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The Associations between Serum Brain-Derived Neurotrophic Factor, Potential Confounders, and Cognitive Decline: A Longitudinal Study

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Abstract

Brain-derived neurotrophic factor (BDNF) plays a role in the maintenance and function of neurons. Although persons with Alzheimer's disease have lower cortical levels of BDNF, evidence regarding the association between circulating BDNF and cognitive function is conflicting. We sought to determine the correlates of BDNF level and whether BDNF level was prospectively associated with cognitive decline in healthy older adults. We measured serum BDNF near baseline in 912 individuals. Cognitive status was assessed repeatedly with the modified Mini-Mental Status Examination and the Digit Symbol Substitution test over the next 10 years. We evaluated the association between BDNF and cognitive decline with longitudinal models. We also assessed the association between BDNF level and demographics, comorbidities and health behaviors. We found an association between serum BDNF and several characteristics that are also associated with dementia (race and depression), suggesting that future studies should control for these potential confounders. We did not find evidence of a longitudinal association between serum BDNF and subsequent cognitive test trajectories in older adults, although we did identify a potential trend toward a cross-sectional association. Our results suggest that serum BDNF may have limited utility as a biomarker of prospective cognitive decline.

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Introduction

Brain-derived neurotrophic factor (BDNF) is one of a family of neurotrophins which play an important role in the development, maintenance and function of neurons [1–4]. In humans, BDNF levels are lower in AD patients than controls in the hippocampus and cortical regions associated with AD; in the healthy adult mouse, BDNF levels are high in brain regions associated with Alzheimer's disease (AD) [5–9]. BDNF also circulates in the blood, where it appears to be stored in platelets and is released through physical or chemical platelet activation [10]. While BDNF can cross the blood-brain barrier, the extent to which blood derived BDNF levels are a reasonable proxy for BDNF in the central nervous system is unclear [11,12].

The research community hoped that circulating BDNF could provide a less invasive, inexpensive biomarker for dementia; but so

far, there is conflicting evidence in the literature regarding the association between BDNF and cognition decline and dementia. Some studies indicate serum BDNF is higher in controls than persons with mild cognitive impairment (MCI), AD or dementia [13–15], others indicate the opposite direction of association [16], and some studies show no association [17–19]. Confounding may explain some of these discrepancies – many studies examining associations between BDNF and cognitive status used basic tests of group differences with minimal controls for confounding and few have examined the longitudinal association between BDNF and cognitive change. To our knowledge, only two other studies have examined cognitive change and blood based BDNF; one study in AD patients found lower BDNF predicted more rapid cognitive decline but the other study found no association in a group of healthy older adults [20,21]. Additional studies with careful controls for potential confounders are necessary.

In this analysis, our goal was two-fold. First, we aimed to elucidate correlates of BDNF, as they show some inconsistencies in the literature. Substantial evidence supports an association between depression and lower serum BDNF but evidence regarding correlations between BDNF and basic characteristics like sex, age, and BMI is equivocal. Second, we aimed to evaluate the association between BDNF and cognitive decline, especially after consideration of potential confounders using a large prospective cohort of cognitively healthy older adults with cognitive performance evaluated at multiple time points over a lengthy follow-up. The cohort is biracial (black and white) which allows examination of possible differences in BDNF by race, which other studies have lacked sufficient diversity to address.

Methods

Ethics Statement

The Institutional Review Boards at all clinical sites (University of Pittsburgh, University of Tennessee- Memphis) and the University of California – San Francisco coordinating center approved the study protocol and all participants signed informed consent.

Participants

Participants were enrolled in the Health, Aging and Body Composition (Health ABC) study, a prospective cohort of 3075 community-dwelling black and white older adults living in Memphis, Tennessee and Pittsburgh, Pennsylvania. Participants were recruited from a random sample of Medicare-eligible adults living within designated zip codes. Participants were eligible if they reported no difficulties performing activities of daily living, walking a quarter mile, climbing 10 steps without resting, were free from life-threatening cancers, and planned to remain in the study area for at least 3 years. All patients signed an informed consent form that was approved by the institutional review boards of each clinical site and the data coordinating center. Serum BDNF levels were measured in a subset of 1000 participants, selected by stratified random sample, excluding those with possible dementia (3MS <80 at baseline). The BDNF measurements for 77 participants were missing for technical reasons, and 11 participants were missing cognitive testing at included time points leaving an analytical sample of 912.

Measures

Serum BDNF was measured at the first follow-up visit (year 2). Fasting blood samples were obtained by standard venipuncture with serum was stored at -70°C and shipped directly to the analytical laboratory in Minneapolis, MN. Assays were performed by R&D Systems' Analytical Testing Service, employing an enzyme-linked immunosorbent assay method. The detection limit for this assay is 1250 pg/mL. The mean inter-assay coefficient of variation is 9.2% and the mean coefficient of variation within assay is 6.5%. Covariates examined were assessed at year 2 where available. If unavailable, assessments from an adjacent year were used (e.g., platelet count was measured in year 3, drinking/smoking behavior measured at baseline). Participants were considered depressed if they scored greater or equal to 16 on the Center for Epidemiologic Studies Depression Scale (CES-D) at baseline or reported treatment for depression at baseline (medication verified). Potential covariates included demographic characteristics, cardiovascular comorbidities, Apolipoprotein $\epsilon 4$ carrier status, BDNF Val66Met Met(A) carrier status (SNP rs6265), health behaviors, and platelet count, which was previously found to be an important predictor of BDNF [22]. Seasonal

fluctuation in serum BDNF has been reported; we examined December – May vs June – November differences, based on previously published results [23]. Literacy was measured with the Rapid Estimate of Adult Literacy in Medicine (REALM), an instrument for which individuals read common health-related words aloud (e.g., germs, prescription, diagnosis) [24]. Cognition was assessed using repeated measurement of the Modified Mini-Mental State Examination (3MS) (tested at years 3, 5, 8, 10, and 11) and the Digit Symbol Substitution Test (DSST) (tested at years 5, 8, 10, and 11) [25,26]. The 3MS test evaluates global cognitive function and ranges from 0 to 100; the DSST mainly evaluates executive function and has a range of 0–90.

Statistical Analysis

After examining the distribution of the BDNF measurements and finding a limited number of extreme values, we trimmed the data to exclude the top and bottom 1% to reduce the possibility of influential observations outside of the typical range unduly impacting the model results. This excluded ten values at or above 47.3 ng/mL (range: 47.3–63.1) and ten values at or below 5.5 ng/mL (range: 2.3–5.5), resulting in a final data range of 5.6 ng/mL – 47.25 ng/mL. The association between BDNF and participant demographic and comorbid characteristics was characterized using BDNF tertiles and tested with Chi-square tests and ANOVA, where appropriate. We constructed a multivariate linear regression model using covariates with $p < 0.20$ to examine the association between predictors and BDNF after simultaneous adjustment.

We assessed the relationship between BDNF and cognitive test scores using linear mixed-effects regression models with random slopes and intercepts. BDNF and platelet count were standardized so that coefficients could be interpreted as change in cognition associated with a one standard deviation increase in BDNF or platelet count. We examined the relationship in both unadjusted and adjusted models. The adjusted model contained covariates which differed significantly between BDNF tertiles ($p \leq 0.05$), covariates which were significant predictors of BDNF in the adjusted model ($p \leq 0.05$), and other covariates known to be important in modeling cognitive scores (study site, age, APOE $\epsilon 4$, race, literacy, and education).

Results

Correlates of BDNF

BDNF measurements were generally normally distributed with a small number of extreme values in the right tail. As such, the data was trimmed to exclude the top and bottom 1% of BDNF observations. BDNF tertiles were significantly associated with platelet count, sex, race, Met carrier (Val66Met genotype), history of MI, and smoking (Table 1). Females, blacks, smokers, and those with high platelet counts had higher BDNF. History of myocardial infarction (MI) and carrying the rarer Met allele was associated with lower BDNF. Although depression did not quite meet the $p < 0.20$ cut-off from Table 1 ($p = 0.22$), we included it in the multivariate model predicting BDNF because of its strong association with BDNF in the literature. We found that race, depression, and platelet count were significant predictors of BDNF after simultaneous adjustment in a multivariate model. BDNF levels in blacks were 1.81 ng/mL higher than whites (95% CI: 1.19, 2.43) and were 2.84 ng/mL lower in participants with depression (95% CI: -5.17 , -0.51). A one standard deviation increase in platelet count was associated with a 1.81 ng/mL increase in BDNF levels (95% CI: 1.19, 2.43). The BDNF estimate for females was 1.04 ng/mL higher, although it was no longer

significant (95% CI: -0.17, 2.24). Smoking, history of MI, and Met allele carrier status were no longer significant after adjustment for other factors.

Cognitive Decline

The median number of 3MS observations per person was 4 (range: 1–5); the median number of DSST observations per person was 3 (range: 1–4). In both unadjusted and adjusted analyses, we found no significant association between BDNF and performance on either cognitive test, cross-sectionally or longitudinally (Table 2). There was a slight trend toward higher cross-sectional performance on DSST associated with higher BDNF, but it was not significant ($p = 0.09$). Performing the same analysis using the log of BDNF, rather than trimmed BDNF values, resulted in the same conclusions. There were no significant interactions between race and BDNF for either cognitive test. A recent study on the association between dementia treatment and serum BDNF found that treatment increased BDNF levels in persons with AD [27]. Four participants in this sample were prescribed dementia medications throughout the follow-up period; results excluding

these individuals did not differ appreciably from the primary results.

Discussion

Serum BDNF showed strong associations with race, platelet count, and depression after adjusting for other factors, and weak associations with a number of other participant characteristics. We found that blacks had higher levels of BDNF than whites, a unique finding which has not been addressed in the literature so far. It is unclear whether the race differential in BDNF is due to unmeasured factors differing between blacks and whites or true biological differences. The strong association between BDNF and platelet count is consistent with theory that BDNF in serum is derived directly from platelets. Age did not differ significantly among serum BDNF tertiles which is in concordance with results from some studies [13–16,28–30] and in conflict with others [19,31,32], although two of the studies which found an age effect included a wider age range than our study. Initially, we found that sex differed significantly between BDNF tertiles but sex was not a

Table 1. Distribution of participant characteristics by BDNF tertile.

	BDNF Tertiles			p-value
	Low (N = 296)	Middle (N = 297)	High (N = 299)	
BDNF (ng/mL)				
Mean (SD)	15.1 (3.6)	23.1 (1.9)	32.5 (5.0)	
Range	5.56 – 19.95	19.97 – 26.49	26.51 – 47.25	
Continuous	Mean (SD)	Mean (SD)	Mean (SD)	p-value
3MS (year 3)	90.2 (7.0)	89.4 (7.4)	89.4 (7.7)	0.33
DSST (year 5)	32.0 (14.7)	31.9 (14.3)	32.2 (13.8)	0.97
Age (years)	74.9 (2.9)	74.9 (2.9)	75.2 (2.9)	0.46
Platelet count (thousands/uL)	205.9 (55.9)	221.2 (54.1)	235.7 (63.8)	<0.001
Categorical	% (N)	% (N)	% (N)	p-value
Female	47.0 (139)	54.9 (163)	63.9 (191)	<0.001
Black	44.6 (132)	54.9 (163)	61.5 (184)	<0.001
Education (<HS)	31.8 (94)	35.5 (105)	38.7 (115)	0.21
Literacy (<9 th grade)	28.7 (85)	29.6 (88)	35.1 (105)	0.19
APOE ε4	30.5 (85)	30.7 (85)	30.3 (87)	1.00
Val66Met Met(A) carrier	25.2 (69)	17.8 (48)	18.9 (53)	0.07
History of MI	25.3 (75)	17.2 (51)	18.4 (55)	0.03
Hypertension	62.5 (185)	59.3 (176)	60.9 (182)	0.72
CVD	11.5 (34)	10.4 (31)	11.4 (34)	0.91
High Cholesterol	48.9 (136)	55.1 (158)	57.2 (162)	0.12
Diabetes	40.5 (120)	38.7 (115)	37.1 (111)	0.69
Depression	8.1 (24)	6.1 (18)	4.7 (14)	0.22
Obese	23.0 (68)	28.6 (85)	29.1 (87)	0.17
Current smoker	6.4 (19)	11.8 (35)	11.4 (34)	0.05
Drinking (>1 drink/day)	6.4 (19)	6.1 (18)	7.7 (23)	0.71
Walking (>150 mins/week)	30.5 (90)	28.3 (84)	24.4 (73)	0.24
Season (Winter/Spring vs Summer/Fall)	35.1 (172)	32.5 (297)	32.5 (299)	0.40

P-values come from ANOVA and Chi-square tests, as appropriate. BDNF = brain derived neurotrophic factor, 3MS = Teng Modified Mini-Mental Status Exam, DSST = Digit Symbol Substitution Test, CVD = cerebrovascular disease, MI = myocardial infarction.

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Table 2. Estimates of the association between BDNF and cross-sectional and longitudinal cognitive test scores (change in test score per 1 SD increase in BDNF).

	N	Cross-sectional		Longitudinal	
		Per 1 SD increase in BDNF	p-value	Per 1 SD increase in BDNF	p-value
3MS					
Unadjusted	892	-0.44 (-0.97, 0.09)	0.11	-0.02 (-0.10, 0.07)	0.71
Adjusted	792	0.10 (-0.41, 0.61)	0.70	-0.02 (-0.11, 0.07)	0.73
DSST					
Unadjusted	892	0.09 (-1.20, 1.39)	0.89	-0.07 (-0.20, 0.06)	0.31
Adjusted	792	1.08 (-0.18, 2.33)	0.09	-0.08 (-0.22, 0.06)	0.27

Unadjusted model contained: BDNF (standardized), time, BDNF*time.

Adjusted model contained: BDNF (standardized), race, sex, history of myocardial infarction, smoking, platelet count (standardized), APOE ε4, age at enrollment, education < high school, literacy < 9th grade, depression, time, and all covariate interactions with time.

Regression model estimates and 95% confidence intervals from linear mixed effects models are provided.

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significant predictor of BDNF in a model adjusted for other covariates.

We did not identify any longitudinal associations between BDNF and cognition; this is in agreement with the other longitudinal study of blood based BDNF in cognitively normal older adults [21]. However, a longitudinal study using CSF-derived BDNF found that lower baseline CSF BDNF levels were significantly associated with greater annual decline on several tests of memory and category fluency in cognitively normal older adults [33]. This contrast may suggest that blood based measures of BDNF are not representative of CSF BDNF levels. A study comparing CSF and serum BDNF measurements in a sample of AD patients found that they were not correlated ($r^2 = 0.03$, $n = 27$); however, a study comparing blood and BDNF levels in pigs and rats found significant positive correlation between the two in both pigs and rats ($r^2 = 0.41$, 0.44 , respectively) [13,34]. Further study will be required to better understand the relationship between cortical, cerebrospinal fluid, and circulating levels of BDNF in humans. The trend toward a cross-sectional association and the lack of longitudinal association, combined with previous published work, may suggest that changes in BDNF occur contemporaneously with cognitive decline, as opposed to preceding decline. This would limit the utility of BDNF as a biomarker for early detection but does not preclude the potential for BDNF to play a useful diagnostic role.

Comparison of our raw and adjusted analyses demonstrates that confounders may have a profound effect on the estimate of the relationship between BDNF and cognition, particularly cross-sectionally. Many previous studies chose to use nonparametric group tests due to concerns that the BDNF distribution showed departures from normality. While extreme values and departures from normality are a concern, our results suggest that confounding may be a much greater concern, especially in the presence of an adequate sample size.

Strengths of this analysis include the prospective nature of the study, large biracial sample, and ability to control for multiple potential confounders. This analysis was limited by data collection occurring at different time points for some variables, such as platelet count. The negative impact of this is likely minimal as no

covariate assessment occurred more than a year from BDNF blood draw and comparisons of platelet counts measured in years 3 and 11 suggest high intra-person correlation of platelet count over time (Spearman correlation coefficient 0.73). It is possible that our relatively healthy sample of older adults without dementia at baseline lacked sufficient variability in BDNF to detect associations reported elsewhere, although the serum BDNF range exhibited in our data is consistent with other similar studies. There is evidence that treatment for depression raises BDNF levels [35]. Our depression definition was partially based on treatment for depression, which makes it difficult to untangle the separate associations with depression and antidepressants. It is possible that our estimate of the association between BDNF and depression was attenuated due to the mixture of treated and untreated individuals. We were limited to evaluating two cognitive tests with repeated assessment in this sample (DSST and 3MS), which may not provide a complete picture of cognitive status and change.

After adjustment for confounding, our analysis provides no evidence that serum BDNF is prospectively associated with decline on DSST or 3MS. We found that serum BDNF was associated with a number of characteristics which also associated with dementia, which suggests that future studies should control for these potential confounders.

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Author Contributions

Conceived and designed the experiments: TH ES SS CR KY JN. Analyzed the data: JN KY. Wrote the paper: JN KY ES TH SS CR.

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