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ORIGINAL RESEARCH

Growth Differentiation Factor 15 and NT-proBNP as Blood-Based Markers of Vascular Brain Injury and Dementia

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BACKGROUND: GDF15 (growth differentiation factor 15) and NT-proBNP (N-terminal pro-B-type natriuretic peptide) may offer promise as biomarkers for cognitive outcomes, including dementia. We determined the association of these biomarkers with cognitive outcomes in a community-based cohort.

METHODS AND RESULTS: Plasma GDF15 (n=1603) and NT-proBNP levels (n=1590) (53% women; mean age, 68.7 years) were measured in dementia-free Framingham Offspring cohort participants at examination 7 (1998–2001). Participants were followed up for incident dementia. Secondary outcomes included Alzheimer disease dementia, magnetic resonance imaging structural brain measures, and neurocognitive performance. During a median 11.8-year follow-up, 131 participants developed dementia. On multivariable Cox proportional-hazards analysis, higher circulating GDF15 was associated with an increased risk of incident all-cause and Alzheimer disease dementia (hazard ratio [HR] per SD increment in natural log-transformed biomarker value, 1.54 [95% CI, 1.22–1.95] and 1.37 [95% CI, 1.03–1.81], respectively), whereas higher plasma NT-proBNP was also associated with an increased risk of all-cause dementia (HR, 1.32; 95% CI, 1.05–1.65). Elevated GDF15 was associated with lower total brain and hippocampal volumes, greater white matter hyperintensity volume, and poorer cognitive performance. Elevated NT-proBNP was associated with greater white matter hyperintensity volume and poorer cognitive performance. Addition of both biomarkers to a conventional risk factor model improved dementia risk classification (net reclassification improvement index, 0.25; 95% CI, 0.05–0.45).

CONCLUSIONS: Elevated plasma GDF15 and NT-proBNP were associated with vascular brain injury on magnetic resonance imaging, poorer neurocognitive performance, and increased risk of incident dementia in individuals aged >60 years. Both biomarkers improved dementia risk classification beyond that of traditional clinical risk factors, indicating their potential value in predicting incident dementia.

Key Words: biomarker ■ dementia ■ vascular cognitive impairment

Identifying novel biomarkers predictive of increased dementia risk can further our understanding of the complex biological pathways underlying dementia, identify future potential therapeutic targets, and improve overall dementia risk prediction. The plasma biomarkers, GDF15 (growth differentiation factor 15) and NT-proBNP (N-terminal pro-B-type natriuretic peptide), offer promise as potential biomarkers for adverse cognitive outcomes. GDF15, also known as macrophage inhibitory cytokine-1, is a member of the transforming growth factor-b superfamily. Within the nervous system, GDF15 is synthesized by lesioned neurons,

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CLINICAL PERSPECTIVE

What Is New?

- This is the first study to report an association between elevated plasma levels of growth differentiation factor 15 and cognitive decline and incident dementia.
- Our results validate findings from previous studies reporting an association between elevated circulating NT-proBNP (N-terminal pro-B-type natriuretic peptide) levels and risk of incident dementia in a community-based sample of cognitively healthy adults.
- Elevated plasma levels of both biomarkers were also cross-sectionally associated with evidence of vascular injury on magnetic resonance imaging brain (namely, white matter disease hyperintensity volume).

What Are the Clinical Implications?

- Both growth differentiation factor 15 and NT-proBNP improved dementia risk classification beyond that of traditional clinical risk factors, such as hypertension and vascular disease.
- Growth differentiation factor 15 and NT-proBNP offer promise as potential biomarkers for predicting the risk of vascular cognitive impairment and dementia.

Nonstandard Abbreviations and Acronyms

ApoE4	apolipoprotein E4
DM	diabetes mellitus
FHS	Framingham Heart Study
WMHV	white matter hyperintensity volume

microglial cells, and choroid plexus, and plays regulatory roles in inflammation and proapoptosis and antiapoptosis in injured tissues.[1–3] It is also believed to be a marker of vascular stress and endothelial dysfunction,[4] and has previously been associated with cardiovascular outcomes, including myocardial infarction.[5,6] Previous studies have reported an association between elevated plasma GDF15 levels and poorer cognitive performance, a trend toward greater short-term cognitive decline, and an increased burden of white matter disease as well as lower total brain volume.[7–9] However, it is unknown if GDF15 is predictive of an increased risk of cognitive decline over a longer duration of follow-up or with clinically confirmed dementia.

NT-proBNP, a marker of ventricular distention, has previously been associated with an increased risk of adverse cognitive outcomes, including incident dementia.[10–17] However, some of the prior studies on dementia were limited by small sample sizes, use of registry-based diagnoses rather than clinically confirmed dementia, and inclusion of older individuals (aged >75 years) or those with diabetes mellitus (DM), in whom the prevalence of cardiac failure is higher. Validating the results of prior studies[13,17] in a community-based cohort of cognitively healthy adults would further support a potential role of NT-proBNP in dementia risk stratification.

In the present study, we determined the associations of plasma GDF15 and NT-proBNP levels, individually and jointly, with neurocognitive performance, structural magnetic resonance imaging (MRI) brain measures predictive of dementia, and clinically confirmed all-cause and Alzheimer disease (AD) dementia, in a community-based, prospective cohort.

METHODS

Anonymized data and materials have been made publicly available at the National Heart, Lung, and Blood Institute and can be accessed at https://biolincc.nhlbi.nih.gov/home/ and https://www.ncbi.nlm.nih.gov/gap/.

Study Sample

The Framingham Offspring cohort, recruited between 1971 and 1975, is a large, community-based cohort longitudinally followed up for the development of vascular risk factors, cognitive decline, stroke, and dementia for >40 years.[18] Participants are examined approximately every 4 years from study entry. For this study, we included Framingham Offspring cohort participants attending the seventh examination cycle (1998–2001) who had plasma biomarkers measured at this examination, who were free of a diagnosis of dementia, and who had data available on dementia status on follow-up. We excluded individuals aged <60 years at baseline (examination 7) because of the negligible number of incident dementia cases in our cohort before the age of 60 years. The Figure shows the flow of cohort participants. All participants provided written informed consent. The study protocols and consent forms were approved by the institutional review board at the Boston University Medical Center.

Outcome Measures

Our primary outcome measure was incident all-cause dementia developing at any time after the seventh examination and before December 2014. A diagnosis of dementia was based on a review of

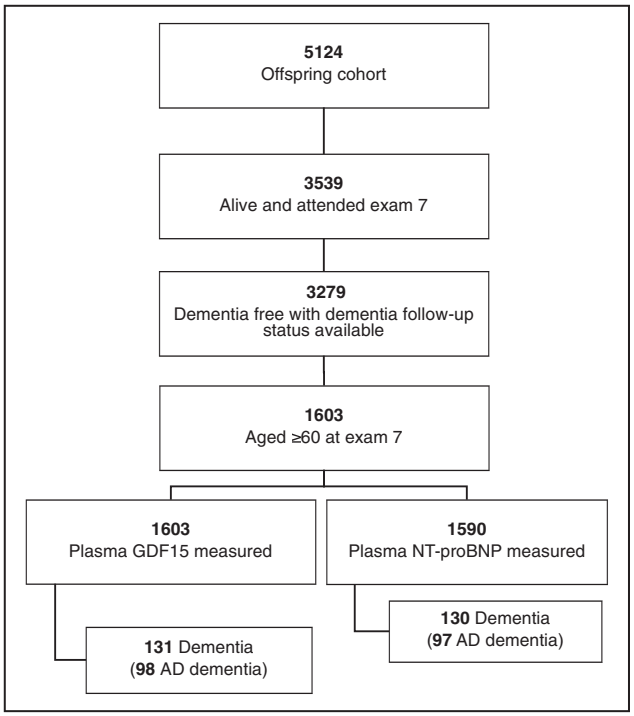

Figure. Flow of cohort participants.
AD indicates Alzheimer disease; GDF15, growth differentiation factor 15; and NT-proBNP, N-terminal pro-B-type natriuretic peptide.

available neurological examination records, neuropsychological assessments, results of neuroimaging investigations, hospital/nursing home/outpatient clinic records, information from family interviews, and autopsy results (when available) by a committee that included at least one neuropsychologist and one neurologist. Dementia was diagnosed according to *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition*, criteria, necessitating impairment in memory and at least one other domain of cognitive function, as well as documented functional disability. AD dementia was included as a secondary outcome measure, and the diagnosis was based on the criteria of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association for definite, probable, or possible AD.[19] Additional secondary outcomes included cognitive performance at examination 7 and annualized change in performance on select neuropsychological tests between examinations 7 and 8, including the trail making test (parts B and A), Hooper visual organization test, visual reproductions delayed recall, similarities test, and logical memory delayed recall and a weighted global cognitive test score as a measure of general cognition. We also evaluated structural MRI brain measures at examination 7, including white matter hyperintensity volume (WMHV), covert brain infarcts, total brain volume, and hippocampal volume as secondary outcomes. Further details on outcome measures, including neurocognitive testing and MRI brain measures, have previously been published and are included in Data S1.[20,21]

Laboratory Measurements of GDF15 and NT-proBNP

GDF15 and NT-proBNP were measured as part of the Systems Approach to Biomarker Research in Cardiovascular Disease Initiative.[22] We selected GDF15 and NT-proBNP from this biomarker panel for inclusion in this study on the basis of a priori evidence of a biologically plausible association with cognitive decline and dementia. Blood plasma samples were obtained at the baseline clinical visit (examination 7). Fasting blood samples were drawn in the early morning from the antecubital vein of participants who had been lying supine for 10 minutes. Samples were centrifuged immediately and stored at −80°C until assays were performed. GDF15 and NT-proBNP were assayed using a modified ELISA sandwich approach, multiplexed on a Luminex xMAP platform (Sigma-Aldrich, St. Louis, MO). More detailed assay methods have previously been published.[22] For GDF15, the lower detection limit was 40 pg/L and the upper detection limit was 20 600 pg/mL. The interassay coefficient of variation ranged from 6.8% to 11.9%. For NT-proBNP, the lower and upper detection limits were 9.71 and 30 100 pg/mL, respectively. The interassay coefficient of variation ranged from 10.3% to 13.3%.

Covariates

We adjusted for baseline demographics and clinical covariates (measured at examination cycle 7), which have previously been associated with risk of dementia[23] (variables were selected on the basis of clinical importance and evidence of prior associations), including age, sex, education (self-reported and categorized as no high school degree, high school degree but no college degree, some college but no degree, and college degree or higher), systolic blood pressure/use of antihypertensive medication, apolipoprotein E4 (ApoE4) carrier status (a carrier was defined as E2/E4, E3/E4, or E4/E4; a noncarrier was defined as E2/E2, E2/E3, or E3/E3), body mass index, current smoking, estimated glomerular filtration rate, prevalent DM, and prevalent cardiovascular disease (CVD; which included peripheral vascular disease [including intermittent claudication]; coronary artery disease [including coronary insufficiency, angina, and myocardial infarction]; cerebrovascular disease [including transient ischemic attack and stroke]; and congestive heart failure]).

Statistical Analysis

To evaluate GDF15 and NT-proBNP as continuous variables (primary analysis), we natural logarithmically transformed and standardized biomarker values to normalize their distributions and facilitate comparisons. We also calculated tertiles of plasma GDF15 and NT-proBNP, comparing the top tertiles with the bottom tertile given an apparent threshold effect at this cutoff for GDF15 using cubic spline plots. We used multivariable-adjusted Cox proportional hazards models to estimate the association between plasma GDF15 and NT-proBNP levels (natural logarithmically transformed values and tertiles) and risk of incident all-cause dementia and AD dementia. Participants were followed up from baseline (examination 7) to the time of the incident event. Participants without incident events were followed up to the time of death or date the participant was last confirmed to be event free (up until December 2014). The assumption of proportional hazards was upheld, and results were reported as hazard ratios (HRs) with corresponding 95% CIs. Model 1 adjusted for age and sex; model 2 (primary model) additionally adjusted for education, systolic blood pressure, use of antihypertensive medication, ApoE4 carrier status, body mass index, current smoking, estimated glomerular filtration rate, prevalent DM, and prevalent CVD; and model 3 additionally adjusted for plasma biomarkers (ie, we adjusted for NT-proBNP in the GDF15 model, and vice versa). We evaluated model discrimination (C-statistic and integrated discrimination improvement) and improvement in risk prediction (net reclassification improvement index) for incident all-cause and AD dementia, following the addition of individual and combined biomarkers to the models.

We used logistic and linear regression models to evaluate the cross-sectional associations between plasma biomarker levels and MRI-based structural brain measures, including covert brain infarcts, WMHV, total brain volume, and hippocampal volume, adjusting for age, age squared (given age and brain volume show a nonlinear association), sex, time from blood draw to MRI brain, systolic blood pressure, use of antihypertensive medication, body mass index, current smoking, estimated glomerular filtration rate, prevalent DM, and prevalent CVD. We fit linear regression models to examine (1) the cross-sectional association between plasma biomarker levels and neuropsychological test performance at examination 7 and (2) the association between plasma biomarkers and annualized change in neuropsychological performance between examinations 7 and 8, adjusting for the covariates listed above. We tested for an interaction for all-cause and AD dementia according to ApoE4 carrier status. We completed sensitivity analyses excluding individuals with a history of stroke (n=42) and those with prevalent congestive heart failure (n=26), as well as using clinical cutoffs (NT-proBNP, 0–<125, 125–<300, and ≥300 pg/mL), similar to those used in a prior recent study.[17] Finally, we completed a summary meta-analysis of combined results from the FHS (Framingham Heart Study) and Hisayama cohorts,[17] adjusting for age and sex, using a fixed effects models because of the low level of heterogeneity between studies (I^2=29% for dementia analysis and 20% for AD dementia analysis). Results were considered significant if P<0.05 for the main analyses and P<0.10 for tests for interactions. Analyses were conducted using SAS v9.4 (SAS Institute Inc, Cary, NC).

RESULTS

For the primary outcome analysis of dementia, the study cohort included 1603 participants for GDF15 and 1590 participants for NT-proBNP. The mean age of participants was 68.7 years (SD, 5.7 years), and 52.7% were women (Table 1). Compared with those in the bottom tertile of GDF15, participants in the top tertile had a higher prevalence of vascular risk factors and disease, including CVD, atrial fibrillation, and prior stroke. Participants in the top tertile of NT-proBNP, compared with the bottom tertile, similarly had an increased vascular risk profile (Table 1). Baseline characteristics according to clinical cutoffs for NT-proBNP (NT-proBNP, 0–<125, 125–<300, and ≥300 pg/mL) are presented in Table S1.

GDF15, NT-proBNP, and Dementia

During a median (quartile 1–quartile 3) 11.8 (7.1–13.3) year follow-up, 131 (8.2%) participants were diagnosed with all-cause dementia, 98 of whom were diagnosed with AD dementia. On multivariable proportional-hazards analysis, adjusting for education status, vascular risk factors, and ApoE4 status, GDF15 was associated with an increased risk of all-cause dementia (HR, 1.54; 95% CI, 1.22–1.95) and AD dementia (HR, 1.37; 95% CI, 1.03–1.81) per SD increment in natural log-transformed biomarker value. After accounting for NT-proBNP, the association was further attenuated but remained significant for all-cause dementia but not AD dementia. Results were consistent on tertile analysis (Tables 2 and 3). There was an interaction according to ApoE4 allele carrier status and risk of AD dementia (P=0.06), with an increased risk in those without the ApoE4 allele (HR, 2.02; 95% CI, 1.22–3.36). NT-proBNP was associated with an increased risk of dementia (HR, 1.32; 95% CI, 1.05–1.65) but not AD dementia (HR, 1.23; 95% CI,

Table 1. Baseline Characteristics

Variable	Overall (n=1603)	GDF15			NT-proBNP		
		Tertile 1 (n=533)	Tertile 2 (n=536)	Tertile 3 (n=534)	Tertile 1 (n=528)	Tertile 2 (n=531)	Tertile 3 (n=531)
Age, mean (SD), y	68.7 (5.7)	68.0 (5.7)	68.6 (5.7)	69.5 (5.6)	68.3 (5.8)	68.6 (5.7)	69.1 (5.6)
Women	844 (52.7)	304 (36.0)	281 (33.3)	259 (30.7)	216 (25.8)	313 (37.4)	308 (36.8)
Systolic blood pressure, mean (SD), mm Hg	132.3 (19.2)	132.0 (19.0)	132.4 (18.8)	132.6 (19.9)	130.2 (15.6)	132.3 (18.7)	134.4 (22.2)
BMI, median (quartile 1–quartile 3), kg/m^2	27.4 (24.7–30.9)	27.1 (24.6–29.9)	27.3 (24.7–30.7)	28.0 (24.8–31.7)	28.2 (25.3–31.1)	27.5 (24.8–30.4)	26.7 (23.9–30.6)
GDF15, median (quartile 1–quartile 3), pg/mL	766.0 (602.0–1010.0)	552.0 (488.0–633.0)	749.0 (661.0–879.0)	1165.0 (936.0–1540.0)	716.5 (577.5–916.5)	746.0 (593.0–974.0)	831.0 (656.0–1150.0)
NT-proBNP, median (quartile 1–quartile 3), pg/mL	272.5 (130.0–538.0)	224.0 (116.0–412.0)	265.5 (128.0–495.0)	364.5 (155.5–798.5)	95.0 (63.3–130.0)	265.0 (192.0–366.0)	752.0 (487.0–1190.0)
Education							
No high school degree	101 (6.5)	22 (21.8)	31 (30.7)	48 (47.5)	28 (27.7)	30 (29.7)	43 (42.6)
High school degree	527 (33.8)	158 (30.0)	183 (34.7)	186 (35.3)	179 (34.2)	172 (32.9)	172 (32.9)
Some years of college	452 (29.0)	148 (32.7)	158 (35.0)	146 (32.3)	150 (33.4)	158 (35.2)	141 (31.4)
College degree	478 (30.7)	188 (39.3)	155 (32.4)	135 (28.2)	155 (32.8)	160 (33.9)	157 (33.3)
Antihypertensive medication	702 (43.9)	187 (26.6	219 (31.2)	296 (42.2)	189 (27.1)	217 (31.1)	292 (41.8)
Current smoker	135 (8.4)	14 (10.4)	36 (26.7)	85 (63.0)	44 (32.8)	55 (41.0)	35 (26.1)
ApoE4 allele	356 (22.5)	110 (30.9)	110 (30.9)	136 (38.2)	102 (28.9)	116 (32.9)	135 (38.2)
Prevalent CVD	303 (18.9)	54 (17.8)	95 (31.4)	154 (50.8)	57 (19.1)	71 (23.8)	171 (57.2)
Atrial fibrillation	95 (5.9)	17 (17.9)	34 (35.8)	44 (46.3)	12 (12.6)	14 (14.7)	69 (72.6)
Stroke	42 (2.6)	7 (16.7)	9 (21.4)	26 (61.9)	11 (26.8)	9 (20.0)	21 (51.2)
CHF	26 (1.6)	0 (0.0)	6 (23.1)	20 (76.9)	2 (7.7)	1 (3.9)	23 (88.5)
eGFR, median (quartile 1–quartile 3), mL/min	77.5 (66.3–87.2)	81.7 (71.6–89.3)	78.2 (67.7–87.4)	70.6 (56.7–83.6)	79.8 (68.4–88.4)	78.2 (67.3–87.1)	74.3 (63.4–85.8)
Diabetes mellitus	263 (16.6)	59 (22.4)	59 (22.4)	145 (55.1)	92 (35.4)	75 (28.9)	93 (35.8)

Data are given as number (percentage), unless otherwise indicated. Baseline demographic and clinical characteristics were defined at examination 7. Stroke and CHF are included in prevalent CVD. The mean (SD) for natural log-transformed GDF15 was 6.7 (0.4); and it was 5.6 (1.1) for NT-proBNP. ApoE4 indicates apolipoprotein E4; BMI, body mass index; CHF, congestive heart failure; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; GDF15, growth differentiation factor 15; and NT-proBNP, N-terminal pro-B-type natriuretic peptide.

Table 2. GDF15 and NT-proBNP and Risk of Incident Dementia

Biomarker	Model 1		Model 2		Model 3	
	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
GDF15						
Per SDU increase	1.57 (1.30–1.90)	<0.0001	1.54 (1.22–1.95)	0.0004	1.45 (1.13–1.85)	0.003
T2 vs T1	1.26 (0.78–2.02)	0.35	1.33 (0.82–2.16)	0.25	1.28 (0.79–2.10)	0.32
T3 vs T1	2.49 (1.61–3.87)	<0.001	2.36 (1.45–3.83)	<0.001	2.16 (1.31–3.56)	0.003
NT-proBNP						
Per SDU increase	1.40 (1.14–1.71)	0.001	1.32 (1.05–1.65)	0.02	1.25 (0.99–1.56)	0.06
T2 vs T1	0.89 (0.56–1.41)	0.61	0.79 (0.49–1.28)	0.34	0.75 (0.47–1.21)	0.24
T3 vs T1	1.82 (1.20–2.75)	0.005	1.62 (1.03–2.55)	0.04	1.43 (0.90–2.28)	0.13

Model 1, adjusted for age and sex. Model 2, adjusted for age, sex, education, systolic blood pressure, use of antihypertensive medication, body mass index, current smoking, estimated glomerular filtration rate, prevalent diabetes mellitus, prevalent cardiovascular disease, and apolipoprotein E4 carrier status. Model 3, model 2+adjustment for GDF15 (NT-proBNP analysis) and NT-proBNP (GDF15 analysis). GDF15 and NT-proBNP were natural logarithmically transformed and standardized. GDF15 indicates growth differentiation factor 15; HR, hazard ratio; NT-proBNP, N-terminal pro-B-type natriuretic peptide; SDU, SD unit; T1, tertile 1; T2, tertile 2; and T3, tertile 3.

0.95–1.61) per SD increase in natural log-transformed value (Tables 2 and 3). There was also a significant interaction with ApoE4 allele carrier status (*P*=0.085), such that the risk of AD dementia associated with NT-proBNP was greater in people without the ApoE4 allele (HR, 2.00; 95% CI, 1.17–3.42), in whom vascular factors may play a larger role. Excluding those with prior stroke and those with a history of congestive heart failure did not significantly alter the results (Tables S2 and S3). In analyses based on clinical cutoffs (NT-proBNP levels of 125–<300 and ≥300 pg/mL, compared with a reference of <125 pg/mL), results were consistent although did not reach significance, likely because of the smaller proportion of individuals with lower NT-proBNP levels in our cohort (Table S4). In a summary meta-analysis of the FHS and Hisayama cohorts combined, NT-proBNP was also associated with an increased risk of dementia (HR, 1.42; 95% CI, 1.30–1.56) and AD dementia (HR, 1.31; 95% CI, 1.16–148) per average SD increment in natural log-transformed biomarker value (Table S5).

Risk Prediction for Dementia

The C-statistic for the model of conventional risk factors for dementia was 0.81 (95% CI, 0.77–0.84), with no significant change following the addition of GDF15, NT-proBNP, or both to the model. However, addition of GDF15 and NT-proBNP resulted in a relative integrated discrimination improvement of 15% (95% CI, 7%–24%) compared with the base model. Following the addition of both biomarkers to a conventional risk factor model, 18% of individuals with dementia were correctly assigned a higher predicted risk, whereas 7% of

Table 3. GDF15 and NT-proBNP and Risk of Incident AD Dementia

Biomarker	Model 1		Model 2		Model 3	
	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
GDF15						
Per SDU increase	1.48 (1.18–1.86)	<0.001	1.37 (1.03–1.81)	0.03	1.28 (0.96–1.72)	0.09
T2 vs T1	0.97 (0.56–1.68)	0.92	1.01 (0.58–1.77)	0.96	0.99 (0.56–1.75)	0.98
T3 vs T1	2.37 (1.45–3.85)	<0.001	2.07 (1.20–3.56)	0.009	1.95 (1.12–3.42)	0.02
NT-proBNP						
Per SDU increase	1.34 (1.06–1.70)	0.02	1.23 (0.95–1.61)	0.12	1.19 (0.91–1.55)	0.21
T2 vs T1	0.94 (0.56–1.58)	0.81	0.80 (0.47–1.38)	0.43	0.78 (0.45–1.33)	0.36
T3 vs T1	1.60 (0.98–2.61)	0.06	1.37 (0.80–2.32)	0.25	1.23 (0.71–2.13)	0.45

Model 1, adjusted for age and sex. Model 2, adjusted for age, sex, education, systolic blood pressure, use of antihypertensive medication, body mass index, current smoking, estimated glomerular filtration rate, prevalent diabetes mellitus, prevalent cardiovascular disease, and apolipoprotein E4 carrier status. Model 3, model 2+adjustment for GDF15 (NT-proBNP analysis) and NT-proBNP (GDF15 analysis). GDF15 and NT-proBNP were natural logarithmically transformed and standardized. AD indicates Alzheimer disease; GDF15, growth differentiation factor 15; HR, hazard ratio; NT-proBNP, N-terminal pro-B-type natriuretic peptide; SDU, SD unit; T1, tertile 1; T2, tertile 2; and T3, tertile 3.

individuals without dementia were correctly assigned a lower predicted risk (overall net reclassification improvement, 0.25; 95% CI, 0.05–0.45) (Table S6).

GDF15, NT-proBNP, and Structural Brain Measures

Elevated GDF15 was associated with lower total brain volume (–0.38±0.06; *P*<0.001), hippocampal volume (–0.003±0.002; *P*=0.046), and greater WMHV (0.07±0.03; *P*=0.02) per SD unit increment in natural log-transformed biomarker value. Results were consistent when GDF15 was assessed by tertiles. Elevated plasma NT-proBNP was associated with increased WMHV (0.05±0.02; *P*=0.048; per SD unit increment) but not with other structural MRI brain measures (Table 4).

GDF15, NT-proBNP, and Neurocognitive Performance

Elevated circulating GDF15 levels were cross-sectionally associated with poorer global cognitive performance (ß±SE, –0.08±0.02; *P*=0.002), as well as poorer performance on individual tests of abstract reasoning (similarities, –0.20±0.10; *P*=0.04), visual memory (visual reproductions delayed recall, –0.24±0.09; *P*=0.009), and visuospatial processing (Hooper visual organization test, –0.03±0.01; *P*=0.02), per SD increment in natural log-transformed GDF15 value. Higher circulating NT-proBNP was also cross-sectionally associated with poorer global cognitive performance (–0.04±0.02; *P*=0.03) as well as poorer performance on visual reproductions delayed recall (–0.16±0.08; *P*=0.04), per SD increment in natural log-transformed NT-proBNP value (Table S7). Elevated plasma GDF15 levels were also associated with greater annualized decline in performance on tests of global cognition, logical memory, executive function, and visuospatial processing (Table S8).

DISCUSSION

In our study, we observed that elevated plasma levels of GDF15 and NT-proBNP were associated with an increased risk of incident dementia over a median 12-year follow-up. Both biomarkers improved dementia risk classification beyond that of traditional clinical risk factors.

Elevated plasma GDF15 and NT-proBNP were also associated with increased WMHV and poorer cognitive performance, whereas GDF15 alone was associated with lower total brain volume and cognitive decline.

There exists an important need for valid, reliable biomarkers for vascular cognitive impairment and dementia.[24,25] Such biomarkers could be used to monitor disease severity and progression, identify disease at a preclinical stage and serve as surrogate outcomes in early-phase clinical trials of vascular cognitive impairment and dementia, and improve our understanding of underlying disease mechanisms. Both GDF15 and NT-proBNP offer promise as potential biomarkers for predicting the risk of vascular cognitive impairment and dementia. We observed an increased risk of clinical all-cause dementia and AD dementia in association with elevated plasma GDF15 levels. Although previous studies have reported an association between elevated plasma GDF15 levels and poorer cognitive performance and a trend toward short-term (2-year) cognitive decline,[8] to our knowledge, this is the first study to report an association between plasma GDF15 and both cognitive decline in cognitively normal middle-aged and older adults and incident clinical dementia and AD dementia. We also found that elevated plasma GDF15

Table 4. GDF15, NT-proBNP, and MRI Markers of Structural Brain Injury

	TBV, %		Hippocampal Volume, %		WMHV, %*		Covert Brain Infarcts	
Biomarker	β±SE	*P* Value	β±SE	*P* Value	β±SE	*P* Value	OR (95% CI)	*P* Value
GDF15								
Per SDU increase†	–0.38±0.06	<0.001	–0.003±0.002	0.046	0.07±0.03	0.02	1.14 (0.92–1.40)	0.23
T2 vs T1	0.05±0.10	0.61	0.002±0.002	0.55	–0.03±0.05	0.60	0.92 (0.63–1.35)	0.53
T3 vs T1	–0.40±0.11	<0.001	–0.002±0.003	0.59	0.12±0.05	0.02	1.05 (0.70–1.56)	0.62
NT-proBNP								
Per SDU increase†	–0.03±0.05	0.51	0.0001±0.001	0.92	0.05±0.02	0.048	1.08 (0.90–1.30)	0.39
T2 vs T1	0.11±0.10	0.30	–0.0003±0.003	0.90	0.02±0.05	0.70	1.32 (0.90–1.92)	0.18
T3 vs T1	–0.11±0.11	0.29	–0.001±0.003	0.82	0.10±0.05	0.045	1.13 (0.76–1.68)	0.91

Model, adjusted for age, age squared, sex, time from blood draw to MRI brain, systolic blood pressure, use of antihypertensive medication, body mass index, current smoking, estimated glomerular filtration rate, prevalent diabetes mellitus, and prevalent cardiovascular disease. GDF15 indicates growth differentiation factor 15; MRI, magnetic resonance imaging; NT-proBNP, N-terminal pro-B-type natriuretic peptide; OR, odds ratio; SDU, SD unit; T1, tertile 1; T2, tertile 2; T3, tertile 3; TBV, total brain volume; and WMHV, white matter hyperintensity volume.

*Natural log transformed.

†Natural log transformed and standardized.

levels were associated with greater annualized decline in performance on tests of global cognition, logical memory, executive function, and visuospatial processing, suggesting that longer follow-up of this cohort may result in even stronger associations with incident dementia.

GDF15 is believed to be a marker of vascular stress and impaired endothelial function.[4] Elevated circulating GDF15 has previously been associated with cardiovascular outcomes, including myocardial infarction,[5,6] suggesting that GDF15 levels may reflect an elevated vascular risk profile. Indeed, in our cohort, individuals in the top tertile of GDF15 had a higher prevalence of vascular risk factors, including current smoking, DM, CVD, and stroke, compared with those in the bottom tertiles. In addition, we observed a cross-sectional association between elevated plasma GDF15 levels and greater white matter disease burden, which has also previously been reported at a different examination cycle in the Framingham cohort.[7] However, after accounting for the effects of vascular risk factors as well as NT-proBNP, the association between plasma GDF15 levels and dementia and AD dementia remained significant. In addition, we observed no association between covert brain infarct volume and GDF15 levels. Thus, it is unlikely that increased vascular risk factor burden and subclinical vascular brain injury solely account for these associations. Interestingly, we observed an interaction between circulating GDF15 levels and risk of AD dementia, but not overall dementia, according to ApoE4 carrier status, with an increased risk of AD dementia in those without the ApoE4 allele compared with those with this allele, suggesting that the association with clinical disease in these people is not mediated through ApoE-related pathways. GDF15 is also known to play a role in inflammation and apoptosis in damaged tissues.[1–3] Elevated GDF15 may be a marker of a proinflammatory environment predisposing to increased white matter disease and subsequent dementia. In a Drosophila model of the neurodegenerative condition, Huntington disease, GDF15 was reported to be upregulated under conditions of stress. Use of pharmacological inhibition and RNA interference to normalize GDF15 function resulted in marked protection against stress-induced apoptosis as well as Huntington disease–related neurodegeneration (slowing of neuronal loss), suggesting GDF15 may be a potential therapeutic target to modulate the risk of other neurodegenerative diseases, including dementia and AD dementia.[26]

We also observed an association between elevated circulating NT-proBNP levels and risk of clinically confirmed incident dementia but not AD dementia. Previous studies have reported similar associations in an older population and in patients with DM[11,12] as well as in 2 larger cohorts of community-dwelling individuals.[13,17] In the Hisayama cohort, serum NT-proBNP levels of ≥300 pg/mL, compared with ≤54 pg/mL, were associated with an increased risk of all-cause dementia, after accounting for age and sex (HR, 3.02; 95% CI, 2.08–4.37).[17] In our study, plasma NT-proBNP levels of ≥300 pg/mL, compared with <125 pg/mL, were not associated with risk of all-cause dementia after accounting for age and sex, although the trend approached significance (HR, 1.66; 95% CI, 0.95–2.92). Given the small number of individuals with NT-proBNP ≤54 pg/mL in our study (n=100; 6% of the sample), we were unable to adopt the same reference category used in the Hisayama cohort. Furthermore, >50% of the individuals in our cohort had NT-proBNP levels ≥300 pg/mL, compared with 11.6% in the Hisayama cohort, further precluding a direct comparison of our results. However, our findings do validate the results of these previous studies among a community-based population of cognitively healthy adults whose dementia diagnoses were confirmed on the basis of clinical assessments, medical record review by a behavioral neurologist, and rigorous application of standardized criteria. In addition, in a combined summary meta-analysis of our results with those of the Hisayama cohort, results remained significant with minimal variation in effect sizes attributable to differences between studies.

NT-proBNP is a marker of left ventricular distention in patients with heart failure and is associated with an increased risk of cardiovascular events, including ischemic stroke and atrial fibrillation. Thus, the association between NT-proBNP and dementia risk is likely mediated through increased vascular risk and subclinical vascular disease. Indeed, in our cohort, participants in the top tertile of NT-proBNP, compared with the bottom tertile, had higher baseline systolic blood pressures, and were more likely to be on antihypertensive medication or have a history of atrial fibrillation, CVD, or stroke. Furthermore, we found that NT-proBNP was associated with MRI brain measures predictive of vascular dementia, including a greater burden of white matter disease, as well as with poorer performance on tests of visual memory and global cognition. The association between plasma NT-proBNP levels and both dementia and AD dementia was attenuated, and no longer significant, after accounting for vascular risk factors and GDF15 levels, also supporting a vascular-mediated hypothesis. In 2 previous studies,[13,17] elevated serum NT-proBNP was associated with an increased risk of AD dementia; thus, it is also possible that our study was underpowered to detect an association with AD dementia. Plasma NT-proBNP is readily available in a clinical setting and offers potential prognostic value as a candidate biomarker for more accurately predicting risk of dementia in cognitively healthy adults, including those without congestive heart failure, although appropriate clinical cutoffs need to be determined.

When looking at the relative integrated discrimination improvement index, we found that addition of GDF15 and NT-proBNP resulted in a relative integrated discrimination improvement index of 15% compared with the base model, indicating that measurement of these biomarkers provides incremental predictive value beyond that of conventional risk factors. Addition of both biomarkers to conventional risk factors improved dementia risk classification, in that 18% of individuals with dementia were correctly assigned a higher predicted risk of dementia, whereas 7% of individuals without dementia were correctly assigned a lower predicted risk, supporting a potential role for circulating GDF15 and NT-proBNP as biomarkers for predicting dementia risk.

Our study has several strengths, including a population confirmed to be free of clinical dementia at the baseline examination, use of intensive surveillance procedures to detect new cases of dementia or cognitive decline, comprehensive phenotyping of the cohort, a large number of individuals with measured plasma biomarker values, and a relatively long duration of follow-up. An important limitation is the predominantly White population, which limits the generalizability of our findings to other ethnicities. In addition, our analyses did not adjust for a history of sleep apnea or cancer. Finally, we were unable to account for changes in plasma biomarker values over time, as data on repeated measures were not available.

CONCLUSIONS

Elevated plasma GDF15 and NT-proBNP are associated with a greater burden of white matter disease, increased brain atrophy (GDF15 alone), poorer cognitive performance, and an increased risk of clinically confirmed dementia and AD dementia (GDF15 alone) in individuals aged >60 years. Both biomarkers improve dementia risk classification beyond that of traditional clinical risk factors. GDF15 and NT-proBNP may be useful circulating biomarkers for vascular brain injury and dementia in the general population.

ARTICLE INFORMATION

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Disclosures

None.

Supplementary Materials

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SUPPLEMENTAL MATERIAL

Data S1.

Supplemental Methods

Outcomes

Primary outcome*:* Starting at examination five, all participants were systematically screened for the development of dementia via the Mini-Mental State Examination (MMSE) and annual health status updates, and starting from examination seven, all participants were invited to complete an MRI brain and neuropsychological testing. If a participant, family member, or Framingham study physician was concerned about cognitive impairment, or the Mini-Mental State Examination (MMSE) score was below the education-based cutoff, three points lower than the preceding examination, or five points lower than the participant's previous highest recorded score, more in-depth cognitive testing was performed.[27] Participants with suspected cognitive impairment who did not meet diagnostic criteria for dementia underwent additional yearly neuropsychological assessments between the scheduled Offspring examinations.

Secondary outcomes: Removal of non-brain tissues: The skull is removed using an atlas-based method[28] followed by human quality control to provide generally minor cleanup if needed. Structural MRI brain images are then nonlinearly registered performed by a cubic B-spline deformation[29] to a minimal deformation template (MDT) synthetic brain image.[30] Image intensity inhomogeneity correction: B1 field inhomogeneity is a common problem that limits the precision of image segmentation. We utilize a template-based iterative method for correcting field inhomogeneity bias.[31] Gray matter, white matter and CSF measurement: our segmentation algorithm is based on an expectation-maximization (EM) algorithm that iteratively refines its segmentation estimates to produce outputs that are most consistent with the input intensities from the native-space T1 images along with a model of image smoothness.[32, 33] The segmentation yielded by these appearance models alone is refined using a Markov random field (MRF) model based on an adaptive priors model.[33] The MRF-based segmentation at the final iteration is used as the final output segmentation. Total brain volume (TBV) is defined as supratentorial brain volume as a percentage of the intracranial volume determined from coronal sections. White matter hyperintensity (WMH) is performed on a combination of FLAIR and 3D T1 images using a modified Bayesian probability structure based on a previously published method of histogram fitting.[34] Prior probability maps for WMH were created from more than 700 individuals with semi-automatic detection of WMH followed by manual editing. Likelihood estimates of the native image are calculated through histogram segmentation and thresholding. All segmentation is initially performed in standard space resulting in probability likelihood values of WMH at each voxel in the white matter. These probabilities are then thresholded at 3.5 SD above the mean to create a binary WMH mask. Further segmentation is based on a modified Bayesian approach that combines image likelihood estimates, spatial priors and tissue class constraints. The segmented WMH masks are then back-transformed on to native space for tissue volume calculation. Volumes are log-transformed to normalize population variance. The automatic hippocampal segmentation method employs a standard atlas based diffeomorphic approach,[35] with the minor modification of label refinement. We further modified this approach to include the European Alzheimer's Disease Consortium-Alzheimer's Disease Neuroimaging Initiative harmonized hippocampal masks using the following approach: 1) Subject image pre-processing with extraction of intracranial cavity, non-uniformity correction, tissue classification as discussed above; 2) Atlas Registration of all EADC-ADNI hippocampal masks[36-40] to each subject; 3) Atlas Fusion utilizing multi-atlas label fusion;[41, 42] and 4) Intensity-based label refinement. Covert brain infarcts (CBI): the presence of MRI infarction was determined from the size, location and imaging characteristics of the lesion. The image analysis system allowed for superimposition of the subtraction image, the proton density image and the T2 weighted image at three times magnified view to assist in interpretation of lesion characteristics. Signal void, best seen on the T2 weighted image, was interpreted to indicate a vessel. Only lesions 3mm or larger qualified for consideration as cerebral infarcts. Other necessary imaging characteristics included: 1) CSF density on the subtraction image, and 2) if the stroke was in the basal ganglia area, distinct separation from the circle of Willis vessels. Kappa values for agreement amongst the three raters are generally good and range from 0.73 to 0.90.[43, 44] Imaging data was centrally processed at the Imaging of Dementia and Aging (IDeA) laboratory located at UC Davis and analyzed by operators blinded to all participant characteristics including cognitive performance on neuropsychological testing.

The global cognitive performance outcome was created using principal component analysis and forcing a single score solution, combining weighted loadings for the individual cognitive tests described above.

Table S1. Baseline characteristics according to clinical cut-offs for NT-proBNP.

Variable No. (%)	**NT-proBNP** **0 to <125 pg/mL** **(n=377)**	**NT-proBNP** **125 to <300 pg/mL** **(n=468)**	**NT-proBNP** **≥300 pg/mL** **(n=745)**
Age, y, mean (SD)	66.4 (4.8)	67.5 (5.3)	70.6 (5.8)
Women	137 (36.3)	253 (54.1)	447 (60.0)
Systolic blood pressure, mmHg, mean (SD)	129.7 (15.7)	130.3 (17.3)	134.9 (21.3)
BMI, kg/m^2, median (Q1, Q3)	28.4 (25.8-31.8)	27.4 (24.8-30.8)	26.9 (24.1-30.4)
GDF15, pg/mL, median (Q1, Q3)	687.0 (878.0, 2640.0)	696.5 (579.0, 906.5)	858.0 (671.0, 1150.0)
Education			
No high school degree	21 (5.8)	20 (4.4)	60 (8.3)
High school degree	124 (34.0)	152 (33.5)	247 (34.0)
Some years of college	102 (28.0)	142 (31.3)	205 (28.2)
College degree	118 (32.3)	140 (30.9)	214 (29.5)
Anti-hypertensive medication	128 (34.0)	177 (37.9)	393 (52.8)
Current smoker	35 (9.3)	46 (9.9)	53 (7.1)
ApoE4 allele	75 (20.2)	97 (21.2)	181 (24.5)
Prevalent CVD	34 (9.0)	48 (10.3)	217 (29.1)
Atrial fibrillation	6 (1.6)	14 (3.0)	75 (10.1)
Stroke	7 (1.9)	7 (1.5)	27 (3.6)
CHF	2 (0.5)	1 (0.2)	23 (3.1)
eGFR, ml/min, median (Q1, Q3)	81.5 (70.4-90.0)	79.9 (69.6, 88.5)	72.8 (62.6, 84.5)
Diabetes mellitus	60 (16.2)	67 (14.5)	133 (18.0)

GDF15, growth differentiation factor 15; NT-proBNP, N-terminal prohormone of brain natriuretic peptide; SD, standard deviation; CVD, cardiovascular disease; APOE E4, apolipoprotein E4 allele; CHF, congestive heart failure; eGFR, estimated glomerular filtration rate.

Table S2. GDF15 and NT-proBNP and risk of incident dementia and AD, excluding those with prior stroke.

	Dementia						Alzheimer's disease					
	Model 1		Model 2		Model 3		Model 1		Model 2		Model 3	
Biomarker	**HR (95% CI)**	**P-value**	**HR (95% CI)**	**P-value**	**HR (95% CI)**	**P-value**	**HR (95% CI)**	**P-value**	**HR (95% CI)**	**P-value**	**HR (95% CI)**	**P-value**
GDF15												
Per SDU increase	1.57 (1.30-1.91)	<0.001	1.57 (1.23-1.99)	<0.001	1.46 (1.14-1.88)	0.003	1.47 (1.16-1.85)	0.001	1.39 (1.05-1.85)	0.02	1.30 (0.97-1.74)	0.08
T2 versus T1	1.25 (0.77-2.02)	0.36	1.34 (0.82-2.19)	0.25	1.25 (0.76-2.05)	0.38	0.93 (0.53-1.61)	0.79	0.99 (0.57-1.75)	0.98	0.94 (0.53-1.66)	0.83
T3 versus T1	2.46 (1.57-3.86)	<0.001	2.38 (1.46-3.89)	<0.001	2.11 (1.27-3.51)	0.004	2.26 (1.38-3.71))	0.001	2.08 (1.21-3.59)	0.008	1.91 (1.09-3.35)	0.02
NT-proBNP												
Per SDU increase	1.47 (1.19-1.80)	<0.001	1.39 (1.10-1.75)	0.005	1.31 (1.04-1.65)	0.02	1.41 (1.10-1.80)	0.006	1.30 (0.99-1.70)	0.06	1.24 (0.95-1.63)	0.11
T2 versus T1	1.04 (0.65-1.67)	0.87	0.99 (0.61-1.62)	0.97	0.94 (0.57-1.53)	0.79	1.09 (0.64-1.87)	0.74	1.00 (0.57-1.74)	0.99	0.96 (0.55-1.67)	0.89
T3 versus T1	1.97 (1.27-3.05)	0.002	1.79 (1.12-2.86)	0.02	1.57 (0.96-2.54)	0.07	1.74 (1.04-2.89)	0.03	1.51 (0.87-2.61)	0.14	1.35 (0.77-2.39)	0.30

GDF15, growth differentiation factor 15; NT-proBNP, N-terminal prohormone of brain natriuretic peptide; HR, hazard ratio; CI, confidence interval; ; T1, tertile 1; T2, tertile 2; T3, tertile 3

Model 1: adjusted for age and sex.

Model 2: adjusted for age, sex, education, systolic blood pressure, use of antihypertensive medication, body mass index, current smoking, estimated glomerular filtration rate, prevalent diabetes mellitus, prevalent cardiovascular disease and ApoE4 carrier status

Model 3: Model 2 + adjustment for GDF15 (NT-proBNP analysis) and NT-ProBNP (GDF15 analysis)GDF15 and NT-proBNP were natural logarithmically transformed and standardized

Table S3. GDF15 and NT-proBNP and risk of incident dementia and AD, excluding those with CHF.

	Dementia						Alzheimer's disease					
	Model 1		Model 2		Model 3		Model 1		Model 2		Model 3	
Biomarker	**HR (95% CI)**	**P-value**	**HR (95% CI)**	**P-value**	**HR (95% CI)**	**P-value**	**HR (95% CI)**	**P-value**	**HR (95% CI)**	**P-value**	**HR (95% CI)**	**P-value**
GDF15												
Per SDU increase	1.59 (1.31-1.92)	<0.001	1.54 (1.21-1.95)	<0.001	1.44 (1.13-1.84)	0.004	1.49 (1.19-1.88)	<0.001	1.37 (1.03-1.81)	0.03	1.28 (0.96-1.70)	0.10
T2 versus T1	1.20 (0.75-1.94)	0.45	1.28 (0.78-2.08)	0.33	1.22 (0.75-2.01)	0.43	0.94 (0.54-1.64)	0.83	0.99 (0.56-1.74)	0.97	0.95 (0.54-1.69)	0.87
T3 versus T1	2.51 (1.62-3.91)	<0.001	2.38 (1.46-3.88)	<0.001	2.18 (1.32-3.60)	0.002	2.41 (1.48-3.93)	<0.001	2.14 (1.24-3.68)	0.006	2.00 (1.14-3.50)	0.02
NT-proBNP												
Per SDU increase	1.41 (1.15-1.74)	0.001	1.35 (1.07-1.71)	0.01	1.28 (1.02-1.62)	0.04	1.36 (1.07-1.74)	0.01	1.28 (0.98-1.68)	0.07	1.24 (0.94-1.62)	0.13
T2 versus T1	0.91 (0.57-1.44)	0.68	0.81 (0.50-1.32)	0.39	0.77 (0.47-1.24)	0.28	0.97 (0.57-1.63)	0.90	0.82 (0.47-1.42)	0.48	0.79 (0.46-1.36)	0.39
T3 versus T1	1.84 (1.20-2.81)	0.005	1.70 (1.07-2.69)	0.03	1.50 (0.94-2.41)	0.09	1.68 (1.02-2.75)	0.04	1.48 (0.87-2.54)	0.15	1.35 (0.77-2.34)	0.29

GDF15, growth differentiation factor 15; NT-proBNP, N-terminal prohormone of brain natriuretic peptide; HR, hazard ratio; CI, confidence interval; T1, tertile 1; T2, tertile 2; T3, tertile 3

Model 1: adjusted for age and sex.

Model 2: adjusted for age, sex, education, systolic blood pressure, use of antihypertensive medication, body mass index, current smoking, estimated glomerular filtration rate, prevalent diabetes mellitus, prevalent cardiovascular disease and ApoE4 carrier status.

Model 3: Model 2 + adjustment for GDF15 (NT-proBNP analysis) and NT-ProBNP (GDF15 analysis)

GDF15 and NT-proBNP were natural logarithmically transformed and standardized

Table S4. Risk of incident dementia and AD dementia by NT-proBNP clinical cut-offs.

	Dementia				Alzheimer's disease dementia			
	Model 1		Model 2		Model 1		Model 2	
NT-proBNP	**HR (95% CI)**	**P-value**	**HR (95% CI)**	**P-value**	**HR (95% CI)**	**P-value**	**HR (95% CI)**	**P-value**
0-124.9 pg/mL	ref		ref		ref		ref	
125-299.9 pg/mL	1.14 (0.61-2.12)	0.68	1.32 (0.69-2.52)	0.41	1.27 (0.62-2.63)	0.51	1.42 (0.67-3.00)	0.36
≥300 pg/mL	1.66 (0.95-2.92)	0.08	1.63 (0.89-2.99)	0.11	1.57 (0.80-3.07)	0.20	1.48 (0.73-3.03)	0.28

Model 1: adjusted for age and sex.
Model 2: adjusted for age, sex, education, systolic blood pressure, use of antihypertensive medication, body mass index, current smoking, estimated glomerular filtration rate, prevalent diabetes mellitus, prevalent cardiovascular disease and ApoE4 carrier status.
NT-proBNP was natural logarithmically transformed and standardized.

Table S5. Summary meta-analysis.

	FHS		Hisayama		Combined meta-analysis		
	N	HR (95% CI)	N	HR (95% CI)	N	HR (95% CI)	p
Dementia	1590	1.40 (1.14, 1.71)	1635	1.43 (1.29, 1.59)	3225	1.42 (1.30, 1.56)	<0.001
AD Dementia	1590	1.34 (1.06, 1.70)	1635	1.30 (1.13, 1.49)	3225	1.31 (1.16, 1.48)	<0.001

Model adjusted for age and sex.
NT-proBNP was natural logarithmically transformed and standardized.

Table S6. Model discrimination and risk reclassification following addition of GDF15 and NT-proBNP.

	Dementia				Alzheimer's disease dementia			
	C-statistic (95% CI)	Relative IDI (95% CI)	Overall NRI (95% CI)	*NRI, events NRI, nonevents	C-statistic (95% CI)	Relative IDI Statistic (95% CI)	Overall NRI Statistic (95% CI)	*NRI, events NRI, nonevents
Model 2	0.81 (0.77-0.84)	-	-	-	0.85 (0.81-0.88)	-	-	-
Model 2 + GDF15	0.82 (0.78-0.85)	0.11 (0.04-0.18)	0.27 (0.06-0.48)	0.18 0.09	0.85 (0.81-0.88)	0.06 (0.02-0.11)	0.29 (0.06-0.52)	0.22 0.07
Model 2 + NT-proBNP	0.81 (0.77-0.85)	0.05 (0.01-0.09)	0.21 (0.02-0.40)	0.19 0.02	0.85 (0.81-0.88)	0.02 (-0.004-0.05)	0.18 (-0.03-0.40)	0.19 -0.01
Model 2 + GDF15 and NT-proBNP	0.82 (0.78-0.85)	0.15 (0.07-0.24)	0.25 (0.05-0.45)	0.18 0.07	0.85 (0.81-0.88)	0.08 (0.03-0.14)	0.18 (-0.06-0.41)	0.16 0.02

IDI, integrated discrimination improvement; NRI, net reclassification improvement; GDF15, growth differentiation factor 15; NT-proBNP, N-terminal prohormone of brain natriuretic peptide; CI, confidence interval.
Model 2: adjusted for age, sex, education, systolic blood pressure, use of antihypertensive medication, body mass index, current smoking, estimated glomerular filtration rate, prevalent diabetes mellitus, prevalent cardiovascular disease and ApoE4 carrier status.
GDF15 and NT-proBNP were natural logarithmically transformed and standardized
* Proportion of events correctly reclassified
Proportion of non-events correctly reclassified
† Versus model 2

Table S7. GDF15, NT-proBNP and neuropsychological test performance.

	Global cognition (weighted score units)		Similarities (n correct)		Visual Reproductions (n correct after delay)		Logical Memory (n correct after delay)		Trail Making B-A (min)†		Hooper Visual Organization Test**	
Biomarker	β±SE	p-value	β±SE	p-value	β±SE	p-value	β±SE	p-value	β±SE	p-value	β±SE	p-value
GDF15												
Per SDU increase*	-0.08±0.02	0.002	-0.20±0.10	0.04	-0.24±0.09	0.009	0.04±0.10	0.72	-0.01±0.01	0.12	-0.03±0.01	0.02
T2 versus T1	0.03±0.04	041	0.01±0.16	0.95	0.12±0.16	0.45	0.33±0.17	0.05	0.01±0.01	0.50	0.02±0.03	0.50
T3 versus T1	-0.11±0.04	0.01	-0.26±0.17	0.13	-0.35±0.17	0.04	0.13±0.19	0.49	-0.02±0.01	0.17	-0.04±0.03	0.12
NT-proBNP												
Per SDU increase*	-0.04±0.02	0.03	-0.14±0.08	0.07	-0.16±0.08	0.04	-0.04±0.08	0.65	-0.01±0.01	0.31	-0.01±0.01	0.39
T2 versus T1	0.01±0.04	0.76	0.11±0.16	0.50	0.03±0.16	0.85	0.10±0.17	0.57	-0.002±0.01	0.86	0.03±0.03	0.31
T3 versus T1	-0.07±0.04	0.09	-0.17±0.17	0.30	-0.38±0.16	0.02	0.03±0.18	0.87	-0.01±0.01	0.33	-0.02±0.03	0.36

GDF15, growth differentiation factor 15; NT-proBNP, N-terminal prohormone of brain natriuretic peptide; SDU, Standard deviation units; SE, Standard error. Model: adjusted for age, age squared, sex, education, time from blood draw to neuropsychological testing, systolic blood pressure, use of antihypertensive medication, body mass index, current smoking, estimated glomerular filtration rate, prevalent diabetes mellitus and prevalent cardiovascular disease.
*Natural log transformed and standardized
†Natural log transformed to restore normality (higher scores indicate better performance)

Table S8. GDF15, NT-proBNP and annualized change in neuropsychological test performance.

	Global cognition		Similarities		Visual Reproductions		Logical Memory		Trail Making B-A		Hooper Visual Organization Test	
Biomarker	β±SE	p-value	β±SE	p-value	β±SE	p-value	β±SE	p-value	β±SE	p-value	β±SE	p-value
GDF15												
Per SDU increase*	-0.06±0.02	0.008	-0.03±0.02	0.14	-0.03±0.02	0.17	-0.05±0.02	0.046	0.02±0.01	0.002	-0.06±0.02	0.0002
T2 versus T1	-0.03±0.04	0.51	-0.02±0.03	0.54	-0.02±0.03	0.43	-0.02±0.04	0.67	-0.004±0.01	0.75	-0.03±0.02	0.23
T3 versus T1	-0.11±0.04	0.009	-0.04±0.03	0.23	-0.03±0.03	0.31	-0.05±0.04	0.24	0.02±0.01	0.11	-0.09±0.03	0.001
NT-proBNP												
Per SDU increase*	-0.02±0.02	0.26	-0.02±0.02	0.35	0.02±0.01	0.29	-0.02±0.02	0.25	-0.003±0.01	0.60	-0.03±0.01	0.02
T2 versus T1	-0.02±0.04	0.67	0.003±0.03	0.92	0.003±0.03	0.93	-0.01±0.04	0.75	-0.005±0.01	0.69	-0.03±0.02	0.28
T3 versus T1	-0.04±0.04	0.30	-0.02±0.03	0.46	0.04±0.03	0.21	-0.04±0.04	0.28	-0.004±0.01	0.76	-0.06±0.03	0.02

GDF15, growth differentiation factor 15; NT-proBNP, N-terminal prohormone of brain natriuretic peptide; SDU, Standard deviation units; SE, Standard error.
Model: adjusted for age, age squared, sex, education, time from blood draw to neuropsychological testing, systolic blood pressure, use of antihypertensive medication, body mass index, current smoking, estimated glomerular filtration rate, prevalent diabetes mellitus and prevalent cardiovascular disease.
*Natural log transformed and standardized
†Natural log transformed