Axonal injury partially mediates associations between increased left ventricular mass index and white matter damage

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Abstract

Background and purpose: Left ventricular (LV) mass index is a marker of subclinical LV remodeling that relates to white matter damage in aging, but molecular pathways underlying this association are unknown. This study assessed if LV mass index related to cerebrospinal fluid (CSF) biomarkers of microglial activation (sTREM2 [soluble triggering receptor expressed on myeloid cells 2]), axonal injury (NFL [neurofilament light]), neurodegeneration (total-tau), and amyloid-β, and whether these biomarkers partially accounted for associations between increased LV mass index and white matter damage. We hypothesized higher LV mass index would relate to greater CSF biomarker levels, and these pathologies would partially mediate associations with cerebral white matter microstructure.

Methods: Vanderbilt Memory and Aging Project participants who underwent cardiac magnetic resonance, lumbar puncture, and diffusion tensor imaging (n=142, 72±6 years, 37% mild cognitive impairment [MCI], 32% APOE-ε4 positive, LV mass index 51.4±8.1 g/m2, NFL 1070±588 pg/mL) were included. Linear regressions and voxel-wise analyses related LV mass index to each biomarker and diffusion tensor imaging metrics, respectively. Follow-up models assessed interactions with MCI and APOE-ε4. In models where LV mass index significantly related to a biomarker and white matter microstructure, we assessed if the biomarker mediated white matter associations.

Results: Among all participants, LV mass index was unrelated to CSF biomarkers (P>0.33). LV mass index interacted with MCI (P=0.01), such that higher LV mass index

related to increased NFL among MCI participants. Associations were also present among APOE-ε4 carriers (P=0.02). NFL partially mediated up to 13% of the effect of increased LV mass index on white matter damage.

Conclusions: Subclinical cardiovascular remodeling, measured as an increase in LV mass index, is associated with neuroaxonal degeneration among individuals with MCI and APOE-ε4. Neuroaxonal degeneration partially reflects associations between higher LV mass index and white matter damage. Findings highlight neuroaxonal degeneration, rather than amyloidosis or microglia, may be more relevant in pathways between structural cardiovascular remodeling and white matter damage.

1. Introduction

Left ventricular (LV) hypertrophy (LVH) is a pathologic increase in the mass of the left ventricle that is associated with cognitive decline[1] and structural brain changes[2] among aging adults. Prior to overt LVH, increased LV mass index (LV mass/body surface area) is an imaging biomarker reflecting subclinical remodeling of the ventricular wall.[3] Increased LV mass index is associated with cognitive decline,[4] white matter macrostructure damage,[5,6] and white matter microstructure damage,[7] even in the absence of LVH.[7] However, the mechanisms underlying associations between increased LV mass and white matter changes remain unknown.

Multiple pathways to white matter injury could account for the associations between LV mass index and white matter damage. First, increased LV mass index is associated with cerebral small vessel disease,[2,8] an increased risk of stroke,[9] and hypertension.[10] These pathologies contribute to a neuroinflammatory state,[11] which is a common etiology of white matter damage in older adults[12] (e.g., a cerebrospinal fluid (CSF) biomarker of microglial activation, soluble triggering receptor expressed on myeloid cells 2 (sTREM2), is associated with white matter damage[13]). Small vessel disease is also associated with axonal injury,[14] which can be assessed by CSF neurofilament light (NFL),[15] a protein comprising large caliber myelinated axons.[15] Unsurprisingly, CSF NFL concentrations are also associated with white matter damage.[16,17]

In addition to small vessel disease, increased LV mass index has been associated with smaller grey matter volumes.[6] Neurodegeneration can disrupt adjacent white matter via Wallerian degeneration[18] and may be an additional pathway through

which LV mass index affects white matter. Finally, associations between LV mass index and white matter microstructural damage are stronger in those with mild cognitive impairment (MCI),[7] a prodromal stage of Alzheimer's disease (AD), as compared to normal cognition (NC), suggesting that AD pathology may partially account for the association. Plus, amyloid-β (Aβ), a pathological hallmark of AD, has been associated with white matter damage.[16] Thus, neuroinflammation, axonal injury, non-specific neurodegeneration, or Aβ may each represent a pathway through which LV mass index affects white matter microstructure.

The current study first examines the association between LV mass index and CSF markers of neuroinflammation (sTREM2), neuroaxonal injury (NFL), non-specific neurodegeneration (total-tau (t-tau)), and Aβ aggregation, and then assesses whether CSF biomarkers partially mediate our previously reported link between LV mass index and white matter microstructure among older adults.[7] CSF sTREM2,[13] NFL,[16] t-tau,[19] and Aβ[16] are each associated with white matter microstructural damage, so we hypothesized that higher LV mass index would relate to greater CSF evidence of these same pathological processes. We also hypothesized these pathologies would partially mediate associations between LV mass index and white matter microstructure. We tested whether cognitive diagnosis (NC versus MCI) or *APOE-ε4* carrier status, the largest genetic susceptibility risk factor for AD,[20] modified these associations, as associations between higher LV mass index and white matter microstructural damage are most prominent among individuals with MCI[7] and *APOE-ε4* independently damages white matter in aging adults.[21]

2. Materials and Methods

2.1 Study Cohort

The Vanderbilt Memory and Aging Project[22] is a longitudinal observational study investigating vascular health and brain aging. As part of a comprehensive screening, participants were required to be 60 years of age or older and excluded for a cognitive diagnosis other than NC, early MCI,[23] or MCI,[24] MRI contraindication, history of neurological disease (e.g., multiple sclerosis, clinical stroke), heart failure, major psychiatric illness, head injury with loss of consciousness >5 minutes, or a systemic or terminal illness affecting longitudinal participation. At enrollment, participants completed a comprehensive examination, including (but not limited to) fasting blood draw, physical examination, clinical interview, medication review, echocardiogram, cardiac magnetic resonance, multi-modal brain MRI, and an optional lumbar puncture. Participants were excluded from the current study for missing LV mass, covariate, or outcome data. See **Figure 1** for inclusion/exclusion details. The protocol was approved by the Vanderbilt University Medical Center Institutional Review Board. Written informed consent was obtained from participants prior to data collection. Due to consent restrictions in data sharing, only a subset of data is available for purposes of reproducing results or replicating procedures. Data, analytic methods, and study materials can be obtained by contacting the corresponding author.

2.2 CMR

As previously published, CMR was acquired at Vanderbilt University Medical Center using a 1.5T Siemens Avanto system. LV and right ventricular volume and

function were assessed using a breath-hold, electrocardiogram synchronized, cine steady-state free precession sequence with the following parameters: TR=180ms, TE=1.1ms, flip angle=80°, field of view=300-340mm, and 156x192 matrix. Trained analysts blinded to clinical information (JGT, SN) used QMass MR 7.6 Enterprise Solution to define LV endocardial and epicardial contours at end systole (ES) and end diastole (ED). LV ES and ED volumes were calculated using Simpson's rule. LV mass was calculated at ED by summing the myocardial area for each slice, multiplying by slice thickness plus slice gap, and multiplying by 1.05g/mL. LV mass index was defined as LV mass/body surface area.

2.3 Lumbar Puncture & Biochemical Analysis

A subset of participants completed an optional fasting lumbar puncture at enrollment (n=155, **Figure 1**). CSF was collected with polypropylene syringes using a Sprotte 25-gauge spinal needle in an intervertebral lumbar space. Samples were immediately mixed and centrifuged. Supernatants were aliquoted in 0.5 mL polypropylene tubes and stored at 80°C. Samples were analyzed in single batch using an in house immunoassay with electrochemiluminescence detection to determine the levels of sTREM2.[25] Commercially available enzyme-linked immunosorbent assays were used to measure NFL (UmanDiagnostics), t-tau (INNOTEST hTAU) and Aβ (INNOTEST b-AMYLOID$_{(1-42)}$. Board-certified laboratory technicians processed data blinded to clinical information (Palmqvist et al., 2014). Intra-assay coefficients of variation were <10%.[25]

2.4 Brain MRI

Participants were scanned at the Vanderbilt University Institute of Imaging Science on a 3T Philips Achieva system (Best, The Netherlands) using an 8-channel SENSE reception coil array as part of a multi-modal acquisition protocol. DTI data were acquired along 32 diffusion gradient vectors (TR/TE=10000/60ms, spatial resolution=2x2x2mm^3, b-value=1000s/mm^2) and post-processed through an established tract-based spatial statistics (TBSS) pipeline using the Functional Magnetic Resonance Imaging of the Brain (FMRIB) Software Library (FSL) version 4.1.4 (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSL).[26]

As previously published, the diffusion tensor model was fit using FMRIB's Diffusion Toolbox, and fractional anisotropy (FA), mean diffusivity, radial diffusivity, and axial diffusivity values were calculated. All FA images were non-linearly registered, merged into a 4D image, and a mean image was created. The mean image was used to generate a mean skeleton to which a threshold was applied to exclude voxels that did not overlap among ≥80% of participants. For each individual metric, all participant data were merged into one 4D file that was projected onto the original mean FA skeleton.

2.5 Analytical Plan

Covariates have been defined previously[7] and were selected *a priori* for their potential to confound analytical models. Linear regression models with ordinary least square estimates related LV mass index individually to CSF sTREM2, NFL, t-tau, and Aβ concentrations (pg/mL), adjusting for age, sex, education, race/ethnicity, FSRP (excluding points assigned to age and LV hypertrophy (LVH)), cognitive diagnosis, and

APOE-ε4 status. To replicate our previously reported findings[7] in this smaller sample, voxel-wise analyses using general linear models and FSL randomise with 5000 permutations related LV mass index (g/m^2) to FA, mean diffusivity, radial diffusivity, and axial diffusivity, adjusting for identical covariates. Excluding the small subset of participants with early MCI, all models were repeated evaluating an *LV mass x cognitive diagnosis* interaction term followed by stratification by cognitive diagnosis (NC, MCI). Models were repeated evaluating an *LV mass x APOE-ε4 status* interaction term followed by stratification by *APOE*-ε4 status (carrier, non-carrier). To determine if hypertension was driving associations, *LV mass x hypertension* interactions were also tested. Multiple comparison correction was performed using a false discovery rate for non-voxel-wise models and cluster enhancement permutation[27] for voxel-wise models. The threshold for statistical significance was set *a priori* as corrected p-value<0.05, and sensitivity analyses, removing participants with LVH, prevalent cardiovascular disease (CVD), or atrial fibrillation, were performed.

For models in which LV mass index was significantly related to CSF biomarkers and DTI metrics in clusters over 100 voxels, analyses tested whether the CSF biomarker mediated associations between LV mass index and white matter microstructure (see **Figure 2**). To calculate the total effects, data from DTI clusters significantly related to LV mass index were extracted and the average value for each DTI metric in the cluster was calculated for every participant. The total effects were then calculated as linear models relating LV mass index to the average DTI metric value in every cluster, adjusting for age, sex, education, race/ethnicity, FSRP (excluding points assigned to age and LVH), cognitive diagnosis, and *APOE*-ε4 status. The average

direct effects were calculated as linear models relating LV mass index to the average

DTI metric value in every cluster, adjusting for identical covariates and the CSF

biomarker. The average mediation effects were calculated as the total effect minus the

average direct effect, and the proportion mediated effects were calculated as the

average mediation effect divided by the total effect. The proportion mediated was

interpreted as the percent of the outcome mediated by CSF NFL. For all models, the

threshold for statistical significance was set *a priori* as corrected p-value<0.05 and

analyses were conducted in R version 3.5.2 (www.r-project.org).

3. Results

3.1. Participant Characteristics

The sample included 142 participants (72±6 years, 68% male, 92% non-Hispanic White). LV mass index ranged 32.6 to 69.3 g/m^2. CSF sTREM2 ranged 660 to 10084 pg/mL, CSF NFL ranged 268 to 4025 pg/mL, CSF t-tau ranged 107 to 1542 pg/mL, and CSF Aβ ranged 289 to 1195 pg/mL. See **Table 1** for participant characteristics, stratified by NC, early MCI, and MCI.

3.2. LV Mass Index & CSF Biomarkers

Among the entire sample, LV mass index was unrelated to all CSF biomarker concentrations (p-values>0.33). LV mass index interacted with cognitive diagnosis on CSF NFL (p=0.01), such that higher LV mass index was associated with higher CSF NFL concentrations among MCI participants (β=27.1, p=0.04). However, among NC participants, higher LV mass index was counter-intuitively associated with lower CSF NFL concentrations (β=-14.8, p=0.05). LV mass index did not interact with cognitive diagnosis on any other CSF biomarker (p-values>0.11), and analyses stratified by diagnosis were null (p-values>0.24). LV mass index did not interact with *APOE*-ε4 carrier status on any CSF biomarker (p-values>0.08), but stratified models revealed higher LV mass index was associated with higher CSF NFL concentrations only among *APOE*-ε4 carriers (β=25.4, p=0.02). All other analyses stratified by *APOE*-ε4 status were null (p-values>0.20). LV mass index did not interact with hypertension on any CSF biomarker (p-values>0.28, data not shown). In sensitivity analyses excluding participants with prevalent CVD, atrial fibrillation, or LVH, the associations between LV

mass index and CSF NFL in MCI (p=0.12) and NC participants (p=0.07) were slightly

attenuated. All other associations between LV mass index and CSF NFL remained

below the *a priori* threshold of 0.05. See **Table 2** and **Supplemental Table I** for details.

To better understand the counterintuitive findings between LV mass index and

CSF NFL in NC participants, several potential interactions were explored between LV

mass index and age, sex, Aβ, p-tau, and diabetes. All models were null (p-values>0.13).

3.3 LV Mass Index & DTI Metrics

Consistent with our previously reported findings,[7] among all participants, higher

LV mass index related to higher mean diffusivity (corrected p-values<0.049), radial

diffusivity (corrected p=0.018), and axial diffusivity (corrected p-values<0.023),

indicating greater white matter microstructural damage. Effects were strongest primarily

in the anterior corona radiata and inferior frontal gyrus. When excluding participants with

prevalent CVD, atrial fibrillation, or LVH, associations between LV mass index and axial

diffusivity remained (corrected p-value=0.043). See **Supplemental Table II** for details.

LV mass index did not interact with cognitive diagnosis on any DTI metric

(corrected p-values>0.40). In stratified results, LV mass index was not associated with

any DTI metric among NC participants (corrected p-values>0.22). However, among MCI

participants, higher LV mass index was associated with higher mean diffusivity

(corrected p-values<0.05), radial diffusivity (corrected p-values<0.049), and axial

diffusivity (corrected p-values<0.041), indicating greater white matter microstructural

damage. Effects were strongest in the body of the corpus callosum, middle frontal

gyrus, and inferior frontal gyrus. See **Supplemental Table III** for details. When

excluding participant's with prevalent CVD, atrial fibrillation, or LVH, results were attenuated (corrected p-values>0.12), likely due to decreased power.

LV mass index did not interact with *APOE-ε4* carrier status on any DTI metric (corrected p-values>0.19). Among *APOE-ε4* non-carriers, higher LV mass index related to higher axial diffusivity (corrected p=0.035), primarily in the inferior frontal gyrus. Among *APOE-ε4* carriers, higher LV mass index was related to higher mean diffusivity (corrected p-values<0.05) in the splenium of the corpus callosum and inferior frontal gyrus. When excluding participants with prevalent CVD, atrial fibrillation, or LVH, results were attenuated (corrected p-values>0.07). See **Supplemental Table IV** for details. LV mass index did not interact with hypertension on any DTI metric (corrected p-values>0.38, data not shown).

3.4 Mediation Analysis

Based on the linear regression results above, CSF NFL was assessed as a mediator for the LV mass index and DTI associations among both MCI participants and *APOE-ε4* carriers in certain clusters meeting the assumptions of the mediation model. First, among MCI participants, CSF NFL partially mediated associations between LV mass index and mean, radial, and axial diffusivity. Specifically, CSF NFL accounted for 6% of the effect of LV mass index on mean diffusivity in the corpus callosum, 13% of the effect of LV mass index on radial diffusivity in the anterior corona radiata, and 9% of the effect of LV mass index on axial diffusivity in the inferior fronto-occipital fasciculus. Among *APOE-ε4* carriers, CSF NFL partially mediated associations between LV mass index and mean diffusivity, such that CSF NFL accounted for 13% of the effect of LV

mass index on mean diffusivity in the anterior corona radiata. See **Table 3** for detailed

mediation results.

4. Discussion

Among our community-dwelling cohort of older adults free of clinical stroke, higher LV mass index was associated with greater *in vivo* molecular evidence of neuroaxonal injury (NFL) among individuals with MCI and among *APOE-ε4* carriers. However, among NC participants, higher LV mass index was counter-intuitively associated with less molecular evidence of neuroaxonal injury. Notably, all associations were independent of LVH, prevalent cardiovascular disease, or atrial fibrillation as evidenced by the effects persisting despite exclusion of participants with these conditions in sensitivity analyses. In mediation analyses, we discovered that neuroaxonal injury partially mediates associations between increased LV mass index and white matter microstructural damage among individuals with MCI and among *APOE-ε4* carriers.

This study is among the first to report an association between LV mass index and neuroaxonal injury, and the first to investigate neuroaxonal injury as a mediating pathway between subclinical cardiovascular remodeling and white matter injury. We found that CSF NFL accounts for up to 13% of the association between LV mass index and white matter microstructural damage in MCI and *APOE-ε4* carriers, suggesting neuroaxonal degeneration is one pathway by which increased LV mass detrimentally affects white matter health. As the left ventricle hypertrophies, the pumping efficiency of the myocardium decreases[28] and cerebral blood delivery may become compromised.[5] Resulting cerebral microvascular changes[2,8] may lead to oligemia[29] and neuroaxonal injury,[14] detected as an increase in CSF NFL concentrations. Oligemia alters ion homeostasis,[30] which disrupts neurofilaments[31] and initiates axon compaction,[32]

resulting in white matter damage that can be detected on DTI. This pathway is likely pronounced in individuals with MCI who have extensive pathology underlying their cognitive symptoms,[33] which heightens cerebral vulnerability to subtle microvascular changes and neuroaxonal injury. While *APOE-ε4* is a genetic susceptibility risk factor for AD,[20] it is also a modifier of vascular health[34] and disrupts lipid transport, leading to myelination deficits and white matter compromise.[21] Thus, the effects of subclinical cardiovascular remodeling on white matter microstructure via neuroaxonal degeneration may be exacerbated in *APOE-ε4* carriers.

Importantly, these findings were seen in individuals without clinical stroke, heart failure, or LVH, and results were not driven by hypertension. Our results suggest that subtle changes in cardiovascular health, even as early as mid-life, may have the largest influence on brain health outcomes. Even in the absence of frank white matter disease and heart disease, neuroaxonal injury still accounts for 13% percent of white matter damage due to increased LV mass index. To put this finding into context, small vessel disease accounts for up to 25% of ischemic strokes.[35] Thus, neuroaxonal injury is a significant contributor to pre-clinical white matter disease and should be studied further as a potential pathway through which early cardiovascular changes precipitate or exacerbate brain health outcomes.

While we found that neuroaxonal degeneration partially accounts for associations between higher LV mass index and white matter microstructural damage, there are additional pathways that might account for our results. Notably, we did not detect any associations between LV mass index and biomarkers of neuroinflammation, neurodegeneration, or Aβ aggregation, suggesting LV mass index does not affect white

matter through these pathways. It is possible that a shared upstream mechanism is driving the complex associations among LV mass index, CSF NFL, and white matter damage in individuals with MCI and *APOE*-ε4 carriers. For example, underlying cytoskeletal abnormalities could result in structural changes in both the myocardium (detected as an increase in LV mass on CMR[37]) and white matter microstructural damage (seen on DTI[38]). Future studies are needed to explore additional pathways through which subclinical cardiovascular changes affects abnormal brain aging.

Among individuals with NC, we observed the opposite association, such that lower LV mass index related to higher concentrations of CSF NFL. One potential explanation for this counterintuitive finding is that lower LV mass may indicate that the heart is not pumping as often or with as much force. In that context, there may be reduced cerebral blood flow delivery that contributes to neuroaxonal degeneration,[39] which is consistent with prior work showing suboptimal cardiac function is associated with reduced cerebral blood flow.[40] However, our counterintuitive observation would not survive correction for multiple comparisons, emphasizing the need for future studies and replication.

The current study has several strengths, including a clinically well characterized cohort emphasizing participants free of clinical stroke along with excellent methods for quantifying LV mass index, CSF biomarkers, and cerebral white matter microstructure. The cohort is relatively healthy and free of cardiovascular disease, highlighting that even preclinical changes in cardiovascular structure affect brain health. Additional strengths include comprehensive ascertainment of potential confounders, application of mediation analyses to interrogate these complex associations, and application of a

cluster enhancement permutation procedure in the DTI analyses to correct for multiple comparisons, reducing the possibility of a false positive finding. Finally, core laboratories using quality control procedures analyzed all CMR, CSF, and MRI measurements in batch, and technicians were blinded to clinical information. Despite these strengths, the study is cross-sectional and does not address dependency or directionality. Future work, including longitudinal studies, are needed to further examine neuroaxonal injury as a mediating pathway and the temporal nature of associations reported here. Some observations did not persist after multiple comparison correction, raising the possibility of a false positive finding and emphasizing the need for replication. Also, the cohort was predominantly non-Hispanic White with participants ranging 60 to 92 years of age, thus limiting generalizability.

The current study offers a novel association between subclinical cardiovascular remodeling and greater *in vivo* molecular evidence of neuroaxonal degeneration among individuals with MCI and among *APOE-*ε4 carriers. Further, we discovered that neuroaxonal degeneration partially mediates this association. Results suggest neuroaxonal degeneration may be a critical element of the pathway of injury between structural cardiovascular remodeling and white matter damage. Future research will elucidate whether early intervention on subclinical cardiovascular remodeling may be an important strategy in protecting brain health with increasing age.

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Table 1. Participant Characteristics

	Total n=142	NC n=74	Early MCI n=15	MCI n=53	p-value
Age, years	72±6	72±7	73±6	73±6	0.76
Sex, % male	68	72	80	60	0.24
Race, % Non-Hispanic White	92	93	93	91	0.84
Education, years	16±3	17±2	16±3	15±3	**0.002[‡]**
APOE-ε4, % positive	32	27	13	45	**0.02[†‡]**
FSRP, total score[§]	12±4	11±4	13±3	12±4	0.08
Systolic blood pressure, mmHg	142±16	139±15	148±15	145±18	0.07
Antihypertensive medication usage, %	46	47	40	47	0.87
Diabetes, %	17	11	27	23	0.12
Current smoking, %	1	0	7	2	0.13
Atrial fibrillation, %	4	5	0	2	0.42
Prevalent CVD, %	3	4	0	2	0.60
Left ventricular hypertrophy, %	3	0	7	6	0.10
Left Ventricular Mass Index, g/m^2	51.4±8.1	51.4±8.1	52.6±6.6	51.1±8.6	0.85
CSF sTREM2, pg/mL	3728±1863	3606±1924	4053±2002	3805±1755	0.53
CSF Neurofilament Light, pg/mL	1070±588	924±450	1088±465	1268±724	**<0.001[‡]**
CSF t-tau, pg/mL	434±232	380±177	429±125	511±295	**0.02[‡]**
CSF Aβ, pg/mL	721±241	770±221	817±282	625±228	**<0.001[†‡]**

Note. Values denoted as mean±standard deviation or frequency. Participant characteristics were compared across cognitive diagnosis using Kruskal-Wallis test for continuous variables and chi-square test for categorical variables. [†]Early MCI different than MCI, [‡]NC different from MCI. [§]a modified FSRP score was included in statistical models excluding points assigned to age and LVH (Total=6±2, NC=6±3, Early MCI=7±2, MCI=6±3); Aβ=amyloid beta; *APOE*=apolipoprotein E; CSF=cerebrospinal fluid; CVD=cardiovascular disease; FSRP=Framingham Stroke Risk Profile; MCI=mild cognitive impairment; NC=normal cognition; sTREM2=soluble triggering receptor expressed on myeloid cells 2; t-tau=total tau.

Table 2. LV Mass Index Associations with CSF NFL

Model	n	β	95% Confidence Intervals	p-value
Main Effects	142	5.23	-7.9, 18.35	0.43
Cognitive Diagnostic Interaction	127	31.96	8.35, 55.57	**0.01***
NC Participants	74	-14.78	-29.53, -0.02	**0.05**
MCI Participants	53	27.11	0.86, 53.37	**0.04**
APOE-ε4 Carrier Status Interaction	142	20.50	-2.59, 43.75	0.08
APOE-ε4 Non-Carriers	96	-7.13	-25.02, 10.75	0.43
APOE-ε4 Carriers	46	25.36	4.55, 46.17	**0.02**
Sensitivity Analyses				
Main Effects	130	3.79	-9.35, 16.94	0.57
Cognitive Diagnostic Interaction	116	27.66	4.03, 51.28	**0.02**
NC Participants	68	-13.81	-28.94, 1.32	0.07
MCI Participants	48	21.17	-5.53, 47.87	0.12
APOE-ε4 Carrier Status Interaction	130	19.75	-3.36, 42.87	0.09
APOE-ε4 Non-Carriers	88	-9.34	7.85, 0.28	0.28
APOE-ε4 Carriers	42	25.31	2.84, 47.78	**0.03**

Note. Sensitivity models excluded participants with prevalent CVD, atrial fibrillation, or LVH. β indicates the degree of change in CSF NFL concentration per 1 g/m^2 increase in LV mass index. *indicates p-values that would survive correction for multiple comparisons. *APOE*=apolipoprotein E; CSF=cerebrospinal fluid; CVD=cardiovascular disease; LV=left ventricular; LVH= left ventricular hypertrophy; MCI=mild cognitive impairment; NC=normal cognition; NFL=neurofilament light.

Table 3. Mediation Analysis Results

MCI Participants*

Mean Diffusivity Cluster 1 (8836 mm^3, Body of the corpus callosum)

Outcome	β
Total Effect	2.73×10^{-6}
Average Direct Effect	2.52×10^{-6}
Average Mediation Effect	2.15×10^{-7}
Proportion Mediated	0.06

Radial Diffusivity Cluster 1 (1012 mm^3, Inferior frontal gyrus)

Outcome	β
Total Effect	3.08×10^{-6}
Average Direct Effect	2.71×10^{-6}
Average Mediation Effect	3.70×10^{-7}
Proportion Mediated	0.10

Radial Diffusivity Cluster 2 (469 mm^3, Anterior corona radiata)

Outcome	β
Total Effect	3.46×10^{-6}
Average Direct Effect	3.24×10^{-6}
Average Mediation Effect	2.15×10^{-7}
Proportion Mediated	0.05

Radial Diffusivity Cluster 3 (2) (350 mm^3, Superior corona radiata)

Outcome	β
Total Effect	2.95×10^{-6}
Average Direct Effect	2.54×10^{-6}
Average Mediation Effect	4.09×10^{-7}
Proportion Mediated	0.13

Axial Diffusivity Cluster 1 (1493 mm^3, Middle frontal gyrus)

Outcome	β
Total Effect	3.63×10^{-6}
Average Direct Effect	3.45×10^{-6}
Average Mediation Effect	1.87×10^{-7}
Proportion Mediated	0.04

Axial Diffusivity Cluster 2 (1178 mm^3, Inferior fronto-occipital fasciculus)

Outcome	β
Total Effect	3.81x10^{-6}
Average Direct Effect	3.44x10^{-6}
Average Mediation Effect	3.78x10^{-7}
Proportion Mediated	0.09

***APOE*-ε4 Carriers[†]**

Mean Diffusivity Cluster 1 (7179 mm^3, Splenium of the corpus callosum)

Outcome	β
Total Effect	3.39x10^{-6}
Average Direct Effect	3.18x10^{-6}
Average Mediation Effect	2.13x10^{-7}
Proportion Mediated	0.05

Mean Diffusivity Cluster 2 (1980 mm^3, Inferior frontal gyrus)

Outcome	β
Total Effect	3.23x10^{-6}
Average Direct Effect	3.05x10^{-6}
Average Mediation Effect	2.24x10^{-7}
Proportion Mediated	0.06

Mean Diffusivity Cluster 3 (716 mm^3, Anterior corona radiata)

Outcome	β
Total Effect	3.09x10^{-6}
Average Direct Effect	2.66x10^{-6}
Average Mediation Effect	4.37x10^{-7}
Proportion Mediated	0.13

Note. *Models were adjusted for age, sex, race/ethnicity, education, Framingham Stroke Risk Profile (minus points assigned for age and LVH), and *APOE*-ε4 carrier status. [†]Models were adjusted for age, sex, race/ethnicity, education, Framingham Stroke Risk Profile (minus points assigned for age and LVH) and cognitive diagnosis. *APOE*=apolipoprotein E; LVH=left ventricular hypertrophy; MCI=mild cognitive impairment.

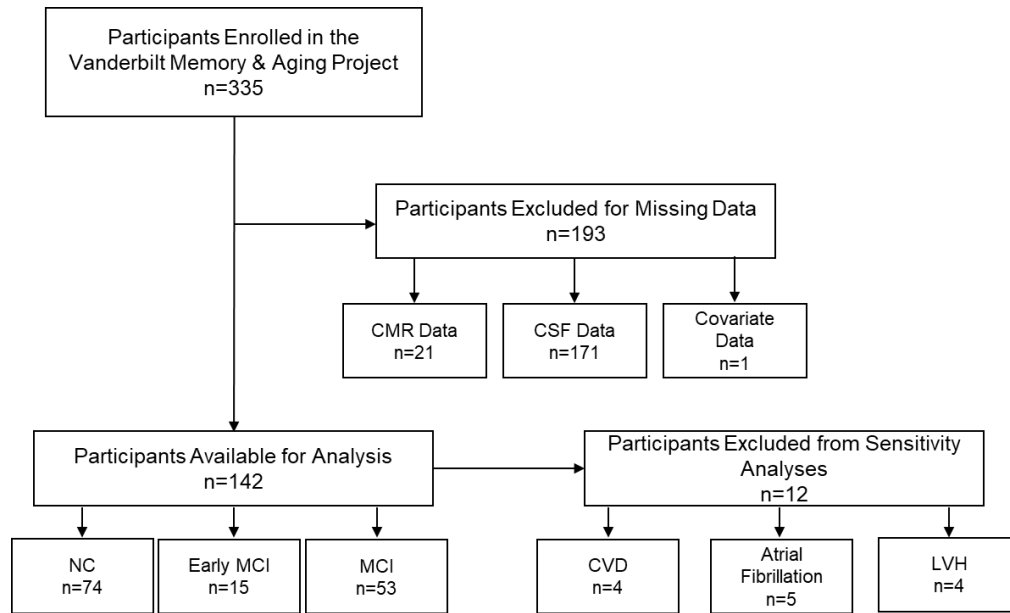**Figure 1**. **Participant Inclusion/Exclusion Details**

Figure 1. Missing data categories are mutually exclusive. Exclusion categories for sensitivity analyses are not mutually exclusive. One participant had both CVD and atrial fibrillation. CMR=cardiac magnetic resonance; CSF=cerebrospinal fluid; CVD=cardiovascular disease; LVH=left ventricular hypertrophy; MCI=mild cognitive impairment; NC=normal cognition.

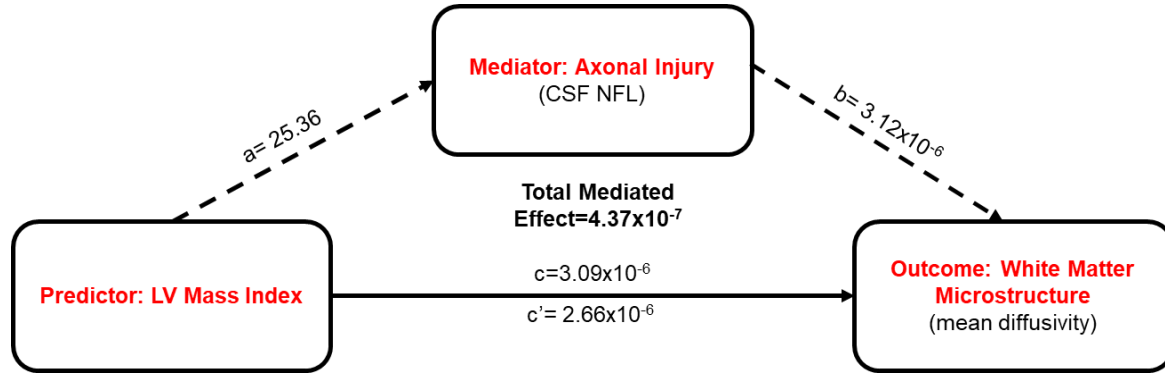**Figure 2. Mediation Example in *APOE*-ε4 Carriers**

Figure 2. Schematic illustrating the mediation analysis for one cluster in *APOE*-ε4 carriers. a) the direct effect of LV mass index on CSF NFL. b) the direct effect of CSF NFL on mean diffusivity in one cluster. c) the total effect of LV mass index on mean diffusivity in one cluster. c) the direct effect of LV mass index on mean diffusivity in one cluster, adjusting for CSF NFL. The total mediated effect was 4.37×10^{-7}. CSF=cerebrospinal fluid; DTI=diffusion tensor imaging. LV=left ventricular; NFL=neurofilament light.

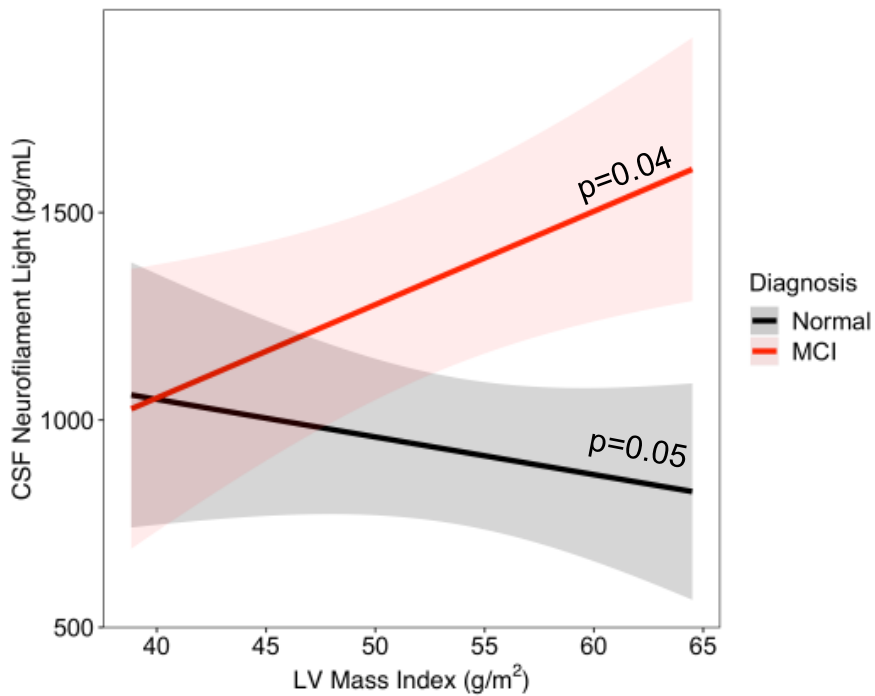**Figure 3. LV Mass Index Interacts with Diagnosis on CSF NFL**

Figure 3. LV mass index x diagnosis interaction on CSF NFL (p=0.01). Lines reflect fitted values of CSF NFL corresponding to LV mass index. Shading reflects the 95% confidence interval. LV=left ventricular; MCI=mild cognitive impairment; NFL=neurofilament light.

Supplemental Material

Supplemental Tables I-IV