SUPPLEMENTAL MATERIAL

Title: Coronary artery calcium assessed years before was positively associated with subtle white-matter injury of the brain in asymptomatic middle-aged men: the Framingham Heart Study

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Number of supplemental materials: 1 method and 3 tables

Supplemental Methods. Diffusion tensor imaging and measures

Supplemental Table I. Average value of peak width of skeletonized mean diffusivity (PSMD, mm^2sec^{-1} x 10^{-4}) from 6 cohort study in middle aged people

Supplemental Table II. Sensitivity analysis without total cranial volume in Model2: the association between CAC and subtle white-matter injury: Framingham Heart Study third-generation cohort (N = 1052)

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Supplemental Methods

MRI setting

DTI was acquired with a 1.5T Siemens Avanto scanner (version syngo MR B15) and was performed using the following parameters: repetition time = 3600 ms, echo time = 94 ms, 25 slices total, field of view = 25 cm, acquisition matrix = 128 × 128, slice thickness = 5 mm with 5 mm gap. Diffusion weighted images were generated using four time repetitions of 30 gradients directions with total gradient diffusion sensitivity of b = 1000 s/mm^2, and one image with b = 0 s/mm^2.
DTI dataset were preprocessed using FSL software tools[45] including correction for eddy current-induced distortions and participant head movements. The brain was masked using BET tool and fractional anisotropy(FA), mean diffusivity(MD), axial diffusivity(AD) and radial diffusivity(RD) maps generated using DTIFIT (FMRIB software library; http://fsl.fmrib.ox.ac.uk/fsl/fslwiki).

Peak Width of Skeletonized Mean Diffusivity

Automatic calculation of PSMD followed the procedure described by Baykara et al[22]. using the freely available script they provided (http://www.psmd-marker.com). Briefly, the DT-MRI data were processed using the standard Tract-based Spatial Statistics (TBSS)[46] pipeline available in FSL with histogram analysis performed on the resulting white matter MD skeletons. First, all participants' FA volumes were linearly and non-linearly registered to the standard space FMRIB FSL 1-mm FA template. Second, a white matter skeleton was created from the mean of all registered FA volumes. This was achieved by searching for maximum FA values in directions perpendicular to the local tract direction in the mean FA volume. An FA threshold of 0.2 was applied to the mean FA skeleton to exclude predominantly non-white matter voxels. Third, MD volumes were projected onto the mean FA skeleton and further thresholded at an FA value of 0.3 to reduce CSF partial volume contamination using the skeleton mask provided by Baykara et al[22]. Finally, PSMD was calculated as the difference between the 95th and 5th percentiles of the voxel-based MD values within each subject's MD skeleton.

FA and Free Water

The model considers two co-existing compartments per voxel: one compartment is a free-water compartment, which models isotropic diffusion with a diffusion coefficient of water at body temperature fixed to 3×10^{-3} mm^2/s. Due to the fast diffusivity and short diffusion time, free-water molecules are expected only at large enough extracellular spaces. The second compartment is the tissue compartment, which accounts for all other molecules, i.e., all intra and extracellular molecules that are hindered or restricted by tissue membranes[47]. The tissue compartment is modeled by a diffusion tensor. The method estimates this tensor, as well as the fractional volume of the free-water compartment in each voxel, resulting in the FW map. For each individual, FW and FW-corrected FA were computed. Individual FA maps obtained from DTIFIT were linearly and non-linearly registered to the standard FSL FA template space (FMRIB 1-mm FA template) using linear and nonlinear transformations. Resulting transformation parameters were applied to individuals FW and FW-corrected FA maps. A white matter mask (WM) was defined by thresholded the FSL FA template at a value of 0.3 to reduce CSF partial volume contamination. For each individual, overall measure of mean FW was computed by superimposing the WM mask onto the respective individual coregistered FW (resp. FW-corrected FA) maps and averaging FW (resp. FW-corrected FA) values within these WM voxels.

Interpretation of DTI measures

FA is a measure of anisotropic water diffusion and reflects the degree of directionality of cellular structures within the fiber tracts and therefore their structural integrity. The decrease in anisotropy could be attributed to axon losses and demyelination leading to loss of white matter integrity from neurodegenerative processes or microvascular pathology. In the pathology of axonal injury and myelin degradation, some studies showed small axonal injury occurs before myelin degradation using DTI measures in animal model[48] and in human research[34]. Axonal injury reduced parallel diffusivity, water directions along with nerve (most components of FA) and myelin degradation increases in perpendicular diffusivity. Axonal injury occurred within 1 week but myelin degradation occurred after several months in the study.

Recently, a method to correct DTI data for extracellular water contamination has been proposed[46]. Resulting “extracted” excessive water, referred to as free water (FW), is modeled by a tensor that is isotropic and reflects the amount of water molecules that are not restricted by their surroundings. FW is independent to water directionality, as

opposed to FA. FW content constitutes a strong biomarker of subtle cerebral injury in association with vascular risk factors in relatively healthy adult individuals[38].

PSMD (peak width of skeletonized mean diffusivity) was developed for a new imaging marker for disease burden in small vessel disease that can be used in clinical routine and readily applied to large samples. PSMD was derived from the removal of prominent cerebrospinal fluid (CSF) signal from MD images. using combined 2 processing techniques for DTI data: skeletonization and histogram analysis. Skeletonization focuses the analysis of MD on the main fiber tracts, thereby largely eliminating CSF contamination. Whole brain histogram analysis is particularly appropriate when dealing with diffuse diseases and when quantifying total disease burden[22]. Previous study showed increases in PSMD were linked to vascular but not to neurodegenerative disease. In longitudinal analysis, PSMD captured SVD progression better than other imaging markers[22].

Supplemental Table I. Average value of peak width of skeletonized mean diffusivity (PSMD, mm^2sec^{-1} x 10^{-4}) from 6 cohort study in middle aged people[49]

Age range	BIL&GIN	SYS	LIFE	1000 BRAINS	UKBiobank	ASPSF
38 to 48	2.24	2.68	2.17	2.49	2.10	2.63
48 to 58		2.82	2.31	2.67	2.12	2.69

BIL&GIN:Brain Imaging of Lateralization study at Groupe d'Imagerie Neurofonctionnelle; SYS cohort: Saguenay Youth Study; ASPSF: Austrian Stroke Prevention Study Family

Supplemental Table II. Sensitivity analysis without total cranial volume in Model 2: the association between CAC and subtle white-matter injury: Framingham Heart Study third-generation cohort (N = 1052)

Method	Group	N	Mean FA				lnFW				lnPSMD			
			Estimate	95%CI	P-value	P for trend	Estimate	95%CI	P-value	P for trend	Estimate	95%CI	P-value	P for trend
	CAC = 0	753	Reference			0.04	Reference			0.09	Reference			0.13
	0 < CAC ≤100	236	-0.117 (-0.263, 0.029)		0.12		0.070 (-0.077, 0.217)		0.35		0.012 (-0.134, 0.158)		0.87	
	CAC >100	63	-0.212 (-0.464, 0.041)		0.10		0.217 (-0.037, 0.471)		0.09		0.263 (0.010, 0.515)		0.04	

Abbreviations: CAC, coronary artery calcium; CFPWV, carotid-femoral pulse wave velocity; FA, fractional anisotropy; FW, free water; PSMD, peak width of skeletonized mean diffusivity; SD, standard error.

In the models, we treated CAC score and CFPWV as continuous and transformed each variable as follows owing to their highly skewed distributions: natural log transformed CAC score +0.1 (lnCAC), and negative inverse CFPWV (niCFPWV). Mean FW and PSMD were log-transformed since they have skewed distributions, which represent lnFW and lnPSMD respectively. Model was adjusted for sex, age at CT/tonometry, age at CT/tonometry squared, and time between at CT/tonometry and MRI, current smoker (yes/no), SBP (mmHg), diabetes (yes/no), medications for hypertension (yes/no), dyslipidemia (yes/no) and total cholesterol (mg/dL).

Supplemental Table III. The association between continuous CAC/CFPWV and subtle white-matter injury stratified by sex: Framingham Heart Study third-generation cohort (N = 1052)

Methods		Mean FA				lnFW			
		Estimate	95%CI	P value	P value for sex interaction	Estimate	95%CI	P value	P value for sex interaction
20^{th}-80^{th} percentile of lnCAC									
Model 1					0.020				0.285
	women	0.011	(-0.189, 0.210)	0.92		—	—	—	
	men	-0.162	(-0.278, -0.046)	0.01		—	—	—	
Model 2					0.043				0.326
	women	-0.011	(-0.210, 0.188)	0.91		—	—	—	
	men	-0.126	(-0.241, -0.011)	0.03		—	—	—	
20^{th}-80^{th} percentile of niCFPWV									
Model 1					0.045				< 0.05
	women	0.037	(-0.122, 0.195)	0.65		0.088	(-0.075, 0.251)	0.29	
	men	-0.063	(-0.224, 0.098)	0.44		0.215	(0.056, 0.375)	< 0.01	
Model 2					0.053				0.084
	women	0.006	(-0.169, 0.181)	0.95		0.033	(-0.152, 0.219)	0.73	
	men	-0.027	(-0.201, 0.147)	0.76		0.194	(0.020, 0.369)	0.03	

Abbreviations: CAC, coronary artery calcium; CFPWV, carotid-femoral pulse wave velocity; FA, fractional anisotropy; FW, free water; PSMD, peak width of skeletonized mean diffusivity.

In the models, we treated CAC score and CFPWV as continuous and transformed each variable as follows owing to their highly skewed distributions: natural log transformed CAC score +0.1 (lnCAC), and negative inverse CFPWV (niCFPWV). Mean FW and PSMD were log-transformed since they have skewed distributions, which represent lnFW and lnPSMD respectively.

* Each 20^{th} to 80^{th} percentile range, 20^{th}–80^{th} percentile of lnCAC = 4.02, 20^{th}–80^{th} percentile of niCFPWV = 41.08

Model 1 was adjusted for sex, age at CT/tonometry, age at CT/tonometry squared, and time between at CT/tonometry and MRI.

Model 2 was further adjusted for current smoker (yes/no), SBP (mmHg), diabetes (yes/no), total cholesterol (mg/dL), medications for hypertension (yes/no), dyslipidemia (yes/no) and total cranial volume (mL)."

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