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Serum BDNF and VEGF levels are associated with Risk of Stroke and Vascular Brain Injury: Framingham Study

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Abstract

Background and Purpose—BDNF, a major neurotrophin and VEGF, an endothelial growth factor have a documented role in neurogenesis, angiogenesis and neuronal survival. In animal experiments they impact infarct size and functional motor recovery after an ischemic brain lesion. We sought to examine the association of serum BDNF and VEGF with the risk of clinical stroke or subclinical vascular brain injury in a community-based sample.

Methods—In 3440 stroke/TIA-free FHS participants (mean age 65±1 yrs, 56% W), we related baseline BDNF and logVEGF to risk of incident stroke/TIA. In a subsample with brain MRI and with neuropsychological (NP) tests available (N=1863 and 2104, respectively; mean age 61±9 yrs, 55% W, in each) we related baseline BDNF and logVEGF to log-white matter hyperintensity volume (IWMHV) on brain MRI, and to visuospatial memory and executive function tests.

Results—During a median follow-up of 10 years, 193 participants experienced incident stroke/TIA. In multivariable analyses adjusted for age-, sex- and traditional stroke risk factors, lower BDNF and higher logVEGF levels were associated with an increased risk of incident stroke/TIA (HR comparing BDNF Q1 versus Q2–4:1.47, 95% CI:1.09–2.00, p=0.012; and HR/SD increase in logVEGF:1.21, 95% CI:1.04–1.40, p=0.012). Persons with higher BDNF levels had less IWMHV ($\beta\pm SE=-0.05\pm 0.02$; p=0.025), and better visual memory ($\beta\pm SE=0.18\pm 0.07$; p=0.005).

Conclusions—Lower serum BDNF and higher VEGF concentrations were associated with increased risk of incident stroke/TIA. Higher levels of BDNF were also associated with less white matter hyperintensity and better visual memory. Our findings suggest that circulating BDNF and VEGF levels modify risk of clinical and subclinical vascular brain injury.

Keywords

BDNF; VEGF; Risk; Stroke; Brain MRI; Subclinical

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None

INTRODUCTION

Cerebrovascular disease (overt and covert) accounts for a significant proportion of brain aging-related mortality and morbidity.^{1, 2} Whereas the burden of cerebrovascular disease may be largely attributed to well established stroke risk factors, genetic and lifestyle differences and structural and functional brain capacities (such as neural density, synaptic connectivity, and the compensatory ability of existing neural networks) may modulate risk and response to vascular brain injury.^{3, 4} Therefore, searching for novel pathways involved in cerebrovascular disease may improve our understanding of disease mechanisms and help facilitate discovery of new molecular targets for stroke therapy and prevention. Brain derived neurotrophic factor (BDNF) and vascular endothelial growth factor (VEGF), both present during early neuronal development, have also been found to have a role in maintaining neuronal plasticity and in neuronal repair by promoting neurogenesis and angiogenesis.^{5, 6}

Low circulating BDNF concentrations have been observed in patients with coronary artery disease (CAD), type 2 DM, metabolic syndrome, acute coronary syndrome⁷ and physical inactivity,⁸ and higher VEGF concentrations in patients with hypertension, type 2 DM, smoking, obesity, and CAD^{9, 10}, all risk factors associated with stroke.¹¹ Interestingly, under hypoxic and ischemic conditions, both BDNF and VEGF and their respective receptors are upregulated in neurons and vascular endothelial cells (ECs).^{12, 13}

To our knowledge, there has to date been no prospective or clinical study that has systematically examined the role of BDNF and VEGF in determining the risk of clinical stroke or subclinical vascular brain injury in a community based population. We proposed to examine an association between serum BDNF and VEGF levels and i) risk of stroke/TIA, ii) brain MRI measures of subclinical vascular injury (WMHV) and iii) performance on executive function and visual memory tasks; the cognitive domains previously associated with vascular risk factors and WMHV.¹⁴

METHODS

Study Sample

The Framingham Study is a prospective, community-based cohort that enrolled 5209 participants in 1948; these participants have had biennial re-examinations.¹⁵ In 1971, the offspring of the Original cohort and their spouses were enrolled in an Offspring cohort, assessed once every four years.¹⁶ The participants from both cohorts have been under ongoing surveillance for incident stroke and TIA since the inception of the study.

Stroke- and TIA- free participants in the Original cohort who attended examination 23 [n=581, 80±4yrs, 64% Women(W)], and Offspring participants who attended examination 7 (n=2859, 62±9yrs, 54% W) were included if they also had available BDNF and VEGF levels with no missing covariate data (Study Sample 1; n=3,440); only 88 persons (3%) were excluded from Study sample 1 for missing covariate data.

Offspring participants who attended examination 7 were also invited to undergo cognitive testing and brain MRI (1999–2005). Among the 3020 persons who attended examination 7 and had available BDNF and VEGF levels, 68 were excluded for missing covariate data. Of the remaining 2952 persons, 2186 had a neuropsychological (NP) battery administered, and 1945 underwent brain MRI. 82 persons were excluded for having stroke, dementia or other neurological illness (such as multiple sclerosis, severe head trauma or brain tumor) that could affect MRI/NP measures; we did not exclude persons with TIA in this analysis. The rest were eligible for investigation of the association between BDNF and VEGF and a)

WMHV and b) executive function and visual memory (Study samples 2a:N=1863, 61±9yrs, 55% W and 2b:N=2104, 61±9yrs, 55% W).

Laboratory Measurements of BDNF and VEGF

Serum BDNF and VEGF concentrations were measured using commercial ELISA assays obtained from R&D Systems (Minneapolis, MN) on previously frozen (stored at -70°C) blood samples drawn in the fasting state from persons who attended the 23rd Original cohort and the 7th Offspring examinations. The intra- and inter-assay coefficients of variation (CVs) for BDNF and VEGF were 4.8 and 7.6%, and for VEGF 3.9 and 8.4%, respectively.

The age and sex-adjusted Pearson correlation coefficient between BDNF and logVEGF was 0.15 ($p<0.001$) for the Original cohort, 0.23 ($p<0.001$) for the Offspring cohort, and 0.22 ($p<0.001$) for both cohorts combined.

Outcome Measures

Stroke/TIA (clinical outcome)—Our protocols for stroke surveillance and for establishing the diagnosis and type of stroke have been previously published.¹¹ Stroke was defined as an acute onset focal neurological deficit of presumed or definite vascular etiology, persisting for more than 24 hours and TIA symptoms lasting for 24 hours. When available we also used imaging studies and other laboratory criteria, noninvasive vascular studies, cardiac evaluations for a source of embolus and also information from autopsy studies; these data were also used to categorize strokes as ischemic or hemorrhagic.

Brain Imaging Measures (subclinical outcome)—MRI acquisitions and measurement techniques and inter-rater reliability scores have been previously published.¹⁷ In summary, digital MRI information once acquired on a 1 or 1.5 Tesla Siemens Magnetom and transferred to the centralized imaging center at the University of California-Davis Medical center, was analyzed by operators blinded to the participant's demographics, exposure to stroke risk factors, and BDNF and VEGF levels. The volume of abnormal WMH was determined by using previously described semi-automated methods, adjusted for total cranial volume (TCV) and log-transformed (log-WMHV). Participants whose log-WMHV was more than 1 SD above the age-specific mean were categorized as having extensive WMH volume (ext-WMHV).

Cognitive Testing—Subjects were administered (typically on the same day as their MRI) a half-hour NP battery using standard administration protocols and trained examiners. Details of the tests administered and normative values for the Framingham Original and Offspring cohorts have been previously published.^{18, 19} In this study we selected cognitive domains previously associated with vascular risk factors and vascular brain injury; visuospatial memory [measured by delayed recall on the visual reproduction test (VR-d) from the Wechsler Memory Scale] and executive function [measured by Trail Making Test B-A]. We transformed Trails B-A using a log-transformation to normalize its distribution and changed its sign so that smaller numbers indicate poorer performance.

Definition of Covariates—We used previously described and validated components of the Framingham Stroke Risk Profile (FSRP),¹¹ as baseline covariates. Educational achievement was defined as: no high-school (HS) degree, HS degree only, or > HS degree (college degree)]. Depressive symptoms were evaluated with the Center for Epidemiological Studies Depression scale (CES-D).²⁰ The physical activity index (PAI) was calculated as a composite score based on information collected from a structured questionnaire.²¹ Both CES-D and PAI were log-transformed for analysis.

Statistical Analysis—BDNF and VEGF were analyzed as continuous variables. VEGF values were log-transformed to normalize the skewed distribution of VEGF. We used Cox-proportional hazards models to relate baseline serum BDNF and logVEGF levels (separately) to risk of incident stroke/TIA in Study Sample 1. We first examined the linear effect of a 1-standard deviation (SD) unit increase/decrease in BDNF and logVEGF on risk of stroke/TIA. We also explored threshold effects comparing the risk across quartiles of BDNF and VEGF. We observed a threshold effect with BDNF only. Therefore, we ran threshold models for BDNF using the lowest quartile, Q1, as the reference and also assessed a threshold model comparing risk for persons in Q1 to persons in Q2–4. We performed a similar set of analyses on a Study Sample 1b with stroke alone as the outcome. Using multivariable linear and logistic regression models we examined the cross-sectional associations between BDNF and logVEGF and markers of brain aging (WMHV, and VR-d and Trails B-A transformed as previously described) using Study Samples 2a and 2b. Results are given as the effect of a 1 SD increase (β /SD or OR/SD) or the difference between Q1 and Q2–4 (β /Q1 or OR/SD).

All primary analyses were adjusted for age as a continuous variable and sex (Model A). In Model A, for the MRI and NP analyses we also adjusted for the time interval between baseline (time when BDNF and VEGF were measured) and date of MRI acquisition and/or NP testing; for the NP analyses we also adjusted for education. In Model B, we additionally adjusted for vascular covariates that are components of the FSRP (systolic blood pressure, antihypertensive therapy, diabetes, smoking status, CVD and AF).

In secondary analyses, we ran Model C examining the association of BDNF and VEGF with MRI and NP after adjusting for total plasma homocysteine (tHcy) and total cholesterol which are biomarkers related to these measures in some but not all studies; these data were available at our baseline examination only for the Offspring, hence we could not adjust the stroke/TIA related analyses for these covariates. In Model D, we adjusted for lifestyle factors adding education level, depression status and physical activity index scores (PAI) to Model B.

To examine the incremental utility of BDNF and VEGF over age-, sex- and components of the FSRP in predicting stroke/TIA, we calculated net reclassification improvement (NRI) in 4 different models adding data on whether or not serum BDNF was in the lowest (Q1) quartile, SD units of change in BDNF and in logVEGF and finally when information on both BDNF and VEGF levels was utilized. We used clinically meaningful risk categories of <5%, 8% and >8% 10-year stroke risks,^{11, 22} defined *a priori* as risks experienced by the average person, at the baseline age of the current sample, in the presence of zero (low risk), one (intermediate risk), or more than one (high risk) of the stroke risk factors included in the FSRP.²³

All analyses were performed using Statistical Analyses System (SAS® Institute Inc).

RESULTS

Study Sample

In Table 1 we present the distribution of clinical and biomarker characteristics for Study Samples 1, 2a and 2b. In supplementary tables (Table I–III) we also provide the distribution of baseline characteristics based on BDNF quartiles (Q1 vs. Q2–4) for the stroke/TIA, the brain MRI and the NP study samples. In the stroke/TIA sample, as compared to those in Q2–Q4, participants in Q1 were significantly older and predominantly women, and had a higher prevalence of atrial fibrillation and lower prevalence of smoking. Similarly, in the brain MRI and the NP samples, participants in the Q1 subset were significantly older and

predominantly women, and had a lower prevalence of smoking (significant only in the brain MRI sample); they also had a higher prevalence of diabetes mellitus, but had lower cholesterol levels.

Association of BDNF and VEGF and Stroke/TIA

BDNF and Stroke/TIA (Table 2)—Over the 10 year follow-up period (median 8 years) we observed 193 incident events [130 strokes (including 111 ischemic, 18 hemorrhagic, and one clinically definite stroke of uncertain subtype) and 63 TIAs] in persons initially free of both stroke and TIA. In multivariable age- and sex- adjusted analysis (Model A) and in analysis adjusted for traditional stroke risk factors (Model B) we observed statistically non significant trends towards lower risk of stroke/TIA with higher BDNF levels (Model A: HR per SD increase in BDNF=0.87/SD, $p=0.07$). In quartile-based analysis adjusted for age and sex, we observed that lower serum BDNF levels were associated with an increased risk of stroke/TIA (HR comparing Q1 to Q2–4=1.51; 95%CI:1.11–2.03, $p=0.008$); this was also true after adjustment for traditional risk factors ($p=0.012$). In Figure 1 we show the cumulative incidence of stroke/TIA in Q1 vs. Q2–4. The results relating BDNF to stroke alone are shown in the online Supplementary table 4.

VEGF and Stroke/TIA (Table 2)—In multivariable analyses we observed statistically significant associations between higher serum logVEGF levels and increased risk of stroke/TIA (HR =1.21/SD; 95%CI:1.04–1.40, $p=0.012$) or stroke alone (HR =1.23/SD; 95%CI: 1.05–1.46, $p=0.013$) as shown in the online Supplementary table 4.

Net Reclassification Improvement (NRI)—In reclassification analyses (supplementary table 5), BDNF and logVEGF alone and the combination of BDNF and logVEGF, when added to a risk assessment model based on traditional stroke risk factors alone (as identified by the FSRP) resulted in significant improvement of risk prediction (NRI for BDNFQ1vsQ2–4 =0.199, 95%CI: 0.023–0.384; NRI for logVEGF=0.234, 95%CI:0.067–0.401, and NRI for BDNF+logVEGF=0.274, 95%CI:0.090–0.449

Association of BDNF and VEGF and White Matter Hyperintensities (Table 3)

The median time interval between BDNF and VEGF collection and brain MRI and NP measures was 0.6 years. In our sample, the mean log-WMHV was -3 ± 1 ml and the prevalence of ext-WMHV was 12%. In Table 3 we present results of multivariable analyses relating serum BDNF and VEGF levels individually to the log-WMHV and to the prevalence of ext-WMH on brain MRI respectively. After adjustment for traditional stroke risk factors we observed that higher serum BDNF levels were strongly associated with less log-WMHV and with lower prevalence of ext-WMH (Model B: $\beta=-0.05\pm 0.02$, $p=0.025$ and OR=0.83, $p=0.009$, respectively). This was also true after adjusting for tHcy and total cholesterol levels (Model C) or education, PAI and CESD (Model D). We did not observe an association between logVEGF and WMH measures.

Association of BDNF and VEGF and Cognitive Measures (Table 4)

The mean cognitive test scores were 8 ± 3 on VR-d and 0.9 ± 0.9 for Trails B-A. In all models lower serum BDNF levels were associated with poorer visual memory, in both continuous and quartile-based analyses (Model B: $\beta/SD=0.18\pm 0.07$, $p=0.005$ and $\beta/Q1$ vs Q2–4 = -0.40 ± 0.15 , $p=0.010$, respectively). We did not observe any association between BDNF and performance on the executive function test. We also did not observe any association between logVEGF levels and either of the cognitive measures.

DISCUSSION

In our community-based, stroke-free middle-aged to elderly sample of 3440 Framingham study participants lower serum BDNF and higher serum VEGF concentrations were associated with increased risk of incident stroke/TIA over a 10 year observation period. Lower serum BDNF levels were also associated with greater white matter hyperintensities (log-WMHV and ext-WMHV) and with poorer visual memory performance (VR-d) in a subset of otherwise stroke-and dementia-free middle-aged persons. The associations were present after adjustment for putative stroke risk factors; suggesting that novel mechanisms associated with circulating levels of BDNF and VEGF and perhaps mediated via BDNF and VEGF pathways modify clinical stroke/TIA risk and the extent of covert vascular brain disease.

BDNF and VEGF levels and Stroke/TIA

To the best of our knowledge this is the first community-based study to report an association between serum BDNF and VEGF levels and risk of stroke/TIA. These associations are novel, but not unexpected.

As mentioned earlier, low BDNF and high circulating VEGF levels have been observed in patients with vascular risk factors associated with atherosclerosis and cerebrovascular disease.^{7–10, 12, 13, 23, 24} Thus, BDNF and VEGF may be biomarkers of these processes or may modify the risk of stroke/TIA by modulating the conventional risk factor pathways. Animal studies have established a positive effect of BDNF and VEGF on infarct size and on long-term potentiation, neuronal remodeling and functional motor recovery after induction of an ischemic brain lesion.^{25, 26} In addition to being a potent angiogenic factor, VEGF has also demonstrated neuroprotective properties: inhibition of apoptosis and stimulation of astrocytes, microglia and cortical neurons growth during embryogenesis and adult neurogenesis.²⁷ However, higher VEGF levels have been observed in persons with obesity, metabolic syndrome, and markers of atherosclerosis such as increased carotid intimo-medial thickness, perhaps as a compensation to their subclinical disease.²⁸

Possible Mechanisms for Association of BDNF and Stroke/TIA—There is experimental evidence that neurons and glial cells act as endogenous sources of BDNF after ischemic and other brain injuries.^{25, 29} It has been shown that BDNF regulates homeostatic interactions between neurons, glial cells and the vasculature, jointly referred to as “neurovascular unit”. Dysfunction of cerebral vascular BDNF signaling therefore may contribute to disruption of the neurovascular unit, hence to an alteration of tissue responses to vascular injury.³⁰ A small molecule BDNF mimetic (LM22A-4) when administered immediately after an ischemic stroke in adult mice lead to increased neurogenesis and improved functional motor recovery.³¹ Studies have also found that exogenous BDNF affects vasodilatation via upregulation of prostacyclin synthesis, and protects against vasoconstriction-related injury and thrombus formation in the walls of an isolated cerebral artery.¹³ Therefore BDNF could reduce stroke risk through its neurotrophic or its vascular effect.

Possible Mechanisms for Association of VEGF and Stroke/TIA—Upregulation of VEGF expression has been observed in the neurons, ECs and astrocytes within border zones of infarcted brain tissue in patients after ischemia.³² Two small clinical studies related higher VEGF levels in patients with acute stroke to better outcomes.^{23, 24} However, this upregulation could result in higher VEGF levels serving as a marker of chronic cerebral ischemia, and hence of increased stroke risk. Whereas there are no prior community-based studies to evaluate the effect of VEGF levels on the risk of incident stroke/TIA, our findings

parallel prior observations that higher VEGF levels are associated with other CVD risk factors and outcomes.^{9, 10, 12, 13} The biological mechanisms by which VEGF may play a role in cerebrovascular disease are thus complex and deserving of further investigation. While VEGF promotes neurogenesis and beneficial angiogenesis, angiogenesis is also a component in the progression of atherosclerosis.^{9, 33} Moreover, in the acute stroke setting the major source of the circulating VEGF may not be the ischemic brain tissue, but rather the transient peripheral leukocytosis.³³ Therefore, the net impact of the proatherogenic and angiogenic roles of VEGF in cerebrovascular disease warrants further investigation.

BDNF and White Matter Hyperintensities

To our knowledge, this is also the first study to examine an association between BDNF and WMH in a community-based, middle-aged, stroke- and dementia-free sample. Although the pathogenesis of WMH is largely unknown, based on available literature our findings have several possible explanations.

WMHs are highly prevalent in persons with vascular risk factors and stroke.^{34, 35} These radiographic lesions are thought to be a consequence of neuronal and/or vascular injury; resulting either from chronic hypoperfusion and ischemia, demyelination, axonal loss and gliosis in the territory of deep, small perforating cerebral arteries, or through neurodegenerative processes involved in usual brain aging and dementia.^{14, 34} In prior reports, WMHs were strongly related to stroke risk factors³⁵ and incident stroke, MCI and dementia.^{14, 36} As mentioned earlier, BDNF is highly expressed in neuronal and vascular tissue.^{25, 27, 37} Low levels have been observed in patients with vascular risk factors,⁷ and in the present study with increased risk of stroke/TIA. However, only a few studies have explored in humans, the association between BDNF (studying genetic polymorphism rather than gene expression or circulating levels) and white matter abnormalities on brain MRI; these studies were restricted to patients with various psychiatric conditions.³⁸ In a cross-sectional study of older subjects with depression, a specific BDNF genotype (the Val66Met allele) was associated with greater WMH volume,³⁸ whereas in a prospective study the same polymorphism appeared to modify the association between stroke and depression.³⁹ In a primate model of lacunar stroke, the production of BDNF was stimulated in activated glial cells at the ischemic border zones. The activation of astrocytes in the white-matter ipsilateral to the injury appeared to contribute to tissue repair and regeneration.⁴⁰ It is likely that the state of hypoperfusion and chronic ischemia in oligemic/ischemic tissue that leads to manifestation of WMH on brain MRI also acts as a trigger for upregulation of BDNF expression. Further experimental and clinical studies are warranted to evaluate the role of BDNF in subclinical vascular brain disease.

BDNF and Cognitive Performance

BDNF plays a critical role in mediating synaptic transmission and synaptic plasticity, a molecular basis for processing new learning and consolidation of short and long term memories.⁴¹ A decline in BDNF levels precedes and parallels the observed decrease in choline acetyltransferase enzyme activity in aging brains, preclinical AD and AD.^{41, 42} In prior reports, higher serum BDNF levels were found to be associated with better performance on neuropsychological testing in healthy older adults.⁴³ Recently, in a prospectively followed aging population (57–79 years), decreased levels of BDNF were also associated with poorer performance on global cognitive testing and in several specific cognitive domains such as verbal memory and language, but not in executive function tests.⁴⁴ Although different cognitive test batteries were used in this prior study (MMSE and CERAD score), our current findings are largely in agreement with their observations. Decline in cognitive function has been associated with higher FSRP and with the presence of ext-WMH.^{14,45} In the present study we also observed an association between low BDNF

levels and a) stroke/TIA and b) WMHs, both previously associated with decline in cognitive function, MCI and dementia.³⁶ In conclusion, our data are consistent with the idea that BDNF pathways are involved in processes of brain aging and cognitive function.

Strengths and Limitations—The prospective community-based setting of our study, ability to adjust for concomitant and pre-stroke levels of vascular risk factors, the ability to study a middle-aged to elderly sample free of stroke and dementia, and the fact that all outcome assessment was done blinded to biomarkers levels and to clinical/subclinical outcomes are some strengths of our study. However, the predominantly European origin of our sample limits the generalizability of our findings. In addition, participants included in the study were not representative of the general population; they had less vascular disease burden than those who were excluded or declined MRI or NP testing. Due to the small number of events in each stroke subtype categories, we used a composite outcome of all strokes as a clinical outcome. A single-occasion measurement of brain MRI and NP outcomes prevents us from examining the association with change in structural and functional measures. There was no CSF data available for BDNF and VEGF, although serum and CSF levels have been shown to be moderately correlated in other studies. Finally, whereas these are exciting and novel observations, but our exploratory analyses needs to be replicated in other prospective studies since the models used (such as the threshold effect for BDNF) were based on our own data and that could result in a risk of model overfitting.

SUMMARY

The results of our study suggest that BDNF and VEGF as novel risk markers may improve stratification of patients at risk for stroke/TIA. Second, as a marker of neuronal function, BDNF may serve as an intermediate biomarker for subclinical vascular disease. More importantly, BDNF may have biological potential to serve as a therapeutic target for primary and secondary prevention of clinical and subclinical vascular brain disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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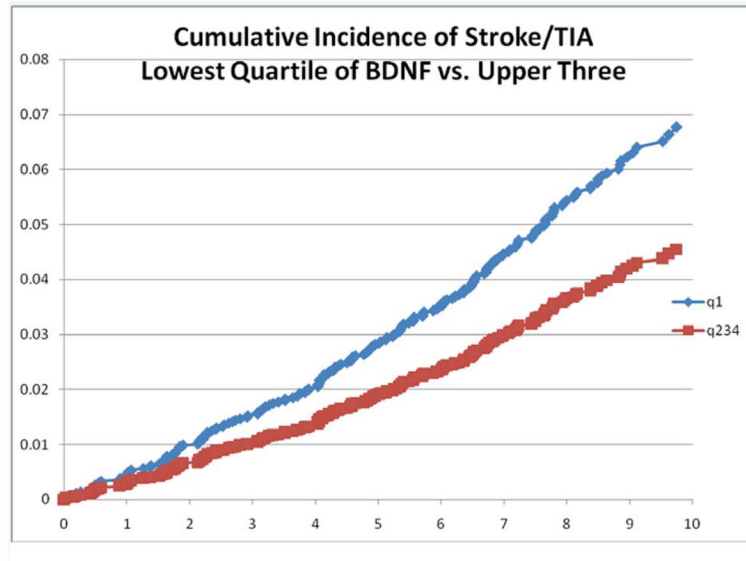


Figure 1. Kaplan-Meier Curves of the Cumulative Probability of Incident Stroke within BDNF quartiles (Q1 vs Q2–4).

Table 1

Baseline Characteristics of the Study Samples

<i>Clinical Characteristics</i>	Incident Stroke/TIA Gen 1 and Gen 2 N=3440	Neuropsychological Test Sample Gen 2 N=2104	MRI Sample Gen 2 N=1863
Women,%	56%	55%	55%
Age, years *	65±11	61±9	61±9
Systolic Blood Pressure, mm Hg *	130±20	126±19	126±19
Anti-hypertensive medication, %	36%	31%	30%
Current smokers, %	11%	11%	11%
Diabetes mellitus, %	12%	11%	12%
Prevalent cardiovascular disease, %	14%	10%	9%
Atrial fibrillation, %	4%	3%	2%
Total cholesterol, mg/dl *	NA	201±37	201±37
Total homocysteine, µmol/L *	NA	7.8 [6.5,9.5]	7.8 [6.5,9.5]
College Degree	NA	39%	39%
Depression (CES-D score)**	NA	3 [1,7]	3 [1,7]
Physical activity index (PAI)	NA	37 [33,41]	37 [33,41]
Growth Factors range			
BDNF, ng/mL	23297 [17959,28841]	23861 [18220,29347]	23853 [18225,29321]
VEGF, ng/mL	290 [165,454]	281 [158,440]	277 [158,432]
Brain MRI Measures			
WMHI (Log) *	N/A	N/A	-3 ± 1
Extensive WMH	N/A	N/A	12%
Cognitive Measures			
VRd	N/A	8±3	N/A
TrB-TrA	N/A	0.67 [0.45–0.98]	N/A

* mean±SD. Values for BDNF and VEGF, tHcy, CES-D, PAI, and TrB-TrA represent median (25th %ile, 75th %ile)

** CES-D=Center for Epidemiological Studies Depression scale

Table 2

Association of serum BDNF and logVEGF and risk of stroke/TIA

Case/N			Stroke/TIA (193/3440)	
			HR [95% CI]	P
BDNF	Per SD	Model A	0.87 [0.75–1.01]	0.07
		Model B	0.87 [0.75–1.01]	0.07
	Q1	Model A	1.00	REF
	Q2		0.78 [0.55–1.12]	0.18
	Q3		0.51 [0.34–0.78]	0.002
	Q4		0.69 [0.47–1.03]	0.07
	Q1vsQ2–Q4		Model A	1.51 [1.11–2.03]
		Model B	1.47 [1.09–2.00]	0.012
logVEGF	Per SD	Model A	1.21 [1.05–1.40]	0.009
		Model B	1.21 [1.04–1.40]	0.012

Model A: adjusted for age and sex

Model B: adjusted for systolic blood pressure, prevalent CVD, diabetes mellitus, atrial fibrillation, current smoking

Table 3

Association of BDNF and VEGF and Brain MRI Measures

		Log(WMHV)		EXT-WMH	
		$\beta \pm SE$	P	OR [95% CI]	P
BDNF	Per SD				
	Model A	-0.04±0.02	0.034	0.84[0.72-0.96]	0.014
	Model B	-0.05±0.02	0.025	0.83[0.71-0.95]	0.009
	Model C ^{††}	-0.05±0.02	0.020	0.83[0.72-0.96]	0.013
	Model D ^{†††}	-0.04±0.02	0.042	0.83[0.72-0.97]	0.015
Q1vsQ2-Q4	Model A	0.09±0.05	0.071	1.28[0.93-1.76]	0.132
	Model B	0.09±0.05	0.067	1.29[0.93-1.78]	0.126
	Model C ^{††}	0.09±0.05	0.060	1.26[0.91-1.74]	0.169
	Model D ^{†††}	0.09±0.05	0.078	1.26[0.90-1.77]	0.171
logVEGF	Per SD				
	Model A	-0.02±0.02	0.352	0.95[0.83-1.10]	0.506
	Model B	-0.02±0.02	0.313	0.95[0.82-1.09]	0.439
	Model C ^{††}	-0.02±0.02	0.313	0.95[0.82-1.09]	0.465
	Model D ^{†††}	-0.02±0.02	0.316	0.93[0.80-1.08]	0.341

* Model A: adjusted for age and sex and time from baseline to MRI
[†] Model B: Model A. additionally adjusted for SBP, CVD, DM, AF and SMK
^{††} Model C: Model B additionally adjusted for total cholesterol and total homocysteine (available only in Offspring; N=1862)
^{†††} Model D: Model B additionally adjusted for education, PAI and CESD (Offspring only; N=1954)

Table 4

Association of BDNF and VEGF and NP Measures

		VRd		Log (TrB-TrA)	
		$\beta \pm SE$	P	$\beta \pm SE$	P
BDNF	Per SD				
	Model A	0.17±0.07	0.010	-0.001±0.004	0.742
	Model B	0.18±0.07	0.005	-0.001±0.004	0.831
	Model C ^{††}	0.18±0.07	0.005	-0.001±0.004	0.810
Q1vsQ2-Q4	Model D ^{†††}	0.14±0.07	0.036	-0.002±0.004	0.631
	Model A	-0.40±0.15	0.010	-0.015±0.010	0.156
	Model B	-0.40±0.15	0.010	-0.014±0.010	0.165
	Model C ^{††}	-0.40±0.15	0.010	-0.014±0.010	0.165
logVEGF	Model D ^{†††}	-0.32±0.16	0.041	-0.016±0.010	0.118
	Model A	-0.02±0.07	0.707	0.002±0.004	0.635
	Model B	-0.01±0.07	0.829	0.003±0.004	0.532
	Model C ^{††}	-0.01±0.07	0.852	0.003±0.004	0.512
	Model D ^{†††}	-0.01±0.07	0.909	0.004±0.004	0.383

* Model A: adjusted for age and sex; time from baseline to NP, and education

[†] Model B: Model A. additionally adjusted for SBP, CVD, DM, AF and SMK

^{††} Model C: Model B additionally adjusted for total cholesterol and total homocysteine (available only in Offspring; N=1862)

^{†††} Model D: Model B additionally adjusted for PAI and CESD (Offspring only; N=1954)