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Authors

Newman, Anne
Patel, Sheena
Kizer, Jorge
et al.

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Research Article

Evaluation of Associations of Growth Differentiation Factor-11, Growth Differentiation Factor-8, and Their Binding Proteins Follistatin and Follistatin-Like Protein-3 With Dementia and Cognition

Anne B. Newman, MD, MPH,[1] Sheena Patel, MPH,[2,3] Jorge R. Kizer, MD,[4,5] Se-Jin Lee, MD, PhD,[6] Shalinder Bhasin, MD,[7] Peggy Cawthon, PhD, MPH,[2,3] Nathan LeBrasseur, PhD,[8] Russel P. Tracy, PhD,[9] Peter Ganz, MD,[10] and Steven R. Cummings, MD[2,3]

[1]Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania, USA. [2]Research Institute, California Pacific Medical Center, University of California, San Francisco, San Francisco, California, USA. [3]Department of Epidemiology and Biostatistics, University of California, San Francisco, San Francisco, California, USA. [4]Cardiology Section, San Francisco Veterans Affairs Health Care System, San Francisco, California, USA. [5]Departments of Medicine, Epidemiology and Biostatistics, University of California, San Francisco, San Francisco, California, USA. [6]Jackson Laboratory and University of Connecticut School of Medicine, Farmington, Connecticut, USA. [7]Research Program in Men's Health, Aging and Metabolism, Boston Claude D. Pepper Older Americans Independence Center, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts, USA. [8]Department of Physical Medicine and Rehabilitation, Mayo Clinic, Rochester, Minnesota, USA. [9]Department of Biochemistry, University of Vermont, Burlington, Vermont, USA. [10]Division of Cardiology, Department of Medicine, University of California, San Francisco, San Francisco, California, USA.

*Address correspondence to: Anne B. Newman, MD, MPH, Graduate School of Public Health, University of Pittsburgh, 130 N. Bellefield Avenue, #310, Pittsburgh, PA 15261, USA. E-mail: anewmana@pitt.edu

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Abstract

Background: Studies using heterochronic parabiosis discovered that circulating factors mediate brain aging in animal models.
Methods: We assessed growth differentiation factors (GDF)-11 and GDF-8 using mass spectrometry and inhibitors follistatin and follistatin-like protein-3 (FSTL-3) with ELISA in the Cardiovascular Health Study (CHS; *N* = 1 506) and the Health, Aging and Body Composition (Health ABC) Study (*N* = 1 237). CLL-11 and beta-2 microglobulin (β2M) were measured with ELISA in a subset of 400 individuals in Health ABC. Associations were assessed with cognitive function, brain magnetic resonance imaging (MRI) findings (CHS only), and incident dementia using correlations, linear regression, and Cox proportional hazards models.
Results: In CHS, levels of GDF-11, GDF-8, and follistatin were not correlated cross-sectionally with the 3MSE or DSST, brain MRI findings of white matter hyperintensity, atrophy, or small infarcts, nor were they associated with incident dementia. FSTL-3 was modestly correlated with poorer cognitive function, greater white matter hyperintensities, and atrophy on MRI, as well as with incident dementia with an adjusted hazard ratio (HR) of 1.72 (95% CI = 1.13, 2.61) per doubling of FSTL-3. FSTL-3 was not associated with cognition or dementia in Health ABC, but GDF-8 was associated with both. The adjusted HR for incident dementia was 1.50 (95% CI = 1.07, 2.10) per doubling of GDF-8.
Conclusions: Total GDF-11 level was not related to cognition or dementia in older adults. Associations of GDF-8 with cognitive outcomes in Health ABC were not expected, but consistent with animal models. Associations of FSTL-3 with cognition, brain abnormalities, and incident dementia in CHS implicate TGFβ superfamily inhibition in the pathogenesis of dementia.

Keywords: Biomarkers, Cognition, Dementia, Epidemiology

Dementia is one of the most feared diseases accompanying the advancing age. Prevention of dementia should consider opportunities to optimize neurological health. Several proteins have been identified in mouse models of heterochronic parabiosis that regulate central nervous system development and health. The term "geroproteins" refers to proteins that may explain the age-accelerating or -rejuvenating effects seen in parabiosis. Of these, growth differentiation factor-11 (GDF-11) has been extensively tested throughout the life course for its effects on the central nervous system including development, maturation, and disease. GDF-11 is a member of the TGFß family and is broadly expressed in the brain (1). In older mice, administration increases blood vessel density and neurogenesis in the brain (2,3). The peripheral administration of recombinant GDF-11 can influence cognition, perhaps through an effect on the vasculature (2,4).

Initially, higher levels of GDF-11 appeared to be protective in other tissues, especially cardiac and skeletal muscle. In mouse studies, GDF-11 reversed cardiac hypertrophy and promoted muscle regeneration following injury (5–7). However, conflicting findings of deleterious effects on cardiac and skeletal muscle health and function have since been reported (5,8–10). The initial finding that suggested GDF-11 declines with advancing age may be due to cross-reactivity between GDF-11 and GDF-8 as they have >90% homology and these may not be adequately distinguished in earlier assays or with an aptamer approach (10). The activity of GDF-11 and GDF-8 is controlled through complex mechanisms of cleavage of the pro-domain and subsequent activation, with binding to receptors inhibited by follistatin and follistatin-like protein-3 (FSTL-3). Follistatin is widely expressed in the brain (11), potentially altering the hypothesized beneficial effect of GDF-11 on the brain and resulting in greater neurodegeneration.

In addition to potential neurotropic effects, GDF-11 or GDF-8 could enhance neurogenic function by rejuvenating vascular networks (12). Given the strong contribution of vascular disease to dementia (11), we hypothesized that GDF-11, possibly GDF-8, follistatin and FSTL-3 might show associations with dementia in humans through a vascular pathway.

Other possible geroproteins include C–C motif chemokine 11 (CCL-11 or eotaxin), a protein involved in inflammatory responses and beta-2 microglobulin (ß2M), a component of major histocompatibility complex i (human leukocyte antigen in humans) which is associated with reduced neurogenesis and lessened memory in young mice (13,14). Higher levels of these proteins are also candidates for impairing cognition or promoting dementia in older people.

To further resolve the associations of geroproteins with cognition in human populations, the **C**omprehensive Evaluation of **A**ging-**R**elated Clinical Outcomes and **G**er**O**proteins (CARGO) study assessed the levels of GDF-8 and 11 with mass spectrometry. We assessed associations of GDF-11, GDF-8, follistatin, and Follistatin-related gene 3 with cognitive function, brain magnetic resonance imaging (MRI), and incident dementia in the Cardiovascular Health Study (CHS) cohort. We hypothesized that GDF-11 would be associated with better cognition and a lower rate of dementia and that its inhibitors, follistatin and FSTL-3, would be associated with a *poorer* cognition and *a higher risk of* dementia. We examined the Health, Aging and Body Composition Study (Health ABC) cohort for replication and, in a subset of 400 Health ABC participants with CCL-11 and ß2M, we explored associations with additional cognitive testing, hypothesizing that they would be associated with poorer *cognition*.

Method

We conducted a cross-sectional and longitudinal study of cognition and dementia in all cases of dementia (n = 154) and a random sample (n = 1 352, total n = 1 506) of individuals with stored serum from the CHS from the 1994–1995 examination (Supplementary Figure 1). The CARGO project was designed to test the associations of these putative markers of aging with a variety of health outcomes: dementia and cognitive function, as reported in this paper; as well as physical function and mobility limitation; and cardiovascular disease outcomes. The size of the random sample was determined by the size of the minimally detectable effect for these outcomes and the number of different biomarkers that we could test in the sample within the funding constraints.

The CHS began in 1989 to determine the natural history of cardiovascular diseases in older adults and enrolled 5 201 men and women (15) at 4 U.S. field centers with 687 Black participants enrolled in 1992–1993 for a total of 5 888 participants. Informed consent was obtained from all participants and studies were approved by IRBs at all participating sites (Universities of Pittsburgh, Tennessee, California (Davis), Johns Hopkins, and Wake Forest.)

In 1992–1994, a brain MRI was obtained in 3 602 participants to assess cerebrovascular disease and, subsequently, dementia was added as an endpoint that was adjudicated by a committee of experts using all follow-up data and standard criteria described later (16). Annual exams including several cognitive tests were conducted between 1992–1993 and 1998–1999, with follow-up every 6 months by phone alternating with annual visits to assess all hospitalizations and self- or proxy report of cardiovascular events. Since 1998–1999, semiannual telephone contact has continued for hospitalizations, medication, and physical function. In 2005–2006, about 1 000 survivors were examined for physical and cognitive function via mostly in-home examinations and followed by phone for function, health events, and mortality. A large repository of serum and plasma is maintained at –80°C at the CHS Core Lab, University of Vermont. Samples had been depleted among the later-enrolled Black cohort, thus we were only able to include a small percentage of the Black participants.

Health ABC was considered as a replication cohort because dementia was determined algorithmically rather than by case-by-case adjudication as was done in the CHS cohort, thus we did not combine the cohorts and endpoints. Health ABC was established in 1997–1998 to study how changes in body composition influenced incident disability and mortality and this was used as the baseline year for this project. Participants were aged 70–79 with no difficulty walking ¼ mile or going up a flight of stairs, and 3 075 men and women (42% Black) were enrolled. Examinations were conducted yearly for the first 6 years of the study, and then in years 8, 10, and 11. The main outcome of Health ABC was incidence of mobility disability. Participants were contacted every 6 months for 17 years for all hospitalizations, and medical records were collected. A repository of serum and plasma, stored at –70°C, is now located in the National Institute on Aging (NIA) Repository in Baltimore. As for the CHS, we evaluated associations in all cases of dementia (n = 245) and a random sample of the noncases (n = 992, total n = 1 237). in a nested case-cohort design.

Assay Methods

We first conducted a double-blind, spiked experiment to select an assay with optimal recovery and no cross-reactivity for the simultaneous measurement of GDF-8 and GDF-11. The details of the Liquid Chromatography with tandem mass spectrometry (LC-MS/

MS) method have been described previously (17). Briefly, serum samples were denatured and reduced in 6 M urea and 20 mM dithiothreitol, alkylated, and after solid phase extraction subjected to tryptic digestion. The tryptic digests were further purified and subjected to LC-MS. The MS consists of a Shimadzu UPLC system with an Aeris Peptide 3.6 µm XB–C18 column, and an ABSciex (Framingham, MA) QTRAP 5500 hybrid triple quadrupole/liner ion trap mass spectrometer operated in positive electrospray ionization mode. Isotope-labeled IPGMVVD^R and NLGLDEHSSES^R peptides were added as internal standards. The standard curve was linear from 0 to 50 ng/mL for GDF-11 and from 0 to 100 ng/mL for GDF-8. The lower limit of quantitation was 0.5 ng/mL for both GDF-8 and GDF-11. The inter-assay coefficient of variation in quality control pools with GDF-11 concentrations of 3.4, 7.4, 12.5, and 52.0 ng/mL were 8.7%, 13.0%, 14.2%, and 12.8%, respectively; inter-assay coefficients of variation at 8.7, 14.1, 17.3, and 51.1 ng/mL of GDF-8 were 15.1%, 12.5%, 16.4%, and 12.0%, respectively. Neither GDF-8 nor IgG1 had detectable cross-reactivity in the GDF-11 assay; similarly, neither GDF-11 nor IgG1 had detectable cross-reactivity in the GDF-8 assay. The accuracy of the assay, determined as the percent recovery in human plasma spiked with 5, 10, and 50 ng/mL of GDF-11 and GDF-8 ranged from 80% to 116% for GDF-11, and 81% to 111% for GDF-8. Follistatin was measured using an Enzyme – Linked Immunosorbent Assay (ELISA) kit (R&D Systems). The detectable range of the assay is ~250–16 000 pg/mL, and the inter-assay CV on pooled serum controls was 6.05%. Follistatin-like 3 was measured using an ELISA kit (R&D Systems, Minneapolis, MN). The detectable range of the assay is ~313–24 622 pg/mL, and the inter-assay CV on pooled serum controls is 2.56%. Beta-2 microglobulin (β2M) was measured using nephlelomtery on the BNII system (Siemens, Malvern, PA). The detectable range of the assay is ~0.68–22.0 mg/L, and the inter-assay CV on pooled serum controls was 4.1%. CCL-11 (also known as eotaxin) was measured using an ELISA kit (R&D Systems). The detectable range of the assay is ~15–1 000 pg/mL, and the inter-assay CV on pooled serum controls was 2.75%. These assays were completed at the Laboratory for Clinical Biochemistry Research, University of Vermont.

Cognitive Function Tests

Cognitive function was regularly assessed in both cohorts with the 3MSE and Digit Symbol Substitution Test (DSST) at baseline and annually or semiannually (18,19). We examined associations between GDF-11, GDF-8, follistatin and FSTL-3, and baseline cognitive function for each test in each cohort.

Additional testing was done as part of each cohort study's neuropsychological batteries and these differed between CHS and Health ABC. In CHS, these tests included the California Verbal Learning Test (20) and Rey-Osterreith complex figure task (21). In Health ABC, these included the Buschke Selective Reminding Test of verbal learning and memory (22), a speed battery consisting of the Boxes and Digit Copying Test to assess motor speed, and the Pattern Comparison and Letter Comparison to assess perceptual speed (23)

Brain MRI

In the CHS cohort, we considered 3 aspects of brain MRI as markers of dementia: the grade of white matter hyperintensity (0–9), the grade of global cerebral atrophy (0–9), and the presence or absence of any small infarcts (<3 mm). Brain MRIs were conducted between 1992 and 1994 using 3 Tesla scanners and read using a standard atlas at a central reading center (24).

Dementia

Incident dementia was examined in each cohort. In CHS, the baseline for these analyses was 5 years after enrollment, and we excluded 100 cases of prevalent dementia from the incidence analysis. At each site, participants in the CHS MRI cohort (n = 3 602) who had a 5-point drop in Modified Mini-Mental State Examination (3MSE), had low education or stroke, all minorities, and a random sample of the remainder had a neurological physical examination, neuropsychological test battery and review of all medical records, brain images from medical records, and medication use. Additionally, all participants at 1 site (Pittsburgh), all of the small number of Black participants, and all participants who had died since the MRI were evaluated. A proxy interview for dementia was used to ascertain functional status and behavior. A dementia adjudication committee of dementia neurologists reviewed this evidence along with the prior annual 3MSE and DSST scores to retrospectively determine the onset of dementia, the date of onset, and subtype (16,25).

In Health ABC, prevalent dementia was an exclusion at enrollment, which was the baseline for these analyses. Incident dementia was determined by an algorithm including any of the following: prescribed dementia medications (galantamine, rivastigmine, memantine, donepezil, and tacrine), hospital records with dementia as a discharge diagnosis, or a decline in 3MS score of more than 1.5 *SD* (race-stratified) from baseline (26).

Cases were defined as reaching the dementia endpoint by the end of the ascertainment period (the year 2000 for CHS and 2015 for Health ABC). The remaining random cohort was followed until the date of death or the last follow-up contact through the end of the ascertainment period.

Potential Confounders

Race and Hispanic ethnicity were ascertained by self-report of one or more of the U.S. census designations in use at the time of enrollment and grouped as White versus Black. Fewer than 1% were other race or Hispanic ethnicity and were included with the Black participants. Education was assessed as years of education, smoking as self-reported past, current or never smoking, and diabetes was assessed by fasting blood sugar ≥126 mg/dL or by medication use. Systolic blood pressure and antihypertensive medications were each included to adjust for hypertension. Body mass index was calculated from measured weight and height in kg/m^2. History of myocardial infarction (MI), angina, and stroke at baseline was determined using self-reported data as well as medication use in Health ABC; in CHS, MI, angina, and stroke prevalence at the 1994–1995 exam was updated from adjudicated events since study inception. ApoE4 allele status was determined using genomic DNA extracted from whole-blood samples and amplified by using polymerase chain reaction. After cleaving and electrophoresis on agarose gels, restriction patterns were determined (27). Apolipoprotein E (APOE) genotypes were grouped as APOE ε4 carriers (ε2/ε4, ε3/ε4, ε4/ε4 genotypes) and noncarriers (ε2/ε2, ε2/ε3, ε3/ε3 genotypes).

Analysis

Cross-sectional associations between protein levels and cognitive tests were examined using spearman correlations and linear regression. Since several cognitive tests, especially the 3MSE, could not be easily transformed to normality, we present the spearman correlations for ease of interpretation. Potential confounders assessed included education (years), Apo e4 (1 or 2 e4 alleles vs none), smoking, hypertension, diabetes, stroke, and coronary heart disease

(CHD) and estimated glomerular filtration rate (eGFR). Interactions were tested for age, gender, and race with *p* values reported for the significance of the interaction. The subset of 400 in Health ABC with CCL-11 and β2M was analyzed similarly. Analyses of this subset and the cognitive tests other than the 3MSE and DSST were exploratory.

Cross-sectional associations between protein levels and brain MRI scores were examined using Spearman correlations and linear regression. Associations between proteins and the presence of small infarcts were examined using logistic regression.

Each protein was examined for differences in the time to dementia event in Cox models with minimal adjustment for age, sex, race, and then also with potential confounders including education, study site, Apo e4 carrier status, smoking, hypertension, diabetes, stroke, MI, and CHD. Models were stratified by cohort. Because of its potential influence on protein clearance, we conducted an additional analysis using eGFR based on cystatin C (28) as a potential confounder or mediator. Interactions were tested for age, gender, and race with *p* values reported for the significance of the interaction. For these analyses, levels of GDF-11, GDF-8, follistatin, and FSTL-3 were each log transformed to improve normality. Results (beta estimates or odds ratios) are reported per doubling in concentration in protein. All analyses were completed in SAS version 9.4 (SAS Institute, Cary, NC).

Results

There were 1 506 participants from the CHS cohort and 1 237 participants from Health ABC included in the cross-sectional analysis (Table 1). The mean age ± *SD* of CHS participants was 76.85 ± 4.76 years, 40.97% were men, and 95.75% were white. The Health ABC group was somewhat younger and more diverse with mean age 73.44 ± 2.87 years; 50.85% were men, 61.36% were White, and 38.64% were Black. They had a similar mean 3MSE scores, whereas Health ABC participants had slightly lower DSST score. Mean levels of GDF-11, GDF-8, and FSTL-3 were slightly higher in CHS. After excluding prevalent dementia, there were 154 incident dementia cases in CHS (*n* = 1 406, 11%) over 11.35 median years of follow-up and 245 incident dementia cases in Health ABC (*n* = 1 237, 19.8%) over 12.5 median years of follow-up. Age-adjusted incidence of dementia was 8.2 per 1 000 person years (95% CI = 6.7, 9.8) in CHS and 17.4 (95% CI = 15.0, 19.7) in Health ABC.

Cognitive Tests

In CHS, levels of GDF-11, GDF-8, and follistatin were not correlated cross-sectionally with either the 3MSE or DSST (Table 2). FSTL-3 was modestly negatively correlated with both cognitive tests in CHS as well as with almost all of the additional cognitive tests administered in a subset (Supplementary Table 1 and Supplementary Figure 2). In linear regression models (not tabulated), FSTL-3 was

Table 1. Characteristics of the Study Populations Sampled From CHS and Health ABC Cohorts

Characteristic	CHS	Health ABC
	(*N* = 1 506)	(*N* = 1 237)
Age, years, mean ± *SD*	76.85 ± 4.76	73.44 ± 2.87
Male, *n* (%)	617 (40.97)	629 (50.85)
White race, *n* (%)	1 442 (95.75)	759 (61.36)
Body mass index, kg/m², mean ± *SD*	26.37 ± 4.25	27.18 ± 4.55
High school graduate, *n* (%)	1 167 (77.59)	948 (76.76)
Current smoker, *n* (%)	108 (7.24)	123 (9.95)
Systolic blood pressure, mean ± *SD*	132.55 ± 20.05	135.04 ± 19.41
Hypertension medication, *n* (%)	761 (50.56)	663 (53.86)
Coronary heart disease history, *n* (%)	361 (23.97)	244 (19.73)
Diabetes history, *n* (%)	228 (16.27)	210 (16.98)
Stroke history, *n* (%)	87 (5.78)	115 (9.3)
APOE ε4 allele positive, *n* (%)	326 (23.66)	356 (30.2)
Dementia		
Incident dementia, *n* (%)	154 (10.95)	245 (19.81)
Incident dementia rate per 1 000 person years	8.2 (95% CI = 6.7, 9.8)	17.4 (95% CI = 15.0, 19.7)
Brain magnetic resonance imaging		
Global brain atrophy-ventricles (0–9), median (IQR)	3 (3, 4)	n/a
White matter hyperintensity, grade (0–9), median (IQR)	2 (1, 3)	n/a
Small infarcts, yes, *n* (%)	233 (15.53)	n/a
Biomarker levels		
GDF-11, ng/mL, mean ± *SD*	3.4 ± 0.83	3.28 ± 0.81
GDF-8, ng/mL, mean ± *SD*	7.56 ±– 2.22	6.73 ± 1.96
Follistatin, pg/mL, mean ± *SD*	1 817.02 ± 1 004.89	1 868.21 ± 874.88
FSTL-3, pg/mL, mean ± *SD*	9 747.76 ± 2 858.7	8 744.62 ± 2334.71
CCL-11 (Eotaxin), pg/mL, mean ± *SD*		139.2 ± 61.27*
Beta-2 microglobulin (β2M), mg/L, mean ± *SD*		2.74 ± 2.77*
Cognitive tests		
Teng Modified Mini-Mental State Examination score, mean ± *SD*	91.52 ± 8.54	90.77 ± 7.66
Digit Symbol Substitution Test score, mean ± *SD*	41.28 ± 13.04	36.57 ± 14.14

Notes: APOE ε4 = apolipoprotein E4; CHS = Cardiovascular Health Study; CCL-11 = C–C motif chemokine ligand 11; FSTL-3: follistatin-like 3; GDF-8 = growth differentiation factor-8; GDF-11 = growth differentiation factor-11; Health ABC = Health, Aging and Body Composition Study; IQR = interquartile range; *SD* = standard deviation.

*CCL-11 and β2M tests were conducted in a subsample of 400 participants.

significantly negatively associated with DSST in adjusted models, such that for every doubling in FSTL-3 concentration, there was a 3.09 (95% CI = 1.96, –4.06) lower DSST score.

Brain MRI

Among the brain MRI measures examined in CHS (Table 3), levels of GDF-11, GDF-8, and follistatin were not associated with atrophy or white matter grade. However, FSTL-3 was moderately associated with both atrophy and white matter grade, although after adjustment for confounders, this was significant only for white matter hyperintensities, where higher FSTL-3 was significantly positively related to greater white matter hyperintensities; every doubling in FSTL-3 concentration, white matter hyperintensity grade was 0.63 (95% CI = 0.35, –0.87) higher after multivariate adjustment. There was a significant positive association of GDF-8 with the presence of small infarcts, OR: 1.66 (95% CI = 1.06, 2.60, Table 3, Model 3). This was attenuated to nonsignificance after further adjustment for eGFR using cystatin C (Table 3, Model 4), based on previous analyses showing significant correlations between several of these assays and eGFR (Supplementary Table 3). Levels of GDF-11, follistatin, and FSTL-3 were not associated with the presence of small infarcts.

Table 2. Spearman Correlations Between Geroproteins and Baseline Modified Mini-Mental State Examination and Digit Symbol Substitutions Tests Scores in CHS and Health ABC

CHS	GDF-11	GDF-8	Follistatin	FSTL-3
Modified Mini-Mental State score	–0.016	–0.024	0.018	–0.167*
n	1 506	1 506	1 506	1 497
Digit Symbol Substitution Test score	–0.045	0.012	–0.008	–0.213*
n	1 456	1 456	1 456	1 447
Health ABC	GDF-11	GDF-8	Follistatin	FSTL-3
Modified Mini-Mental State score	–0.024	–0.073*	–0.010	0.057
n	1 237	1 237	814	1 162
Digit Symbol Substitution Test score	–0.039	–0.058*	0.014	0.038
n	1 229	1 229	808	1 155

Notes: CHS = Cardiovascular Health Study; FSTL-3 = follistatin-like 3; GDF-11 = growth differentiation factor-11; GDF-8 = growth differentiation factor-8.

*$p < .05$.

Incident Dementia

When examining the incidence of dementia in CHS, we considered a doubling in the concentration of GDF-11 and risk for incident dementia, adjusting for age, sex, race, and clinic site. The adjusted HR for incident dementia per doubling GDF-11 was not significantly lower: HR = 0.93 (95% CI = 0.45, 1.91) (Table 4, Model 3). GDF-8 and follistatin were not associated with incident dementia in CHS in either men or women, whereas FSTL-3 was associated. Per doubling of FSTL-3 concentration, the HR for incident dementia in CHS, adjusted for age, sex, race, and clinic site was 1.72 (95% CI = 1.13, 2.61, Model 2) and remained significant after full adjustment with HR of 2.16 (95% CI = 1.17, 3.99, Model 4). The association appeared to be stronger in women (HR = 2.39 [95% CI = 1.46, 3.92]) compared to men (HR = 0.84 [95% CI = 0.38, 1.82]), although the interaction term in the group of men and women combined was not statistically significant ($p_{int} = .422$), so these data were not tabulated. Statistical interactions between GDF-8 and GDF-11 and their inhibitors FST and FSTL-3 were tested and were not significant.

Table 3. Associations Per Doubling in Concentration of GDF-11, GDF-8, Follistatin and FSTL-3 With Global Atrophy Grade (score 0–9), White Matter Hyperintensity Grade (score 0–9), and Presence of Small Infarcts on Brain MRI in CHS, *n* = 1 492

	Model 1	Model 2	Model 3	Model 4
	Beta (95% CI)	Beta (95% CI)	Beta (95% CI)	Beta (95% CI)
Global brain atrophy, score 0–9				
GDF-11	–0.36 (–0.68, 0.24)	–0.41 (–0.71, 0.11)	–0.43 (–0.75, 0.15)	–0.4 (–0.73, 0.21)
GDF-8	–0.09 (–0.43, 0.36)	–0.18 (–0.47, 0.28)	–0.31 (–0.57, 0.16)	–0.31 (–0.57, 0.17)
Follistatin	0.06 (–0.23, 0.28)	0.18 (–0.12, 0.35)	0.2 (–0.12, 0.37)	0.19 (–0.13, 0.37)
FSTL-3	0.77 (0.58, 0.94)*	0.38 (0.06, 0.6)*	0.32 (–0.14, 0.57)	0.36 (–0.17, 0.65)
White matter hyper-intensity, score 0–9				
GDF-11	0.04 (–0.52, 0.54)	–0.01 (–0.51, 0.51)	–0.21 (–0.65, 0.46)	–0.07 (–0.59, 0.54)
GDF-8	0.11 (–0.39, 0.47)	0.08 (–0.39, 0.45)	0.09 (–0.41, 0.48)	0.15 (–0.38, 0.51)
Follistatin	0.16 (–0.19, 0.36)	–0.07 (–0.3, 0.25)	–0.11 (–0.34, 0.24)	–0.14 (–0.36, 0.22)
FSTL-3	0.96 (0.77, 1.14)	0.68 (0.43, 0.88)*	0.63 (0.35, 0.87)*	0.56 (0.15, 0.85)*
Small infarcts, yes				
	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
GDF-11	0.85 (0.49, 1.48)	0.84 (0.48, 1.47)	0.87 (0.46, 1.65)	0.83 (0.43, 1.61)
GDF-8	1.71 (1.2, 2.44)*	1.71 (1.17, 2.51)*	1.66 (1.06, 2.6)*	1.14 (0.65, 1.98)
Follistatin	1.16 (0.78, 1.72)	1.17 (0.78, 1.76)	1.21 (0.77, 1.89)	1.21 (0.76, 1.92)
FSTL-3	0.96 (0.77, 1.19)	0.97 (0.78, 1.22)	0.92 (0.71, 1.18)	0.87 (0.68, 1.13)

Notes: APOE ε4 = apolipoprotein E4; CHS = Cardiovascular Health Study; CI = confidence interval; FSTL-3 = follistatin-like 3; GDF-11 = growth differentiation factor-11; GDF-8 = growth differentiation factor-8; MRI = magnetic resonance imaging; OR = odds ratio. Model 1 is unadjusted; Model 2 adjusted for age, sex, race, and clinic site; Model 3 adjusted for covariates in Model 2 as well as myocardial infarction history, education, smoking status, body mass index, systolic blood pressure, hypertensive medication use, CHD history, diabetes history, stroke history and APOE ε4 carrier; Model 4 is additionally adjusted for estimated glomerular filtration rate using cystatin C.

*$p < .05$.

Table 4. Incident Dementia: Hazard Ratio (95% CI) Per Doubling in Concentration of GDF-11, GDF-8, Follistatin, and FSTL-3 in CHS and Health ABC.

	Model 1	Model 2	Model 3	Model 4
	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)
CHS cohort				
GDF-11	0.8 (0.42, 1.52)	0.76 (0.4, 1.44)	0.93 (0.45, 1.91)	0.96 (0.46, 1.98)
GDF-8	0.59 (0.37, 0.96)*	0.7 (0.44, 1.13)	0.81 (0.47, 1.41)	0.88 (0.5, 1.53)
Follistatin	0.91 (0.72, 1.16)	0.87 (0.68, 1.13)	0.77 (0.57, 1.03)	0.76 (0.56, 1.02)
Follistatin-like 3	2.66 (1.79, 3.96)*	1.72 (1.13, 2.61)*	2.41 (1.47, 3.93)*	2.16 (1.17, 3.99)*
Health ABC cohort				
GDF-11	1.47 (0.88, 2.46)	1.48 (0.85, 2.56)	1.44 (0.84, 2.47)	1.44 (0.84, 2.46)
GDF-8	1.41 (1.03, 1.91)*	1.38 (1, 1.9)	1.5 (1.07, 2.11)*	1.5 (1.07, 2.1)*
Follistatin	1.17 (0.89, 1.53)	1.16 (0.88, 1.53)	1.03 (0.78, 1.36)	1.03 (0.78, 1.36)
Follistatin-like 3	1.37 (0.94, 1.99)	1.27 (0.86, 1.88)	1.3 (0.85, 1.98)	1.51 (0.91, 2.51)

Notes: APOE ε4 = apolipoprotein E4; CHS = Cardiovascular Health Study; CI = confidence interval; FSTL-3 = follistatin-like 3; GDF-11 = growth differentiation factor-11; GDF-8 = growth differentiation factor-8; HR = hazard ratio. Model 1 is unadjusted; Model 2 adjusted for age, sex, race, and clinic site; Model 3 adjusted for covariates in Model 2 as well as myocardial infarction history, education, smoking status, body mass index, systolic blood pressure, hypertensive medication use, CHD history, diabetes history, stroke history and APOE ε4 carrier; Model 4 is additionally adjusted for estimated glomerular filtration rate using cystatin C.

*95% confidence interval excludes 1.

In Health ABC, no associations between GDF-11, follistatin and FSTL-3, and incident dementia were found. GDF-8 was associated with incident dementia in the Health ABC cohort after full multivariate adjustment, including for renal function, with an HR of 1.50 (95% CI = 1.07, 2.10). In Health ABC, levels of GDF-8 were weakly correlated with the 3MSE and DSST (Table 2) as well as with additional cognitive tests assessed in a subset, such that higher GDF-8 was related to poorer cognitive function for most tests (Supplementary Table 2).

CCL-11 and β2M Subset

Of the proteins evaluated in 400 Health ABC participants, β2M was significantly associated with incident dementia with an overall HR for the multivariate-adjusted model of 2.38 (95% CI = 1.36, 4.17). This association was stronger in women with an HR of 3.17 (95% CI = 1.31, 7.64) compared to men (HR = 2.03 [0.90, 4.61]), but the interaction term was not significant (p_{int} = .423). However, after additionally adjusting for eGFR, these relationships were not significant. In this subset, CCL-11 was positively correlated with the performance on the 3MSE and the DSST as well as two of the additional cognitive tests (digit copy and pattern comparison tests). β2M was unrelated to the 3MSE and DSST or any of the additional cognitive tests performed in Health ABC (Supplementary Table 2).

Discussion

Contrary to our hypothesis based on the studies of heterochronic parabiosis, we did not see an association of higher GDF-11 with better cognitive function, fewer brain MRI abnormalities, or lower rates of incident dementia in the CHS cohort. In addition, there also were no associations with GDF-8 or follistatin with cognitive function, brain MRI abnormalities, or incident dementia, except for an association of GDF-8 with small infarcts on brain MRI. In CHS, FSTL-3 was related to poorer cognitive function on multiple tests, brain MRI white matter hyperintensities, as well as incident dementia. The association of FSTL-3 with dementia appeared to be more pronounced in women, though this was not significantly different from the estimate in men.

The associations of FSTL-3 with cognitive outcomes were not replicated in the Health ABC cohort. Although the lack of an association of FSTL-3 with dementia in Health ABC could be due to a less specific definition of dementia, the other associations found with the cognitive tests, 3 MSE and DSST, should be less biased, yet no association was found. Unexpectedly, GDF-8 was positively related to cognitive test performance and incident dementia in Health ABC, with a higher GDF-8 related to poorer performance on the 3MSE and DSST in all as well as with additional tests from the neuropsychological battery in a subset.

Our results cast doubt on the ability of boosting peripheral blood levels of GDF-11 in humans to enhance central nervous system function. Circulating peripheral blood levels of GDF-11 may well be unrelated to tissue levels in the central nervous system. Expression of GDF-11 in cerebrospinal fluid or brain tissue might be more informative of its biological role but may not be in equilibrium across the blood–brain barrier to assess this peripherally. It is also possible that previous studies measuring GDF-11 in animal models are flawed by cross-reactivity with GDF-8. However, we saw no evidence of this in our study as we were able to look at both GDF-8 and GDF-11 simultaneously with no associations of cognition with GDF-11 or GDF-8 in CHS.

The associations of higher FSTL-3 with poorer cognition, greater white matter hyperintensity on MRI, and incident dementia in CHS were quite robust. Taken together, these associations are consistent with a vascular pathway. White matter hyperintensities are related to hypertension and other cardiovascular risk factors (29,30). The association between FSTL-3 and white matter hyperintensity was partly attenuated by poorer kidney function, suggesting either that FSTL-3 is elevated when a clearance by the kidney is reduced or that FSTL-3 is related causally to both renal vascular disease and cerebrovascular disease. Since kidney function and FSTL-3 were assessed concurrently, we cannot tease out the causal pathway to know whether the loss of kidney function is a cause, consequence, or parallel process to the elevation of FSTL-3. This attenuation was not prominent in the associations of FSTL-3 with cognition or dementia, which suggests that elevation of FSTL-3 is not due solely to a reduction in renal clearance and implicates a causal pathway.

Unexpectedly, GDF-8 was related to poorer cognitive function and incident dementia in Health ABC, with higher GDF-8 related to poorer performance on the 3MSE and DSST in all subjects as well as with additional tests from the neuropsychological battery in a subset. These findings contrast with those recently reported by Tanaka et al. (31) who found an inverse relationship between GDF-8 levels and cognitive impairment or dementia. By using 1 322 SOMAmers to analyze proteomic profiles of plasma samples from 997 adults in the InCHIANTI study, these investigators identified GDF-8 as one of only 2 plasma proteins associated with decreased odds of baseline cognitive impairment or dementia. Moreover, higher GDF-8 levels were also associated with a slower decline in performance on the Mini-Mental State Examination as well as larger brain volume and slower brain atrophy as assessed by imaging. A similar association was seen in the ARIC cohort. In 4 110 older adults followed for incident dementia, 38 proteins were associated with dementia. Among the top associations was GDF-11/8, with an HR of 0.39 (95% CI = 0.24, 0.61) (32). Although differences in subject population and/or functional assessments could explain the discrepant findings between our study and those of Tanaka et al. (31) and Walker et al. (32), a major difference between the studies was the assay used to measure GDF-8 levels. In particular, Tanaka et al. and Walker et al. (32) used the SOMAscan assay, which relies on the detection of specific exposed epitopes by individual SOMAmers, whereas our assay is LC-MS based and does not include a capture step dependent on epitope accessibility. An important distinction in this regard is that recognition of aptamers to a given epitope could be influenced by the presence of binding proteins, whereas our assay is agnostic to other proteins that may be bound to the target. Hence, certain protein complexes may be excluded from detection in the SOMAscan assay, whereas our LC-MS assay likely measures total protein, irrespective of whether they exist in complexes with other proteins. This issue is certainly relevant in the case of GDF-8, as it is known that GDF-8 circulates in the blood in complexes with multiple binding partners (33–35).

Given that our study as well as that of Tanaka et al. (31) and Walker et al. (32) only describe associations betweenf circulating GDF-8 levels and cognitive outcomes, it is important to consider 2 issues in attempting to interpret these findings. First, circulating levels of GDF-8 are almost certainly correlated with total skeletal muscle mass throughout the body, as skeletal muscle is by far the primary site of GDF-8 expression (36) and the primary source of circulating GDF-8 protein, at least in mice (37). Hence, associations between functional parameters and GDF-8 levels may actually be reflecting associations between these functional parameters and overall muscle mass. Second, studies in mice have shown that GDF-8 expression is increased in a mouse model of Alzheimer's and that GDF-8 knockdown in the gastrocnemius muscle could mitigate the cognitive deficits in these mice (38). Our finding of higher levels of GDF-8 being associated with poorer cognitive outcomes is certainly consistent with these mouse studies.

The lack of replication of the association of FSTL-3 with dementia found in CHS in the Health ABC cohort may be due to a lower specificity in the diagnosis. Health ABC had an age-adjusted rate of dementia of 17.4 per 1 000 person years that were more than twice that of CHS (8.2 per 1 000 person years) and was higher than reported for this age group in other cohorts, suggesting that the algorithm was overly sensitive and not adequately specific. Discharge codes from hospital admissions may be detecting cases of delirium, perhaps reflecting overall multimorbidity and being less specific for dementia than the full clinical dementia evaluation that was done in CHS. It may also be biased in detecting dementia in hospitalized patients who have greater multimorbidity than in those without inpatient admissions. These issues do not explain the finding in Health ABC of an association of GDF-8 with dementia as GDF-8 was also associated with cognitive tests. These tests were administered to almost everyone, missing only a small percentage who were unable to return to the research clinic for testing. Thus, the consistency of the GDF-8 association with dementia as well as cognition strengthens this finding.

The direct associations of CCL-11 with some cognitive function tests were opposite of the hypothesized direction as CCL-11 is thought to promote cognitive impairment (10,12). The elevation of beta-2 microglobulinemia with incident dementia in Health ABC is consistent with other reports of inflammatory markers in dementia and dementia subtypes (39,40).

Our study leverages 2 large, longstanding, well-characterized cohorts with detailed outcome assessments and well-curated biologic specimens, well-validated assays for total GDF-11 and 8, and partnerships between bench and population scientists. The difference in the findings between the two cohorts calls for additional replication in other studies where the outcomes assessments are more directly comparable. In addition to the difference in the dementia outcome assessment, there are many other differences between the Health ABC and CHS cohorts that could affect the findings. CHS was recruited almost 10 years earlier, were younger (65 and older compared to 70 and older), and had higher indicators of socioeconomic status such as education than Health ABC participants. The proportion of minorities was higher in Health ABC. Health ABC excluded people reporting any difficulty walking ¼ mile or climbing 10 steps, so while they were on average older, they were more highly functional. We considered these factors as potential confounders or effect modifiers, but they do not explain the difference in the findings for GDF-8.

In summary, GDF-11 was not related to better cognition or a lower risk of dementia in older adults in either the CHS or Health ABC cohorts. This suggests that results from heterochronic parabiosis in mice should be confirmed in prospective human studies to test their applicability to people and that boosting the level of GDF-11 in the peripheral circulation would not have central effects. The association of GDF-8 with cognition and dementia in Health ABC was not expected, but consistent with an animal model. The association of FSTL-3 with cognition, brain abnormalities, and incident dementia in CHS implicates TGFβ superfamily inhibition in the pathogenesis of dementia, but more mechanistic work is needed along with additional replication in cohorts with a prospective dementia diagnosis.

Supplementary Material

Supplementary data are available at *The Journals of Gerontology, Series A: Biological Sciences and Medical Sciences* online.

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Conflict of Interest

A.B.N. reports no competing interests. J.R.K. reports stock ownership in stock ownership in Abbott, Bristol Myers-Squibb, Johnson & Johnson, Medtronic, Merck, and Pfizer. P.C. and S.R.C. receive consulting fees from BioAge. S.B. reports receiving research grants from NIA, NCMRR, PCORI, FNIH, AbbVie, Metro International Technology, Transition Therapeutics, and Function Promoting Therapies, LLC; equity interest in Function Promoting Therapies, LLC; and personal consulting fees from OPKO and Aditum. The other authors declare no conflict.

Author Contributions

A.B.N. developed analysis plan, completed analyses, and drafted the manuscript. S.P. completed analyses and critically reviewed the paper. J.R.K., S.-J.L, S.B., P.C., N.L., R.P.T., and P.G. all critically reviewed the manuscript; and S.R.C., R.P.T., and P.C. secured funding for this project.

Data Availability

Data from CHS and Health ABC are available to investigators. For Health ABC, requests for data access may be made to https://healthabc.nia.nih.gov/. For CHS, use of the data is controlled to ensure that only qualified investigators have access, that sensitive CMS data are not released to safeguard privacy, and that proposed research projects conform with study guidelines. Requests for data access for CHS requests for data access from qualified investigators trained in human subject research may be forwarded to CHSDATA@uw.edu.

References

1. Schafer MJ, LeBrasseur NK. The influence of GDF11 on brain fate and function. *GeroScience*. 2019;41(1):1–11. doi:10.1007/s11357-019-00054-6
2. Katsimpardi L, Litterman NK, Schein PA, et al. Vascular and neurogenic rejuvenation of the aging mouse brain by young systemic factors. *Science*. 2014;344(6184):630–634. 10.1126/science.1251141
3. Wu H-H, Ivkovic S, Murray RC, et al. Autoregulation of neurogenesis by GDF11. *Neuron*. 2003;37(2):197–207. doi:10.1016/s0896-6273(02)01172-8
4. Ozek C, Krolewski RC, Buchanan SM, et al. Growth differentiation factor 11 treatment leads to neuronal and vascular improvements in the hippocampus of aged mice. *Sci Rep*. 2018;8(1):17293. doi:10.1038/s41598-018-35716-6
5. Egerman MA, Cadena SM, Gilbert JA, et al. GDF11 increases with age and inhibits skeletal muscle regeneration. *Cell Metab*. 2015;22(1):164–174. doi:10.1016/j.cmet.2015.05.010
6. Sinha M, Jang YC, Oh J, et al. Restoring systemic GDF11 levels reverses age-related dysfunction in mouse skeletal muscle. *Science*. 2014;344(6184):649–652. 10.1126/science.1251152
7. Loffredo FS, Steinhauser ML, Jay SM, et al. Growth differentiation factor 11 is a circulating factor that reverses age-related cardiac hypertrophy. *Cell*. 2013;153(4):828–839. doi:10.1016/j.cell.2013.04.015
8. Smith SC, Zhang X, Zhang X, et al. GDF11 does not rescue aging-related pathological hypertrophy. *Circ Res*. 2015;117(11):926–932. doi:10.1161/CIRCRESAHA.115.307527
9. Harper SC, Johnson J, Borghetti G, et al. GDF11 decreases pressure overload-induced hypertrophy, but can cause severe cachexia and premature death. *Circ Res*. 2018;123(11):1220–1231. doi:10.1161/CIRCRESAHA.118.312955
10. Walker RG, Poggioli T, Katsimpardi L, et al. Biochemistry and biology of GDF11 and myostatin: similarities, differences, and questions for future investigation. *Circ Res*. 2016;118(7):1125–1141; discussion 1142. doi:10.1161/CIRCRESAHA.116.308391
11. Satizabal CL, Beiser AS, Chouraki V, et al. Incidence of dementia over three decades in the Framingham Heart Study. *N Engl J Med*. 2016;374(6):523–532. doi:10.1056/NEJMoa1504327
12. Olson KA, Beatty AL, Heidecker B, et al. Association of growth differentiation factor 11/8, putative anti-ageing factor, with cardiovascular outcomes and overall mortality in humans: analysis of the Heart and Soul and HUNT3 cohorts. *Eur Heart J*. 2015;36(48):3426–3434. doi:10.1093/eurheartj/ehv385
13. Villeda SA, Luo J, Mosher KI, et al. The ageing systemic milieu negatively regulates neurogenesis and cognitive function. *Nature*. 2011;477(7362):90–94. doi:10.1038/nature10357
14. Smith LK, He Y, Park J-S, et al. β2-microglobulin is a systemic pro-aging factor that impairs cognitive function and neurogenesis. *Nat Med*. 2015;21(8):932–937. doi:10.1038/nm.3898
15. Fried LP, Borhani NO, Enright P, et al. The Cardiovascular Health Study: design and rationale. *Ann Epidemiol*. 1991;1(3):263–276. doi:10.1016/1047-2797(91)90005-w
16. Lopez OL, Kuller LH, Fitzpatrick A, et al. Evaluation of dementia in the cardiovascular health cognition study. *Neuroepidemiology*. 2003;22(1):1–12. doi:10.1159/000067110
17. Peng L, Gagliano-Jucá T, Pencina KM, et al. Age trends in growth and differentiation factor-11 and myostatin levels in healthy men, and differential response to testosterone, measured using liquid chromatography-tandem mass spectrometry. *J Gerontol A Biol Sci Med Sci*. 2022;77(4):763–769. doi:10.1093/gerona/glab146
18. Teng EL, Chui HC. The Modified Mini-Mental State (3MS) examination. *J Clin Psychiatry*. 1987;48(8):314–318.
19. Wechsler D. *WAIS-R: Manual: Wechsler Adult Intelligence Scale--Revised*. Harcourt Brace Jovanovich [for] Psychological Corp.; 1981.
20. Delis DC, Friedland J, Kramer JH, et al. *California verbal learning test (CVLT)*. ECPA; 2008.
21. Somerville J, Tremont G, Stern RA. The Boston qualitative scoring system as a measure of executive functioning in Rey-Osterrieth Complex Figure performance. *J Clin Exp Neuropsychol*. 2000;22(5):613–621. doi:10.1076/1380-3395(200010)22:5;1-9;FT613
22. Levin HS, Benton AL, Grossman RG. *Neurobehavioral Consequences of Closed Head Injury*. Oxford University Press; 1982.
23. Salthouse TA. General and specific speed mediation of adult age differences in memory. *J Gerontol Series B Psychol Sci Social Sci*. 1996;51(1):P30–P42. doi:10.1093/geronb/51b.1.p30
24. Bryan RN, Manolio TA, Schertz LD, et al. A method for using MR to evaluate the effects of cardiovascular disease on the brain: the cardiovascular health study. *AJNR Am J Neuroradiol*. 1994;15(9):1625–1633.
25. Fitzpatrick AL, Kuller LH, Ives DG, et al. Incidence and prevalence of dementia in the Cardiovascular Health Study. *J Am Geriatr Soc*. 2004;52(2):195–204. doi:10.1111/j.1532-5415.2004.52058.x
26. Yaffe K, Falvey C, Hamilton N, et al. Diabetes, glucose control, and 9-year cognitive decline among older adults without dementia. *Arch Neurol*. 2012;69(9):1170–1175. doi:10.1001/archneurol.2012.1117
27. Hixson JE, Vernier DT. Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with HhaI. *J Lipid Res*. 1990;31(3):545–548.
28. Inker LA, Schmid CH, Tighiouart H, et al. Estimating glomerular filtration rate from serum creatinine and cystatin C. *N Engl J Med*. 2012;367(1):20–29. doi:10.1056/NEJMoa1114248
29. Longstreth WT, Jr, Manolio TA, Arnold A, et al. Clinical correlates of white matter findings on cranial magnetic resonance imaging of 3301 elderly people: the Cardiovascular Health Study. *Stroke*. 1996;27(8):1274–1282. doi:10.1161/01.str.27.8.1274
30. Bryan RN, Wells SW, Miller TJ, et al. Infarctlike lesions in the brain: prevalence and anatomic characteristics at MR imaging of the elderly -- data from the Cardiovascular Health Study. *Radiology*. 1997;202(1):47–54. doi:10.1148/radiology.202.1.8988191

31. Tanaka T, Lavery R, Varma V, et al. Plasma proteomic signatures predict dementia and cognitive impairment. *Alzheimers Dement (N Y)*. 2020;6(1):e12018.
32. Walker KA, Chen J, Zhang J, et al. Large-scale plasma proteomic analysis identifies proteins and pathways associated with dementia risk. *Nat Aging*. 2021;1(5):473–489. doi:10.1038/s43587-021-00064-0
33. Hill JJ, Davies MV, Pearson AA, et al. The myostatin propeptide and the follistatin-related gene are inhibitory binding proteins of myostatin in normal serum. *J Biol Chem*. 2002;277(43):40735–40741. doi:10.1074/jbc.M206379200
34. Hill JJ, Qiu Y, Hewick RM, et al. Regulation of myostatin in vivo by growth and differentiation factor-associated serum protein-1: a novel protein with protease inhibitor and follistatin domains. *Mol Endocrinol*. 2003;17(6):1144–1154. doi:10.1210/me.2002-0366
35. Zimmers TA, Davies MV, Koniaris LG, et al. Induction of cachexia in mice by systemically administered myostatin. *Science*. 2002;296(5572):1486–1488. 10.1126/science.1069525
36. McPherron AC, Lawler AM, Lee S-J. Regulation of skeletal muscle mass in mice by a new TGF-p superfamily member. *Nature*. 1997;387(6628):83–90. 10.1038/387083a0
37. Lee SJ, Lehar A, Liu Y, et al. Functional redundancy of type I and type II receptors in the regulation of skeletal muscle growth by myostatin and activin A. *Proc Natl Acad Sci USA*. 2020;117(49):30907–30917. doi:10.1073/pnas.2019263117
38. Lin Y-S, Lin F-Y, Hsiao Y-H. Myostatin is associated with cognitive decline in an animal model of Alzheimer's disease. *Mol Neurobiol*. 2019;56(3):1984–1991. doi:10.1007/s12035-018-1201-y
39. O'Bryant SE, Ferman TJ, Zhang F, et al. A proteomic signature for dementia with Lewy bodies. *Alzheimers Dement (Amst)*. 2019;11:270–276. doi:10.1016/j.dadm.2019.01.006
40. Dominici R, Finazzi D, Polito L, et al. Comparison of β2-microglobulin serum level between Alzheimer's patients, cognitive healthy and mild cognitive impaired individuals. *Biomarkers*. 2018;23(6):603–608. doi:10.1080/1354750X.2018.1468825