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Associations of Circulating GDF-15 and ST2 concentrations with Subclinical Vascular Brain Injury and Incident Stroke

Charlotte Andersson, MD, PhD[1,2], **Sarah R. Preis, ScD**[3], **Alexa Beiser, PhD**[1,3,4], **Charles DeCarli, MD**[5], **Kai C. Wollert, MD**[6], **Thomas J Wang, MD**[1,7], **James L. Januzzi Jr., MD**[8], **Ramachandran S Vasan, MD**[1,9], and **Sudha Seshadri, MD**[1,4]

[1]Framingham Heart Study, Framingham, MA, USA

[2]Department of Cardiology, Gentofte Hospital, University of Copenhagen, Denmark

[3]Department of Biostatistics, Boston University School of Public Health, Boston, MA, USA

[4]Department of Neurology, Boston University School of Medicine, Boston MA, USA

[5]University of California at Davis, Sacramento, CA, USA

[6]Division of Molecular and Translational Cardiology, Department of Cardiology and Angiology, Hannover Medical School, Hannover, Germany

[7]Division of Cardiovascular Medicine, Vanderbilt University, Nashville, TN, USA

[8]Cardiology Division, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA

[9]Section of Preventive Medicine and Cardiology, Boston University School of Medicine, Boston, MA, USA

Abstract

Background and Purpose—Growth differentiation factor-15 (GDF-15) and soluble (s)ST2 are markers of cardiac and vascular stress. We investigated the associations between circulating concentrations of these biomarkers and incident stroke and subclinical vascular brain injury in a sample from the Framingham Offspring cohort.

Methods—We followed 3374 stroke- and dementia-free individuals (mean age 59.0±9.7 years, 53% women) attending the Framingham Offspring 6th examination cycle 11.8±3.0 years for incident stroke. A subsample of 2463 individuals underwent brain magnetic resonance imaging and neuropsychological testing approximately 4.0±1.7 years after the 6th examination.

Results—After adjustment for traditional cardiovascular risk factors, B-type natriuretic peptide, high-sensitivity C-reactive protein, and urine albumin levels, higher stress biomarker levels were associated cross-sectionally with lower brain volumes (βs for intracranial volume comparing 4^{rth}

Correspondence to: Charlotte Andersson, MD, Framingham Heart Study, 73 Mt Wayte Avenue, Framingham, MA, 01702, USA, Tel: 617-817-3693, Fax: 508-626-1262, ca@heart.dk.

Disclosures

Dr. Wollert reports grants from Roche Diagnostics, during the conduct of the study. In addition, Dr. Wollert has a patent European Patent 2047275B1 issued. Dr. Januzzi reports personal fees from Critical Diagnostics, personal fees from Roche, during the conduct of the study. Dr. Wang has served on medical advisory boards for Critical Diagnostics and Singulex.

[Q4] vs. 1st biomarker [Q1] quartiles −0.71% for GDF-15, p=0.002, and 0.47% for sST2, p=0.02) and worse performance on the visual reproduction test (βs for Q4 vs. Q1=−0.62 for GDF-15, p=0.009, and −0.40 for sST2, p=0.04). Higher GDF-15 concentrations were also associated with greater log-transformed white-matter hyperintensity volumes (β for Q4 vs. Q1=0.19, p=0.01). Prospectively, a total of 203 (6%) individuals developed incident stroke/transient ischemic attack (TIA) during follow-up. After multivariable adjustment, sST2 remained significantly associated with stroke/TIA, hazard ratio for Q4 vs. Q1 of 1.76, 95% confidence interval 1.06–2.92, p=0.03.

Conclusions—Circulating GDF-15 and sST2 are associated with subclinical brain injury and cognitive impairment. Higher sST2 concentrations are also associated with incident stroke, suggesting potential links between cardiac stress biomarkers and brain injury.

Introduction

Although subclinical vascular brain injury often occurs without (or with very subtle) symptoms, it more than doubles a person's risk of subsequent stroke and dementia.[1, 2] Consequently, it is important to identify those people in order to prevent overt clinical events. Subclinical vascular brain injury may be identified through brain magnetic resonance imaging (MRI) or extensive neuropsychological (NP) testing. However, wide screening of asymptomatic individuals with such tests may not be cost-effective. Therefore, cheap, quick, non-invasive biomarkers that can serve as an initial screening tool are warranted.[3]

Circulating growth-differentiation factor-15 (GDF-15), a marker of cardiac stress and possible endothelial dysfunction,[4] has a strong positive association with prognosis in patients with heart failure,[5] acute chest pain,[6] stable ischemic heart disease,[7] non-ST and ST elevation myocardial infarction,[8, 9] and ischemic stroke.[10, 11] GDF-15 has also been reported to improve prediction of the risk of incident overall cardiovascular disease (CVD), heart failure, and cancer, as well as all-cause and cancer-related mortality in community-based cohorts.[12, 13] Higher GDF-15 concentrations have been noted with increasing age and in smokers, persons with diabetes, hypertension, poor kidney function, and low high-density lipoprotein cholesterol levels.[14] Thus, GDF-15 is a biomarker associated with CVD across the disease and risk factor spectrum.

Higher levels of circulating soluble (s) ST2, another marker of cardiac stress, have been associated with risk of heart failure after acute myocardial infarction,[15] mortality in ST-elevation myocardial infarction patients,[16] mortality in heart failure patients,[17] and with incident overall CVD, heart failure, and all-cause mortality in population-based cohorts.[12] Concentrations of sST2 are positively associated with high age, male gender, hypertension, and diabetes in the community.[18]

Despite much evidence suggesting a role for GDF-15 and sST2 in risk prediction of adverse CVD outcomes in different settings, no prior study has specifically assessed the relations of circulating GDF-15 and sST2 concentrations with cerebrovascular disease. We, therefore, investigated the association of circulating GDF-15 and sST2 levels with the risk of developing clinical stroke prospectively, and with the risk of subclinical vascular brain injury on brain MRI and neuropsychological assessment cross-sectionally in a large community-based sample.

Methods

Study sample

The present analysis was based on participants from the Framingham Offspring Study, which began in 1971 with the enrollment of 5,124 individuals.[19] Of the 3,532 participants who attended the 6th examination cycle (1995–1998), 3,456 had available biomarker measurements for GDF-15 and sST2. We constructed two study samples for our analysis, each a subset of the 3,456 participants with available biomarker data. After the 7th examination cycle (1999–2005) all participants were invited to undergo a brain MRI scan and concurrent neuropsychological test (NP) battery. Of the 3,456 participants with biomarker data, 2,591 completed the MRI/NP testing (characteristics of people with and without MRI are shown in Online Supplemental Table I; in general, people with MRI data had a less adverse cardiovascular risk profile). Of these, we excluded an additional 61 participants for prevalent stroke, 34 for prevalent dementia, and 33 for other neurological conditions (such as brain tumors or multiple sclerosis, which could influence the MRI measures), resulting in a sample size of 2463 for our cross-sectional analysis of MRI/NP outcomes. For our analysis of the stroke/TIA outcome, of the 3,456 participants with biomarker data, we excluded 76 participants with prevalent stroke and 6 participants without follow-up information, resulting in a sample size of 3,374 for these prospective analyses.

Biomarker Measurements

The GDF-15 and sST2 biomarkers were measured on previously unthawed fasting blood samples that had been centrifuged and stored at −80°C. GDF-15 concentrations were obtained from a precommercial immunoassay on a Cobas e 411 analyzer (Roche Diagnostics, Switzerland), and sST2 concentrations were measured using an enzyme-linked immunosorbent assay (Presage ST2; Critical Diagnostics, San Diego, CA). Details of these laboratory analytic methods have been previously described.[20–22]

MRI and Neuropsychological Test Outcome Measures

A full description of the MRI measures, including inter-observational variability has been published previously.[23] Experts blinded to demographic and clinical information analyzed all images in a core laboratory using custom-written software. Total cerebral brain volume (TCBV) was calculated as the ratio between total cerebral brain volume and total intracranial volume. A lower ratio therefore indicates relative brain atrophy. Cumulative volume of white matter hyperintensity lesions (WMHIV) was quantified by an automated method and divided by cerebral brain volume. Because of a skewed distribution, WMHIV was natural log (ln) transformed in our analyses.

Participants were also invited to complete a battery of neuropsychological tests (1999–2001) on the same date as the brain MRI. The tests used and scoring system have been detailed previously.[24] In accordance with previous studies,[25, 26] we related the circulating biomarkers to scores on two cognitive tests known to be sensitive to vascular brain injury, i.e., visual reproduction-delayed recall (VRd) and the Halstead-Reitan Trail Making Tests on which we used the difference between scores on the Trails B and Trails A tests as a

measure of executive function subtracting out the elements of the score related to attention, visual scanning, and motor tracking that were common to both Trails B and A.[27]

Incident Stroke/Transient Ischemic Attack

All individuals have been followed with clinical examinations every 3–6 years and are under continuous surveillance for incident cardiovascular disease, stroke, dementia and mild cognitive impairment. All potential stroke events have been reviewed by a stroke end-point review committee comprised of at least two neurologists. We defined incident stroke as an acute onset focal deficit of presumed or definite vascular etiology of >24 hours duration. We defined a transient ischemic attack (TIA) as a similar clinical presentation but lasting for a duration 24 hours.

Covariate Measurements

Information of covariates was obtained at examination cycle 6. We used variables included in the Framingham Stroke Risk Score Profile (FRSP) to adjust for established risk factors for vascular brain injury. These included systolic blood pressure, use of antihypertensive medications, diabetes, current smoking, CVD (coronary heart disease, heart failure, or intermittent claudication), and atrial fibrillation.[28] The FRSP score has previously been associated with smaller brains and poorer cognitive function in the present cohort as well as with incident stroke in the Original cohort.[27–29] We categorized highest achieved educational status into four groups: <high school degree, high school degree only, some college, or ≥college degree. Other biomarkers, including high-sensitivity C-reactive protein (hs-CRP), B-type natriuretic peptide (BNP), and urine albumin were measured at the 6th examination cycle.

Ethics

All study participants provided written, informed consent and the study protocol was approved by the institutional review board for human research at the Boston University Medical Center.

Statistical analyses

Any variable with a skewed distribution was natural logarithmically (ln)-transformed. Linear regression models were constructed to examine the association between the GDF-15 and sST2 biomarkers and the following outcomes: 1) brain MRI measures (total cerebral brain volume (TCBV) and WMHIV), 2) neuropsychological test measures (VRd and Trails B – Trails A). Cox regression models were performed to assess the longitudinal associations of GDF-15 and sST2 with incident total stroke/transient ischemic attack and with ischemic stroke, using calendar time as the time scale and after confirming that the assumption of proportionality of hazards was met. The GDF-15 and sST2 biomarkers were entered into the models both as continuous variables and as quartiles (in separate analyses). A test for linear trend across biomarker quartiles was conducted by entering an ordinal variable (representing the biomarker quartiles) into the model and calculating a Wald p-value. Three multivariable regression models were constructed. Model 1 adjusted for sex and age (and age squared for MRI measures), education group (for neuropsychological test outcomes) plus time between

examination 6 (where blood was drawn) and MRI/neuropsychological tests (for those analyses). Model 2 additionally adjusted for variables in the FRSP. Model 3 additionally adjusted for other biomarkers, viz., B-type brain natriuretic peptide (BNP), high sensitivity C-reactive protein (hs-CRP), and urine albumin concentrations, because these biomarkers have been shown to be associated with subclinical brain damage and predict incident vascular brain injury and stroke in the present cohort (beyond traditional risk factors).[30] All analyses were performed in SAS version 9.4 (Cary, NC, USA). A 2-sided p-value <0.05 was considered as statistically significant for all tests.

Results

The analysis included 2,463 participants for the neuropsychological test outcomes, 2,128 for the MRI brain measure outcomes, and 3,374 for the incident stroke/TIA outcome.

Approximately 53% of our study sample was women and the overall mean age was 59 (±standard deviation 10) years. Baseline characteristics of the study samples are shown in Table 1. The prevalence of CVD risk factors was rather high, with 27% being treated with antihypertensive medications, 10% having diabetes, 15% being current smokers, and 9% having prevalent CVD.

Brain MRI Measure Outcomes

Higher concentrations of GDF-15 and sST2 were each significantly associated with lower levels of TCBV in all three multivariable models, Table 2. Adjustment for traditional cardiovascular risk factors, as well as BNP, hs-CRP, and urine albumin levels (Models 2 and 3) attenuated the beta coefficients for the biomarkers of interest but they remained statistically significant (βs for TCBV comparing 4^{rth} [Q4] vs. 1st biomarker [Q1] quartiles −0.71% for GDF-15, p=0.002 [p for linear trend= 0.004], and 0.47% for sST2, p=0.02 [p for linear trend= 0.02]). GDF-15 was positively associated with higher ln-WMHIV, β=0.19, p=0.01 for the Q4 vs. Q1 (p for linear trend=0.01) in fully-adjusted models, Table 2. There was no association between sST2 and WMHIV in any of the models evaluated.

Neuropsychological Test Outcomes

Higher concentrations of GDF-15 and sST2 were associated with poorer delayed visual reproduction test results, Table 3: βs for Q4 vs. Q1 =−0.62, p=0.009 for GDF-15 (p for linear trend=0.01), and −0.40, p=0.04 for sST2 (p for linear test=0.10) in fully-adjusted models. In analyses adjusted for age and sex, higher GDF-15 concentrations were associated with lower results on Trails B – Trails A, but after additional adjustment the associations were no longer statistically significant.

Exploratory analyses

In order to better understand the associations of biomarkers with TCBV and neuropsychiatric tests, we investigated if the strength of association between biomarkers and the aforementioned measurements differed between people with and without extensive WMHIV (defined as a WMHIV more than 1.5 standard deviation above the age- and sex-adjusted mean). For TCBV, the association with GDF-15 concentrations was stronger

among those with, compared to those without extensive WMHIV, p for interaction <0.0001, online supplemental Table II. For Trails B – Trails A associations with both of GDF-15 and sST2 levels were stronger among those with compared with those without extensive WMHIV (p for interactions = 0.02 and 0.03, respectively).

Incident Stroke Outcome

During a mean follow-up of 11.8±3.0 years, 203 (6%) participants developed stroke/TIA (130 had ischemic stroke). sST2 and GDF-15 levels were both associated with incident stroke/TIA in models adjusted for age and sex, Table 4. After adjustment for established risk factors, only sST2 remained significantly associated with incident stroke/TIA (hazard ratio 1.76; 95% confidence interval 1.06–2.92, P=0.03 [p for linear trend=0.02]) for Q4 vs. Q1. Analyses restricted to incident ischemic stroke (n=130) yielded similar results (hazard ratio 1.85; 95% CI: 0.98–3.49, P=0.06 for sST2 Q4 vs. Q1 in model 3 [p for linear trend=0.05]).

Discussion

In the present investigation, we observed that higher concentrations of circulating GDF-15 and sST2 were associated with several measures indicative of subclinical brain damage and other concomitant neurodegenerative processes in a sample of middle-aged ambulatory individuals without prior stroke or dementia. sST2 was also observed to be a strong long-term predictor of incident stroke/TIA beyond traditional risk factors plus biomarkers that have previously been documented to predict incident stroke in the Framingham Heart Study (i.e., BNP, hs-CRP, and urine albumin).[30]

Wang et al. have previously investigated the relation of higher GDF-15 and sST2 concentrations with incident heart failure, major coronary events, and a composite CVD outcome (comprising recognized myocardial infarction, coronary insufficiency, coronary heart disease death, heart failure, and stroke) in the Framingham Offspring cohort.[12] Interestingly, both biomarkers could predict events above and beyond established risk factors for all endpoints except for major coronary events. Based on this, it has been hypothesized that GDF-15 and sST2, in ambulatory individuals, relate to cardiac stress/dysfunction rather than atherosclerotic manifestations and subclinical inflammation.

GDF-15, subclinical vascular brain injury, and incident stroke

Several lines of evidence also suggest that GDF-15 may be associated with endothelial dysfunction and small vessel disease. In our exploratory analyses, blood GDF-15 levels showed stronger associations with TCBV and Trails B – Trails A among those with, compared to those without extensive WMHIV, which may suggest that GDF-15 may be primarily reflective of cerebral microvascular disease in population-based settings (since extensive WMHIV is a marker of microvascular disease). GDF-15 has also been associated with microvascular disease in non-cerebral vascular beds. In the Prospective Investigation of the Vasculature in Uppsala Seniors circulating GDF-15 concentrations were directly associated with endothelium-dependent vasodilation in resistance vessels.[4] By comparing endothelial function in wild type and GDF-15 knocked out mice GDF-15 was recently demonstrated to directly modulate the endothelial-dependent NO synthase pathway in the

aortas of mice.[31] Our observations are also in agreement with other lines of evidence suggesting a significant role of GDF-15 in vascular brain injury. For instance, higher GDF-15 levels have previously been reported to be strongly related to worse functional status in the acute phase of ischemic stroke in humans.[10] Mouse models of cerebral ischemia have also demonstrated upregulation in GDF-15 expression (possible by neurons)[32] secondary to vascular brain damage.[33] In contrast, GDF-15 does not seem to be expressed in mice with normal brain circulation.[33] In vivo, GDF-15 appears to prevent certain types of neural losses in rats,[34] and a recent study of elderly community-based individuals (mean age 78–81 years) demonstrated an inverse association of higher GDF-15 concentrations with cognitive function.[35] Because subclinical vascular brain damage increases the risk of dementia and is associated with a steeper decline in cognitive function among elderly individuals, one mechanism that could link high GDF-15 levels with impaired cognitive function may be via its association with subclinical vascular brain injury.[36, 37] In this context, blood GDF-15 levels were recently reported to also be associated positively with gray matter volume decline over a two-year period in an elderly community-based sample.[38]

Somewhat surprising, yet in line with the prior study by Wang et al. (showing no association of GDF-15 with incident coronary events),[12] we observed no association of blood GDF-15 levels with incident stroke/TIA in our sample. This is also in agreement with results from a previous Swedish population-based sample of men (mean age 71 years). Adjusted for multiple risk factors, GDF-15 concentrations were not associated with stroke or stroke-related mortality in this latter study.[39]

sST2 and Subclinical Vascular Brain Injury and Incident Stroke

We observed statistically significant associations between higher concentrations of sST2 and lower TCBV and poorer delayed visual reproduction test result. We also observed an increased risk of stroke with higher sST2 levels after adjustment for established stroke risk factors (although the endpoint with overt stroke was only borderline statistically significant). sST2 has previously predominantly been studied in the setting of heart failure and to the best of our knowledge no prior study has investigated the relation of sST2 with subclinical vascular brain damage and incident stroke. In point of fact, reports relating sST2 to the central nervous system are sparse. Experimental studies have demonstrated that sST2 may be produced by astrocytes and endothelial cells, and can activate microglia and enhance phagocytosis, which could both be important responses to vascular injury.[40]

As noted above, neither GDF-15 nor sST2 concentrations have previously been shown to be significantly associated with coronary events in Offspring cohort.[12] In contrast to GDF-15, we observed an association of sST2 with incident stroke in our study. Whether sST2 is more specific than GDF-15 for vascular brain damage and inflammation is not known. Our observation could also relate to the strong association of sST2 with incident heart failure, because heart failure and atrial fibrillation (which is common in patients with heart failure) are strong risk factors for incident stroke. Circulating sST2 has, however, not been linked to incident atrial fibrillation in the present study sample.[21] We also previously reported concentrations of sST2 predict incident systolic blood pressure in this cohort; despite

adjustment for prevalent hypertension in our models, it may be this explains the stronger links to cerebrovascular disease than GDF-15.[41]

Strengths and Limitations

The main strength of our study was the comprehensive phenotypic data available in the Framingham Offspring cohort, which enabled us to study both subclinical and clinical vascular brain injury in relation to biomarkers. Another strength is that incident stroke events have been adjudicated and are accurate. However, some limitations merit consideration. The number of strokes in the present investigation was rather limited, and therefore a type II statistical error cannot be excluded. Also, the stroke subtypes could not be separately analyzed due to the small sample sizes for individual subtypes. For the MRI/NP analyses, the study sample comprised people who had attended both the 6th examination cycle and had undergone MRI/NP testing an average of 4 years later. Further, the temporal differences between MRI/NP testing and biomarker assays may have lead to regression dilution bias, which may have underestimated the true strength of the association of biomarkers and brain measures. Finally, the sample was predominantly white and middle-aged, and the generalizability of our findings to other age groups and other races is unknown.

Conclusions and clinical implications

Circulating concentrations of GDF-15 and sST2 are associated with subclinical brain damage cross-sectionally, and sST2 is also associated with incident stroke/TIA prospectively after adjustment for established risk factors. Our study should be viewed as only hypothesis-generating, but if confirmed, these associations could point to novel stroke and vascular brain injury prediction models and prevention strategies targeting these pathways.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Study sample characteristics

	Study Sample	
	MRI/NP Outcomes (N=2463)*	**Stroke/TIA Outcome (N=3374)**
Women	1310 (53.2)	1791 (53.1)
Age at examination 6 (years)	58.3 (9.4)	59.0 (9.7)
Age at MRI (years)	62.1 (9.4)	---
Age at NP (years)	62.5 (9.5)	---
Time between exam 6 and MRI (years)	4.0 (1.7)	---
Time between exam 6 and NP (years)	4.2 (2.1)	---
Time between exam 6 and stroke occurrence, death, or censoring (years)	---	11.8 (3.0)
Systolic blood pressure (mmHg)	127 (18)	128 (19)
Hypertension treatment	618 (25.2)	924 (27.5)
Diabetes	236 (9.7)	352 (10.6)
Smoking	352 (14.3)	511 (15.2)
History of CVD	204 (8.3)	311 (9.2)
History of atrial fibrillation	55 (2.2)	101 (3.0)
Left ventricular hypertrophy	9 (0.4)	21 (0.6)
Education group		
<High school degree	89 (3.6)	164 (4.9)
High school degree	1453 (59.0)	1944 (57.6)
≥College degree	921 (37.4)	1133 (33.6)
Missing	0 (0.0)	133 (3.9)
BNP, pg/mL	7.8 (4.0, 16.8)	8.4 (4.0, 18.7)
hs-CRP, mg/L	1.95 (0.88, 4.51)	2.02 (0.92, 4.64)
Urine albumin (mg/L)	5.1 (2.9, 10.1)	5.4 (2.9, 10.8)
GDF-15, ng/L	1002 (802, 1292)	1035 (812, 1348)
sST2, ng/mL	20.6 (16.5, 25.6)	20.9 (16.6, 26.0)

Continuous variables are presented as mean (standard deviation) for blood pressure and median (25^{th}, 75^{th} percentile) for biochemistry samples, and discrete variables as numbers (percentages). MRI= magnetic resonance imaging, NP= neuropsychological test, CVD= cardiovascular disease, BNP= B-type natriuretic peptide, GDF-15= growth differentiation factor-15.

* A total of 2128 participants had MRI measures.

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Table 2

Linear regression results for the association between biomarkers and brain MRI measures.

Outcome	Biomarker	Effect	Model 1 (N=2127)*		Model 2 (N=2083)†		Model 3 (N=1745)‡	
			Beta (SE)	P-value	Beta (SE)	P-value	Beta (SE)	P-value
Total cerebral brain volume	GDF-15§	Continuous	−1.25 (0.18)	<0.0001	−1.01 (0.19)	<0.0001	−0.86 (0.21)	<0.0001
		Quartiles						
		Q1	0.00 (ref)	---	0.00 (ref)	---	0.00 (ref)	---
		Q2	−0.24 (0.17)	0.15	−0.24 (0.17)	0.16	−0.064 (0.18)	0.73
		Q3	−0.40 (0.18)	0.03	−0.31 (0.19)	0.10	−0.15 (0.21)	0.48
		Q4	−1.18 (0.20)	<0.0001	−0.97 (0.21)	<0.0001	−0.71 (0.23)	0.002
		Test for linear trend		<0.0001		<0.0001		0.004
	sST2§	Continuous	−0.77 (0.19)	<0.0001	−0.58 (0.19)	0.002	−0.40 (0.20)	0.05
		Quartiles						
		Q1	0.00 (ref)	---	0.00 (ref)	---	0.00 (ref)	---
		Q2	−0.21 (0.17)	0.21	−0.10 (0.17)	0.54	−0.010 (0.18)	0.96
		Q3	−0.32 (0.17)	0.07	−0.20 (0.17)	0.24	−0.073 (0.19)	0.70
		Q4	−0.82 (0.18)	<0.0001	−0.65 (0.18)	0.0004	−0.47 (0.20)	0.02
		Test for linear trend		<0.0001		0.0005		0.02
White matter hyperintensities volume§	GDF-15§	Continuous	0.16 (0.059)	0.009	0.11 (0.062)	0.07	0.13 (0.067)	0.048
		Quartiles						
		Q1	0.00 (ref)	---	0.00 (ref)	---	0.00 (ref)	---
		Q2	0.056 (0.054)	0.30	0.044 (0.054)	0.42	0.077 (0.059)	0.19
		Q3	0.083 (0.060)	0.17	0.076 (0.060)	0.21	0.13 (0.066)	0.06
		Q4	0.19 (0.066)	0.004	0.14 (0.069)	0.04	0.19 (0.075)	0.01
		Test for linear trend		0.005		0.04		0.01
	sST2§	Continuous	−0.0087 (0.060)	0.88	−0.038 (0.061)	0.53	−0.0046 (0.065)	0.94
		Quartiles						
		Q1	0.00 (ref)	---	0.00 (ref)	---	0.00 (ref)	---
		Q2	−0.029 (0.054)	0.58	−0.025 (0.053)	0.64	0.033 (0.058)	0.57

Outcome	Biomarker	Effect	Model 1 (N=2127)*		Model 2 (N=2083)†		Model 3 (N=1745)‡	
			Beta (SE)	P-value	Beta (SE)	P-value	Beta (SE)	P-value
		Q3	−0.069 (0.056)	0.22	−0.086 (0.056)	0.12	−0.049 (0.061)	0.42
		Q4	0.021 (0.059)	0.72	−0.0024 (0.059)	0.97	0.036 (0.064)	0.58
		Test for linear trend		0.94		0.67		0.93

*Model 1 is adjusted for age at MRI, age at MRI squared, sex, and time between examination 6 and MRI.

†Model 2 is adjusted for Model 1 covariates plus FSRP components (systolic blood pressure, hypertension treatment, diabetes, smoking, history of CVD, history of AF).

‡Model 3 is adjusted for Model 2 covariates plus BNP, hsCRP, and urine albumin.

§Natural log (ln) transformed

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Table 3

Linear regression results for the association between biomarkers and neuropsychological (NP) test measures.

Outcome	Biomarker	Effect	Model 1 (N=2463)*		Model 2 (N=2397)†		Model 3 (N=2008)‡	
			Beta (SE)	P-value	Beta (SE)	P-value	Beta (SE)	P-value
Visual Reproductions Delayed	GDF-15§	Continuous	−0.78 (0.19)	<0.0001	−0.57 (0.20)	0.004	−0.49 (0.21)	0.02
		Quartiles						
		Q1	0.00 (ref)	---	0.00 (ref)	---	0.00 (ref)	---
		Q2	−0.33 (0.17)	0.05	−0.33 (0.17)	0.06	−0.40 (0.19)	0.03
		Q3	−0.41 (0.18)	0.03	−0.30 (0.19)	0.12	−0.40 (0.21)	0.06
		Q4	−0.93 (0.21)	<0.0001	−0.78 (0.22)	0.0003	−0.62 (0.24)	0.009
		Test for linear trend		<0.0001		0.001		0.01
	sST2§	Continuous	−0.59 (0.19)	0.002	−0.47 (0.19)	0.02	−0.41 (0.20)	0.04
		Quartiles						
		Q1	0.00 (ref)	---	0.00 (ref)	---	0.00 (ref)	---
		Q2	−0.14 (0.17)	0.39	−0.12 (0.17)	0.49	−0.19 (0.18)	0.30
		Q3	−0.16 (0.18)	0.37	−0.11 (0.18)	0.52	−0.08 (0.19)	0.67
		Q4	−0.49 (0.18)	0.008	−0.38 (0.19)	0.04	−0.40 (0.20)	0.048
		Test for linear trend		0.01		0.06		0.10
Trails B – Trails A§	GDF-15§	Continuous	−0.038 (0.013)	0.003	−0.027 (0.014)	0.047	−0.010 (0.015)	0.48
		Quartiles						
		Q1	0.00 (ref)	---	0.00 (ref)	---	0.00 (ref)	---
		Q2	0.002 (0.012)	0.83	0.0054 (0.012)	0.65	0.0094 (0.013)	0.47
		Q3	−0.016 (0.013)	0.20	−0.0045 (0.013)	0.73	−0.0091 (0.014)	0.53
		Q4	−0.035 (0.014)	0.02	−0.024 (0.015)	0.11	−0.0034 (0.016)	0.84
		Test for linear trend		0.008		0.09		0.57
	sST2§	Continuous	−0.025 (0.013)	0.06	−0.018 (0.013)	0.18	−0.0060 (0.014)	0.67
		Quartiles						
		Q1	0.00 (ref)	---	0.00 (ref)	---	0.00 (ref)	---
		Q2	0.011 (0.012)	0.35	0.013 (0.012)	0.26	0.016 (0.013)	0.19

Outcome	Biomarker	Effect	Model 1 (N=2463)*		Model 2 (N=2397)†		Model 3 (N=2008)‡	
			Beta (SE)	P-value	Beta (SE)	P-value	Beta (SE)	P-value
		Q3	−0.0056 (0.012)	0.65	0.00026 (0.012)	0.98	0.008 (0.013)	0.56
		Q4	−0.022 (0.013)	0.08	−0.017 (0.013)	0.19	−0.011 (0.014)	0.45
		Test for linear trend		0.04		0.14		0.41

*Model 1 is adjusted for age at NP, sex, education group (<high school degree, high school degree, ≥ college graduate) and time between examination 6 and NP.

†Model 2 is adjusted for Model 1 covariates plus FSRP components (systolic blood pressure, hypertension treatment, diabetes, smoking, history of CVD, history of AF).

‡Model 3 is adjusted for Model 2 covariates plus BNP, hs-CRP, and urine albumin.

§Natural log (ln) transformed.

Table 4

Cox proportional hazards regression results for the association between biomarkers and stroke.

Outcome	Biomarker	Effect	Model 1 (N=3374)*		Model 2 (N=3268)†		Model 3 (N=2741)‡	
			HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value
Stroke/Transient Ischemic Attack‖	GDF-15§	Continuous	1.92 (1.36–2.73)	0.0003	1.41 (0.98–2.04)	0.07	1.16 (0.75–1.78)	0.51
		Quartiles						
		Q1	1.00 (ref)	---	1.00 (ref)	---	1.00 (ref)	---
		Q2	0.68 (0.39–1.20)	0.18	0.64 (0.36–1.12)	0.12	0.55 (0.29–1.04)	0.07
		Q3	1.43 (0.87–2.36)	0.16	1.17 (0.71–1.94)	0.54	1.17 (0.67–2.01)	0.59
		Q4	1.68 (1.00–2.82)	0.05	1.27 (0.75–2.15)	0.38	1.21 (0.67–2.15)	0.53
		Test for linear trend		0.002		0.06		0.11
	sST2§	Continuous	1.97 (1.30–2.98)	0.001	1.75 (1.15–2.68)	0.009	1.60 (1.01–2.54)	0.046
		Quartiles						
		Q1	1.00 (ref)	---	1.00 (ref)	---	1.00 (ref)	---
		Q2	1.37 (0.86–2.19)	0.18	1.38 (0.86–2.20)	0.19	1.42 (0.85–2.37)	0.18
		Q3	2.01 (1.29–3.11)	0.002	1.86 (1.19–2.91)	0.007	1.73 (1.05–2.84)	0.03
		Q4	2.02 (1.29–3.17)	0.002	1.90 (1.20–3.01)	0.006	1.76 (1.06–2.92)	0.03
		Test for linear trend		0.0006		0.003		0.02
Ischemic stroke#	GDF-15§	Continuous	2.49 (1.66–3.73)	<0.0001	1.74 (1.13–2.68)	0.01	1.45 (0.87–2.42)	0.15
		Quartiles						
		Q1	1.00 (ref)	---	1.00 (ref)	---	1.00 (ref)	---
		Q2	0.41 (0.19–0.92)	0.03	0.37 (0.16–0.84)	0.02	0.39 (0.16–0.95)	0.04
		Q3	1.39 (0.75–2.60)	0.30	1.14 (0.61–2.13)	0.69	1.20 (0.61–2.36)	0.60
		Q4	1.75 (0.92–3.32)	0.09	1.29 (0.67–2.47)	0.44	1.22 (0.59–2.50)	0.59
		Test for linear trend		0.001		0.04		0.12
	sST2§	Continuous	2.34 (1.40–3.91)	0.001	2.04 (1.21–3.43)	0.008	1.77 (1.01–3.12)	0.046
		Quartiles						
		Q1	1.00 (ref)	---	1.00 (ref)	---	1.00 (ref)	---
		Q2	1.36 (0.76–2.45)	0.30	1.42 (0.78–2.57)	0.25	1.47 (0.77–2.80)	0.24

Outcome	Biomarker	Effect	Model 1 (N=3374)*		Model 2 (N=3268)†		Model 3 (N=2741)‡	
			HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value
		Q3	1.95 (1.12–3.40)	0.02	1.86 (1.05–3.30)	0.03	1.82 (0.97–3.41)	0.06
		Q4	2.10 (1.20–3.66)	0.009	2.00 (1.13–3.56)	0.02	1.85 (0.98–3.49)	0.06
		Test for linear trend		0.004		0.01		0.05

Abbreviations: HR, hazards ratio; CI, confidence interval

*Model 1 is adjusted for age at exam 6 and sex.

†Model 2 is adjusted for Model 1 covariates plus FSRP components (systolic blood pressure, hypertension treatment, diabetes, smoking, history of CVD, history of AF).

‡Model 3 is adjusted for Model 2 covariates plus BNP, hs-CRP, and urine albumin.

||Number of events is 203 for model 1, 198 for model 2, and 161 for model 3.

#Number of events is 130 for model 1, 127 for model 2, and 105 for model 3.

§Natural log (ln) transformed.