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Clinical and Environmental Correlates of Serum BDNF: A descriptive study with plausible implications for AD research

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Abstract

Background—Brain derived neurotrophic factor (BDNF) may play a central role in the pathogenesis of Alzheimer's disease (AD) through neurotrophic effects on basal cholinergic neurons. Reduced serum levels of BDNF are observed among AD patients and may predict AD risk. Nevertheless, knowledge about factors associated with its levels in blood is lacking.

Objective—To identify clinical and demographic correlates of serum BDNF levels.

Methods—BDNF was measured from serum collected between 1992–1996 and 1998–2001 in participants from the Original and Offspring cohorts of the Framingham Study, respectively. A cross-sectional analysis was done to evaluate the relationship between clinical measures and BDNF levels using standard linear regression and stepwise models. Analyses were conducted in the total sample and separately in each cohort, and were adjusted for age and sex.

Results—BDNF was measured in 3,689 participants (mean age 65 years, 56% women; 82% Offspring). Cigarette smoking and high total cholesterol were associated with elevated BDNF levels, and history of atrial fibrillation was associated with decreased levels. Elevated BDNF levels were related to greater physical activity and lower Tumor Necrosis Factor- α levels in Offspring.

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Drs Seshadri and Beiser had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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Stepwise models also revealed associations with statin use, alcohol consumption and Apolipoprotein Eε4 genotype.

Conclusion—Serum BDNF correlates with various metabolic, inflammatory and life-style measures which in turn have been linked with risk of AD. Future studies of serum BDNF should adjust for these correlates and are needed to further explore the underlying interplay between BDNF and other factors in the pathophysiology of cognitive impairment and AD.

Keywords

Brain-Derived Neurotrophic Factor; Alzheimer's disease; Cohort Studies; Cross Sectional Analysis

INTRODUCTION

Brain-derived neurotrophic factor (BDNF), a member of the neurotrophins superfamily, has received remarkable attention in Alzheimer's disease (AD) research. It plays central roles in normal brain functions and development, regulates growth and survival of neurons, promotes long term potentiation and modulates synaptic transmission and activity-dependent plasticity [1–5]. BDNF and its receptor, tyrosine receptor kinase B (TrkB), are highly expressed and widely distributed throughout the central nervous system, especially in the cerebral cortex and hippocampus [6, 7] where they are implicated in memory formation and long-term potentiation [8, 9]. In recent years, accumulating evidence suggests that BDNF may play a crucial role in AD pathogenesis. Indeed, AD patients have reduced expression of BDNF in the entorhinal cortex and hippocampus compared to non-demented individuals [10, 11]. Genetic polymorphism in BDNF's gene (specifically Val66Met) has been associated with reduced regional brain volumes as well as poorer cognitive performance [12–14].

Substantial amounts of BDNF are present in peripheral blood, and it is possible that these levels reflect those of CNS because after being produced by neurons and glia, BDNF readily crosses the blood brain barrier [15, 16]. Moreover, BDNF also can be produced by peripheral blood mononuclear cells (PBMC) in amounts that vary in diverse pathological conditions [17]. Accumulating data suggest that reduced circulating BDNF levels are present in MCI and AD patients and are related to poorer cognition, smaller hippocampal volume, and recently also to widespread brain amyloid burden [18].

While this literature places BDNF as a central player in AD pathophysiology, other findings also show, that BDNF does not work in isolation but is likely involved in pathways and interaction networks. Indeed, a recent prospective study by our group found that dementia-free individuals with higher BDNF levels were less likely to develop AD. Interestingly, there was a robust interaction between BDNF serum levels and sex, education and age, such that low BDNF levels were associated with increased AD risk only among women, highly educated individuals and the oldest-old [19]. Effect modification by sex has been previously demonstrated in other studies, [20, 21] and can be explained by BDNF interaction with estrogen [22], progesterone [23, 24], and other sex-related hormones. The complexity of the underlying mechanisms involving BDNF is also indicated by the emerging evidence of common influences of amyloid β and cytokines levels on BDNF production, and the

hypothesis that BDNF levels may at least partly explain the link between inflammation and brain function [25–27]. Furthermore, alterations in BDNF circulating levels are not a special feature of neurodegenerative diseases but have also been linked with cardiovascular disease [28], type 2 diabetes [29] and the metabolic syndrome [30], which, in turn, confer risk for AD.

Taken together, BDNF is considered a promising molecule in AD, and future exploration should take into account possible multifactorial mechanisms such as gene-environment [31] and protein-protein [32] interactions. Moreover, BDNF in AD may serve not only as a biomarker for disease risk and prognosis, but may even have therapeutic effects [33, 34]. Importantly, such effects may be achieved by non-invasive means, as BDNF production can be induced by behavioral factors such as physical activity [35] and caloric restriction [36], however other factors associated with BDNF levels in blood are not fully known. In the current descriptive study, we aimed to identify demographic and clinical correlates of serum BDNF by exploiting data from the Original and Offspring cohorts of the Framingham Study. Our results may assist in clarifying AD mechanisms, enhancing precision of future clinical and observational studies, and finally, may point to modifiable factors which affect BDNF levels and therefore possibly also alter AD risk.

METHODS

Study Participants

The design of the Framingham Heart Study (FHS) cohorts has been previously described [37, 38]. Briefly, in 1948 the Framingham Heart Study began enrollment of 5,209 men and women (aged 28–62 years) in the Original cohort. These participants subsequently underwent biennial medical examinations. In 1971, 5,124 offspring of the Original participants and their spouses were enrolled into the Offspring cohort. These participants underwent examinations approximately every four to eight years. The present analysis uses data from the 23rd Original cohort examination (1992–1996) and the 7th Offspring cohort examination (1998–2001). Of the Original cohort, 1,026 participants attended examination 23 (mean age 82, 33% males) and from the Offspring cohort 3,539 participants attended examination 7 (mean age 62, 46% males).

Measurement of BDNF

Serum BDNF levels were determined using Quantikine ELISA kits (Cat. # DBD00) obtained from R&D Systems (Minneapolis, MN) after a 20-fold dilution in polypropylene tubes with Calibrator Diluent RD6P included in the kits. The sensitivity of the assay is estimated to be at or below 62.5 pg/ml. The intra- and inter-assay coefficients of variation (CVs) were 3.8 – 6.2% and 7.6 – 11.3%, respectively. The reference range is 6.2 – 42.6 ng/ml.

Measurement of clinical variables

At each study examination, FHS participants underwent a physical examination, medical history interview, and laboratory measurements. Body mass index was calculated by dividing the participants' weight (kg) by the square of their height (m²). Waist

circumference and hip circumference were measured in inches according to standardized protocols. Systolic and diastolic blood pressures were measured twice by a study physician and the average of the two measurements was used. Hypertension was defined as a Systolic of ≥ 140 mm Hg or diastolic of ≥ 90 mm Hg or current treatment with anti-hypertensive medication. Current smoking was defined by having smoked at least one cigarette per day for the previous year. Alcohol use was self-reported and converted to ounces per week. Diabetes mellitus was defined as random glucose level of ≥ 200 mg/dL, fasting plasma glucose level of ≥ 126 mg/dL or treatment with insulin or a hypoglycemic agent. Total and HDL cholesterol were measured at random state and after an overnight fast in the Original and Offspring cohorts, respectively. Medication use (statins, anti-hypertensives) was assessed by self-report. History of cardiovascular disease (myocardial infarction, angina, coronary insufficiency, stroke, transient ischemic attack, intermittent claudication, or congestive heart failure) and history of atrial fibrillation were assessed by a review panel. APOE ϵ 4 (yes vs. no) was defined according to whether the participant had at least one or more E4 alleles.

In addition to the above-mentioned risk factors, the following were measured only on participants from the FHS Offspring cohort: Center for Epidemiologic Studies Depression Scale (CES-D), physical activity index, triglycerides, high sensitivity C-reactive protein (CRP), tumor necrosis factor- α (TNF- α), homocysteine, estimated glomerular filtration rate (eGFR), and total calories. Triglycerides were measured using automated enzymatic assays, CRP was measured using high sensitivity assay (BN100 nephelometer; Dade Behring, Deerfield, IL) and TNF- α was measured with commercially available ELISA kits from R&D Systems (Minneapolis, MN). eGFR was calculated using the Modification of Diet in Renal Disease (MDRD) Study equation [39]. Total homocysteine was measured with high-performance liquid chromatography with fluorometric detection. Total calories were assessed using the self-administered food frequency questionnaires (FFQ; Willett questionnaire) [40].

Statistical Analysis

The following variables were natural log-transformed to normalize their distributions: triglycerides, CRP, TNF- α , and homocysteine. Age- and sex-adjusted linear regression models were constructed to estimate differences in each clinical measure per standard deviation of BDNF serum levels. Models were constructed for all study participants as well as separately by cohort. Stepwise selection models using backwards elimination using a p-value cutoff of 0.10 were performed to determine the best fitting model for each outcome. Age and sex were forced into all models. All statistical analyses were done using SAS v.9.4 (Cary, NC). A two-sided p-value of <0.05 was considered statistically significant.

RESULTS

The characteristics of the study sample are presented in Table 1. Overall there were 3,689 participants for whom BDNF was measured (669 Cohort and 3,020 Offspring). The mean age was 65 years and approximately 55% were women.

The results of the age- and sex- adjusted linear regressions relating BDNF to each clinical measure are presented in Table 2. In the total study sample, the following factors were statistically significantly associated with increased BDNF level: current cigarette smoking as well as pack-years of smoking and cigarettes per day. In addition, BDNF levels were positively correlated with total cholesterol levels, and individuals with prevalent atrial fibrillation had lower levels of BDNF. Results were similar when the analysis was restricted to the 3,020 Offspring cohort participants. Among the subset of Original cohort participants, increased BDNF levels were associated with pack-years of smoking, cigarettes per day and total cholesterol. Additionally, Original cohort participants with hypertension had higher BDNF levels compared to those with no hypertension. For the subset of variables that were only measured in the Offspring cohort, elevated BDNF levels were associated with physical activity and with low TNF- α .

Table 3 presents the results of the stepwise selection models of the clinical factors associated with BDNF levels. Among all study participants, the final age- and sex-adjusted model demonstrated an association of increased BDNF levels with cigarette smoking, statin use and higher levels of total cholesterol, while decreased levels were associated with atrial fibrillation, APOE ϵ 4 genotype, and alcohol consumption. Among the Offspring cohort, TNF- α was also included in the model. In these models, older age and male sex were associated with lower serum BDNF levels.

DISCUSSION

Among participants of the Framingham Study, higher mean serum BDNF levels were associated with younger mean age, female sex, smoking, physical activity, higher levels of total cholesterol, statin use and lower mean levels of TNF- α . Individuals with prevalent atrial fibrillation and those who carry at least one ApoE ϵ 4 allele had lower levels compared to those without these risk factors, and BDNF levels were inversely related to alcohol consumption.

Decreased serum BDNF levels were related to older age in the Offspring but not in the older Original cohort. This inconsistency is also reflected in the literature, where some [41, 42] but not all [43, 44] studies show significant age-BDNF associations, and may be explained by between studies' differences in age ranges, non-linear relationship such as a possible floor effect in the oldest old, or possible interactions with age-related factors. Increased levels of serum BDNF among women compared to men have been found in the current study as well as in a sample from the Baltimore Longitudinal Study of Aging [45] but were not evident in other samples [43, 46, 47]. If these findings are validated, they may point to an interaction of BDNF with sex hormones [43, 48]. In AD, some evidence suggests that polymorphism in the BDNF gene is linked with AD risk in women only [21].

The relationship of smoking with increased BDNF levels in the current study is robust, as indicated by the large effect size and by the observed dose-response relationship showing a gradual increase in BDNF with increasing packs per year, as well as with amount of cigarettes per day. The lack of significant findings among smokers from the Original cohort may be due to reduced statistical power since only around 7% of the older, Original cohort

participants were current smokers; current smoking decreases as persons age and develop co-morbidities. Elevated levels of serum BDNF have also been demonstrated among a sample from the Netherlands Study of Depression and Anxiety [48]. However, in contrast to our findings, there was no evidence in the Netherlands Study for association with pack years or amount of cigarettes per day. BDNF was also higher in smokers compared to non-smokers in other studies [43, 46], however unlike in the current study, the association did not always remain significant after adjustment for potential confounders [43]. Although for many years the relationship between smoking and dementia risk has been inconclusive, recent evidence suggests that smoking confers an increased risk [49–51]. An important candidate mechanism promoting AD pathophysiology and increased risk for AD in smokers is smoking-related cerebral oxidative stress [52]. Conversely, data suggest that nicotine is protective against AD neuropathology via activation of nicotinic acetylcholine receptors (nAChRs), and specifically, activation of the $\alpha 7$ nAChR subtype by nicotine influences neuroprotective processes such as neurotransmitter release, cell survival and plasticity [53, 54]. Moreover, early AD is characterized by a significant loss of neurons expressing nAChRs, and amyloid aggregation is frequently pronounced at these receptors [54]. Emerging evidence from in vitro studies suggests that BDNF, through binding to its receptor, TrkB, decreases $\alpha 7$ nAChR responses in the hippocampus interneurons [55]. Thus, the elevated BDNF serum levels among smokers observed in our study may indicate an initiation of a process involving nicotine, nAChRs and amyloid beta, which eventually leads to a higher AD risk. Further research is warranted in order to clarify these complex mechanisms.

In line with our findings, a positive correlation between plasma BDNF and total cholesterol has been demonstrated in participants of the Baltimore Longitudinal Study of Aging, although the latter was apparent in women only [45]. BDNF has been shown to elicit cholesterol biosynthesis in cultured cortical and hippocampal neurons, a process that promotes synapse development [56]. In addition, BDNF regulates the translocation of TrkB receptors to lipid rafts, a process that is essential for BDNF signaling [57]. In light of these observations, the positive association between BDNF serum levels and total cholesterol in our study may reflect a role of BDNF in lipid metabolism and increased AD risk. Alternatively, BDNF may reflect an association with statin use, which in turn has been linked with increased BDNF production [58, 59] and was significantly related to BDNF levels in our models.

Neuroinflammation has a central role in AD pathophysiology [60, 61]. Recent evidence further suggests that immune-system activation, ultimately mediated mainly by glial cells such as microglia and astrocytes, not only follows A β deposition, but may precede AD-like pathology and is sufficient to cause it. Moreover, it is now suggested that immune processes may, at least at a given time point, drive AD pathology independently of A β deposition and sustain increased A β levels, thus exacerbating pathology and culminating in a pathophysiological cycle [60]. Emerging studies have shown that inflammation affects the expression of BDNF within the brain. In rats, for example, peripheral immune activation significantly decreased BDNF mRNA levels in the hippocampus and cortical regions [62–64]. These findings have led to the hypothesis that brain inflammatory response to aging and immune challenge may cause a reduction in BDNF levels, a process that may lead to

impairment in brain plasticity and cognition [25–27]. In this context, our finding that increased levels of TNF- α are related to low BDNF levels further supports this hypothesis, and may indicate that the previous observations may be valid also in humans and when serum rather than brain tissue levels are measured. Future studies are needed to further explore the role of BDNF in neuro-inflammation, and to identify the specific cytokines involved. In our dataset, TNF- α , but not CRP was related to BDNF levels, and indeed, recent evidence shows that inhibition of TNF- α prevents the reduction in the levels of hippocampal BDNF as well as stress induced cognitive decline in rats [65].

Our finding that physical activity is related to increased serum BDNF levels add to the bulk of evidence from animal and humans models showing that exercise enhances hippocampal BDNF gene expression and serum levels [35, 66, 67]. These exercise-induced alterations in BDNF levels may result in improved brain integrity and cognition as implied from animal studies [68], however greater understanding of the underlying mechanisms in humans awaits additional studies.

The observation of lower levels of BDNF in the serum of individuals with a history of atrial fibrillation is intriguing. Indeed, several basic experiments reported that BDNF enhances angiomyogenesis, contributes to survival of endothelial cells during cardiovascular development, provides protection against cardiac remodeling after myocardial infarction and modulates heart contraction force [69–71]. Lower BDNF levels also have been measured in plasma of patients with heart failure [72]. Hence, BDNF may play an important role not only in the central nervous system but also in the cardiovascular system, and may explain the overlap between risk of cognitive impairment and that of heart diseases [73].

ApoE ϵ 4 genotype emerged in the current study as a factor associated with serum BDNF levels in a multivariable model, but not after adjusting for age and sex alone. With regard to AD risk, there is evidence of gene-environment interactions which may be reflected in the current results. For example, physical activity has been shown to be effective in reducing AD risk, however ApoE ϵ 4 carriers may particularly benefit from being physically active [74]. Our findings may also reflect the complex interactions between the ApoE ϵ 4 and BDNF genes, as emerging data show that ApoE ϵ 4 modifies the association between genetic polymorphism in the BDNF gene such that carriers of both risk variants have significantly higher β amyloid burden [75], and poorer cognition [76].

Lastly, alcohol consumption was related to lower levels of serum BDNF in the stepwise regression model of the current study's combined and Offspring samples as well as in others [48, 77, 78]. Although the role of BDNF in alcohol-use disorders is unclear [79], it is postulated that BDNF modulates alcohol-induced neurotoxicity, possibly through blockage of *N*-methyl-d-aspartate receptors which results in decreased BDNF expression and thus increased neurotoxicity [80].

BDNF is a key protein modulating brain plasticity, and may even exert therapeutic effects for AD [81]. The expression of BDNF is tightly controlled, and is influenced by inputs such as neuronal activity [82], exercise [83], and stress [84]. In the current study we identified

several AD-related factors which correlate with BDNF human serum and may act synergistically with BDNF to influence AD risk.

The study's strengths are its moderately large samples of community-dwelling individuals, and the ability to examine BDNF correlates in two independent cohorts of different age groups. Yet, it is important to note that serum BDNF measurements are variable and can be influenced by time of sampling, storage duration, whether it was measured in a fasting state [48], and possibly other yet unknown parameters; we have standardized these to the extent feasible in an epidemiological setting.

Our findings may set the ground for future studies which will further investigate whether BDNF levels lie on the causal pathway between the clinical and life-style measures identified in the current study and the late-life risk for cognitive impairment and AD.

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Table 1

Baseline characteristics of the study sample

	All Participants (N=3689)	Offspring Cohort (N=3020)	Original Cohort (N=669)
Age	65.0 (11.3)	61.6 (9.5)	80.3 (4.1)
Male, n (%)	1620 (43.9)	1375 (45.5)	245 (36.6)
BDNF, ng/mL	23532 (8272)	23742 (8411)	22583 (7544)
Waist-hip ratio	0.9 (0.1)	0.9 (0.1)	1.0 (0.1)
Waist circumference, in	39 (6)	39 (6)	38 (5)
Body mass index, kg/m ²	27.9 (5.2)	28.2 (5.3)	26.6 (4.6)
Systolic blood pressure, mm Hg	130 (20)	127 (19)	143 (22)
Hypertension treatment, n (%)	1355 (36.8)	1024 (33.9)	331 (50.0)
Hypertension, n (%)	1864 (50.6)	1380 (45.7)	484 (72.7)
Current smoking, n (%)	422 (11.5)	374 (12.4)	48 (7.2)
Pack-years of smoking *	17 (23)	17 (23)	18 (23)
Cigarettes per day **	2.0 (6.6)	2.2 (7.0)	1.1 (4.6)
Alcohol, oz/week	2.4 (3.7)	2.6 (3.8)	1.7 (3.0)
Diabetes, n (%)	421 (11.5)	337 (11.2)	84 (12.8)
Total cholesterol, mg/dL	201 (37)	200 (37)	205(38)
HDL cholesterol, mg/dL	53 (17)	54 (17)	50 (17)
Statin use, n (%)	619 (16.8)	571 (18.9)	48 (7.2)
Prevalent CVD (all), n (%)	586 (15.9)	336 (11.1)	250 (37.4)
Prevalent AF, n (%)	210 (5.7)	94 (3.1)	89 (13.3)
APOEε4, n (%)	789 (21.8)	658 (22.2)	131 (19.9)
Depression (CES-D score)	---	5.3 (6.7)	---
Physical activity index	---	37.7 (6.4)	---
Ln Triglycerides, mg/dL	---	4.8 (0.5)	---
Total calories, kcal/day	---	1823 (593)	---
Ln CRP, mg/L	---	0.8 (1.1)	---
Ln TNF-α, pg/mL	---	0.2 (0.5)	---
Ln Homocysteine, μmol/L	---	2.1 (0.3)	---
eGFR, mL/min/1.73 m ²	---	84.7 (19.2)	---

Note: All values are mean (standard deviation) unless otherwise indicated

* Among current and former smokers;

** among current smokers

Abbreviations: BDNF, brain derived neurotrophic factor; HDL, high-density lipoprotein; CVD, cardiovascular disease; AF, atrial fibrillation; APOEε4, Apolipoprotein Eε4; CES-D, center for epidemiologic studies depression scale; CRP=C-reactive protein; TNF-α=tumor necrosis factor α; eGFR=estimated glomerular filtration rate

Table 2

Linear regression results of the association between risk factors and BDNF

Exposure Variable	All Participants (N=3689)		Offspring Cohort (N=3020)		Original Cohort (N=669)	
	Beta (SE)	P-value	Beta (SE)	P-value	Beta (SE)	P-value
Waist-hip ratio	-1058 (1979)	0.59	616 (2236)	0.78	-7211 (4340)	0.10
Waist circumference, in	9 (26)	0.72	17 (29)	0.56	-22 (61)	0.72
Body mass index, kg/m ²	18.2 (26.1)	0.49	16.9 (28.7)	0.56	40.6 (61.7)	0.52
Systolic blood pressure, mmHg	5.7 (7.4)	0.44	3.7 (8.6)	0.67	15.9 (13.7)	0.25
Hypertension treatment	-174.0 (291.9)	0.55	-197.1 (335.0)	0.56	83.9 (588.4)	0.89
Hypertension	119.2 (290.4)	0.68	-85.3 (323.7)	0.79	1408.2 (656.8)	0.03
Current smoking	2286.4 (427.1)	<0.0001	2349.9 (462.8)	<0.0001	2050.6 (1136.6)	0.07
Pack-years of smoking *	31.5 (6.2)	<0.0001	32.7 (7.0)	<0.0001	28.0 (14.2)	0.049
Cigarettes per day **	101.9 (20.6)	<0.0001	100.0 (21.9)	<0.0001	137.0 (64.0)	0.03
Alcohol consumption, oz/week	-46.7 (38.4)	0.22	-41.2 (41.7)	0.32	-61.1 (100.4)	0.54
Diabetes	335.1 (429.2)	0.44	293.4 (488.3)	0.55	552.2 (890.6)	0.54
Total cholesterol, mg/dL	19.4 (3.7)	<0.0001	17.3 (4.2)	<0.0001	32.5 (8.0)	<0.0001
HDL cholesterol, mg/dL	3.2 (8.8)	0.72	1.2 (10.0)	0.90	4.8 (18.6)	0.80
Statin use	140.6 (363.2)	0.70	98.3 (395.7)	0.80	951.6 (1138.3)	0.40
Prevalent CVD	-596.9 (391.6)	0.13	-881.0 (498.7)	0.08	-122.6 (612.0)	0.84
Prevalent AF	-1701.6 (598.6)	0.005	-1984.5 (787.6)	0.01	-1585.3 (875.3)	0.07
APOEε4	-562.5 (329.7)	0.09	-625.0 (367.4)	0.09	-271.4 (739.0)	0.71
Depression (CES-D score)	---	---	-34.9 (23.0)	0.13	---	---
Physical activity index	---	---	51.0 (24.3)	0.04	---	---
Ln Triglycerides	---	---	70.6 (281.4)	0.80	---	---
Total calories, kcal/day	---	---	-0.34 (0.27)	0.21	---	---
Ln CRP, mg/dL	---	---	10.8 (138.3)	0.94	---	---
Ln TNF-α, pg/mL	---	---	-974.3 (357.6)	0.007	---	---
Ln Homocysteine, μmol/L	---	---	823.9 (515.1)	0.11	---	---
eGFR, mL/min/1.73 m ²	---	---	12.4 (8.6)	0.15	---	---

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* Among current and former smokers;
** among current smokers

All analyses are adjusted for age and sex

Abbreviations: BDNF, brain derived neurotrophic factor; HDL, high-density lipoprotein; CVD, cardiovascular disease; AF, atrial fibrillation; APOEε4, Apolipoprotein Eε4; CES-D, center for epidemiologic studies depression scale; CRP, C-reactive protein; TNF-α, tumor necrosis factor α; eGFR, estimated glomerular filtration rate

Table 3

Results for the stepwise selection models (backwards elimination using a cutoff of $p < 0.10$) for BDNF

	Variables in Model	Beta (SE)	P-value
All participants	Age	−48.3 (12.5)	0.0001
	Male sex	−1279.9 (293.9)	<0.0001
	Current smoking	2510.5 (437.9)	<0.0001
	Total cholesterol, mg/dL	21.6 (3.9)	<0.0001
	Statin use	740.1 (375.8)	0.049
	History of AF	−1349.5 (616.7)	0.03
	APOEε4	−661.4 (331.5)	0.046
	Alcohol, oz/week	−83.7 (39.0)	0.03
Offspring Cohort	Age	−43.9 (20.0)	0.03
	Male sex	−2016.3 (393.0)	<0.0001
	Current smoking	2553.1 (581.8)	<0.0001
	Total cholesterol, mg/dL	15.9 (5.2)	0.002
	APOEε4	−890.2 (443.7)	0.04
	Alcohol, oz/week	−83.3 (50.0)	0.096
	Ln TNF-α, pg/mL	−904.9 (384.9)	0.02
Original Cohort	Age	78.4 (74.1)	0.29
	Male sex	644.7 (657.0)	0.33
	Current smoking	2182.3 (1172.7)	0.06
	Total Cholesterol, mg/dL	34.0 (8.3)	<0.0001

Note: Age and sex are forced into the model.

Abbreviations: BDNF, brain derived neurotrophic factor; AF, atrial fibrillation; APOEε4, Apolipoprotein Eε4; TNF-α, tumor necrosis factor α