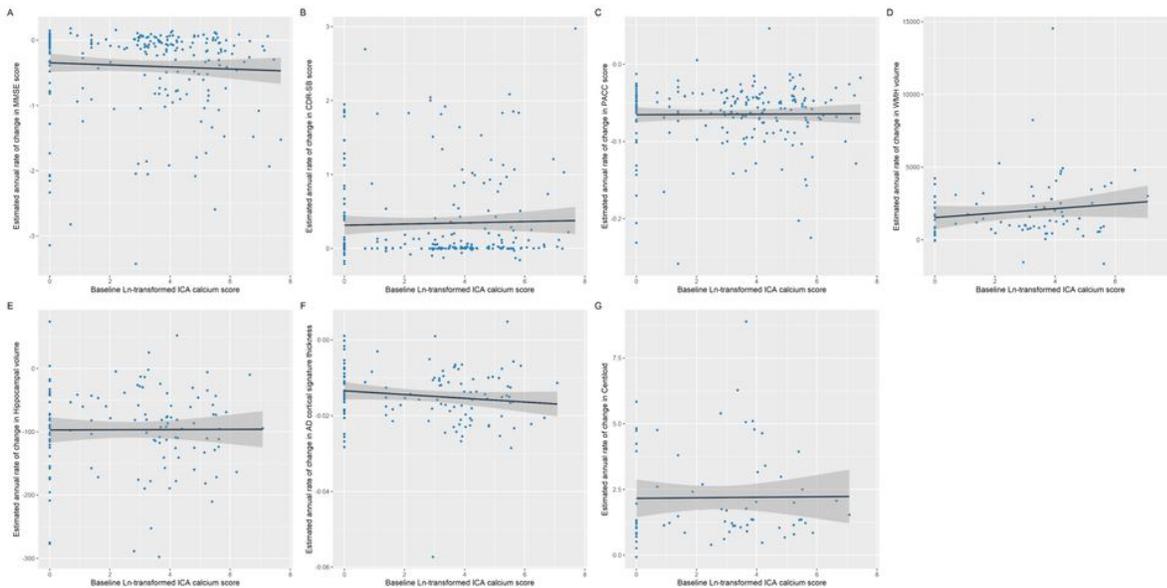
Figures



ADRC: Alzheimer disease research center; CDR: clinical dementia rating scale

Figure 1

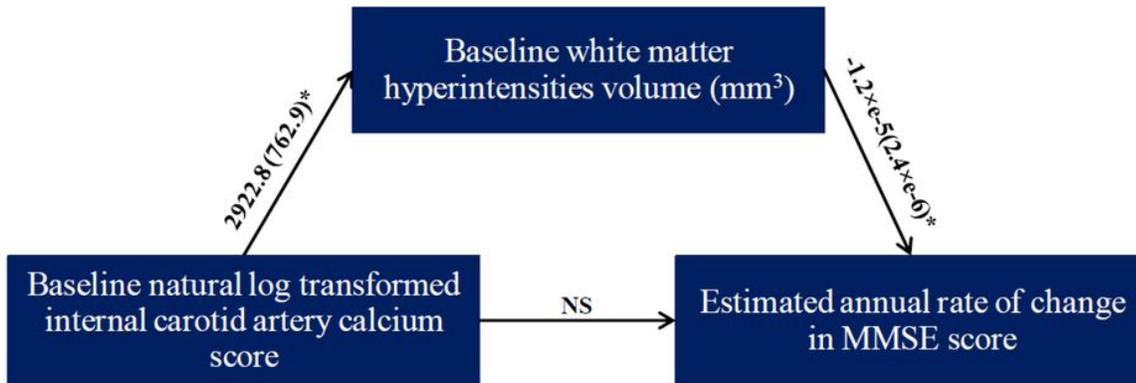
Inclusion criteria for the study population and reasons for exclusion



None of the models reached statistical significance. For p-values see text. ICA: internal carotid artery; MMSE: mini-mental state examination; CDR-SB: CDR Sum of Boxes score; PACC: pre-clinical Alzheimer disease cognitive composite; WMH: white-matter hyperintensities; AD: Alzheimer disease; Centiloid: measure of global amyloid disposition based on conversion of PIB or AV45 PET SUVRs to a standardized scale

Figure 2

Association of the presence of internal carotid artery calcification in baseline with longitudinal cognitive scores and AD imaging biomarkers



Numbers represent the regression coefficient for each model fit. P-value for both significant models was estimated <0.001. MMSE: mini-mental state examination; NS: non-significant

Figure 3

Mediation model between Ln-transformed ICA calcium score and estimated annual rate of change in the MMSE score