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Brain-Derived Neurotrophic Factor Levels in Alzheimer's Disease

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Abstract

Objective: The current search for biomarkers that are diagnostic and/or prognostic of Alzheimer's disease (AD) is of vital importance given the rapidly aging population. It was recently reported that brain derived neurotrophic factor (BDNF) fluctuated according to AD severity, suggesting that BDNF might have utility for diagnostics and monitoring of therapeutic efficacy. The current study sought to examine whether BDNF levels varied according to AD severity, as previously reported.

Method: There were 196 participants (Probable AD n = 98, Controls n = 98) in the Texas Alzheimer's Research Consortium (TARC) Longitudinal Research Cohort available for analysis. BDNF levels were assayed via multiplex immunoassay. Regression analyses were utilized to examine the relation between BDNF levels, MMSE, and CDR scores adjusting for age and gender.

Results: In adjusted models, BDNF levels did not distinguish between AD patients and normal controls and did not significantly predict AD severity or global cognitive functioning.

Conclusions: These findings do not support the notion that BDNF serves as a diagnostic marker for AD or disease severity. It is likely that the most accurate approach to identifying biomarkers of AD will be through an algorithmic approach that combines multiple markers reflective of various pathways.

Keywords

Alzheimer's disease; Biomarkers; BDNF; Dementia Severity; Clinical Dementia Rating

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Introduction

The recent explosion of research in the field of biomarker analysis in Alzheimer's disease (AD) is an attempt to identify early diagnostic and prognostic markers for this disease as well as possible entry points for novel therapeutics to treat and/or prevent the illness. The 1998 Consensus Report of the Working Group on Molecular and Biochemical Markers of Alzheimer's Disease stated that ideal biomarkers should be noninvasive, simple to perform, inexpensive, reliable, able to detect the neuropathological changes of AD, and validated through confirmed cases[1]. The ideal biomarkers to fit this definition would be those gleaned from blood[2].

One family of biomarkers that has received a great deal of attention in AD is the neurotrophic factors (NFTs)[3]. NFTs are small proteins that play key roles in neuronal survival, axonal guidance, cell morphology, as well as memory formation and cognition[4]. Many neurotrophic factors are synthesized in areas impacted by AD neuropathology early in the course of the disease (e.g. entorhinal cortex, hippocampal formation, amygdala). Additionally, axonal transport is essential for NFT signaling, as they are oftentimes synthesized away from their site of action; however, AD and other neurodegenerative dementias are frequently associated with axonal transport failures[4]. Therefore, dysregulation of NFT is expected in neurodegenerative dementias such as AD.

Brain-derived neurotrophic factor (BDNF) is one NFT that has been linked with AD. Postmortem studies have documented decreased BDNF, pro-BDNF, and BDNF mRNA levels in brains of patients diagnosed with AD and Mild Cognitive Impairment (MCI)[5-8]. It has also been suggested that serum BDNF levels are altered in AD. In a sample of patients diagnosed with severe AD (n=60; mean MMSE = 6.9) and vascular dementia (VaD)(n=60; mean MMSE = 6.8), serum BDNF levels were decreased in AD compared to controls (n=33) and VaD[9]. Laske et al[10] analyzed a sample of 30 AD patients and 10 non-demented controls and found that serum BDNF levels were increased during "early" AD (i.e. MMSE Score ≥ 21) when compared to "late" AD (MMSE < 21) and suggested that BDNF be further evaluated as a possible diagnostic marker of early AD. The current study sought to examine the hypothesis that serum BDNF levels vary according to stage of AD severity. Based on previous findings, it was hypothesized that serum BDNF levels would be increased in mild AD (i.e. CDR scores of 0.5-1.0) when compared to controls (CDR = 0) and moderate AD (CDR = 2).

Materials and Methods

Participants

Participants included 198 individuals (99 diagnosed with Probable AD and 99 controls) enrolled into the Texas Alzheimer's Research Consortium (TARC). The methodology of the TARC project has been described in detail elsewhere[11]. All patients met consensus-based diagnosis for probable AD based on NINCDS-ADRDA criteria[12] and controls performed within normal limits on psychometric assessment and were assigned a CDR global score of 0.0. Autopsy-confirmation of clinical diagnosis was not available on any participants. The breakdown of numbers of CDR global scores was as follows: 0 = 99 (controls), 0.5 = 21 (AD), 1 = 44 (AD), 2 = 29 (AD)(5 AD cases were missing CDR scores). The TARC project received Institutional Review Board approval and all participants and/or caregivers signed written informed consent documents.

Measures

In addition to other clinical and neuropsychological measures, each participant was administered the Mini-Mental State Examination (MMSE)[13] and rated on the Clinical Dementia Rating scale (CDR)[14] as part of their clinic evaluation.

Assays

Non-fasting samples were collected in serum-separating tubes during clinical evaluations, allowed to clot at room temperature for one hour, centrifuged, aliquoted, and stored at -80°C in plastic vials. Samples were sent frozen in a single batch to Rules Based Medicine (www.rulesbasedmedicine.com, Austin, TX) where they were thawed for assay without additional freeze-thaw cycles. Rules Based Medicine conducted multiplexed immunoassay via their human Multi-Analyte Profile (human MAP). Multiple proteins, including BDNF, were quantified through multiplex fluorescent immunoassay utilizing colored microspheres with protein-specific antibodies. For BDNF, the least detectable dose (LDD) was 0.029ng/mL, inter-run coefficient of variation was $\leq 7\%$, dynamic range was 0.0028 – 14ng/mL, overall spiked standard recovery was 95%, and cross-reactivity with other human MAP analytes was $< 5\%$. Assays conducted by this company utilizing this platform have been published elsewhere [15-18].

Analyses

Statistical analyses were conducted using SAS version 9.3 (SAS Institute, Inc., Cary, NC). Analyses comparing demographic variables and BDNF levels between clinical groups were carried out using Analysis of Variance (ANOVA) or t-test for continuous variables and chi-squared test for discrete variables. Analyses examining the relation between BDNF levels MMSE and CDR scores were carried out using linear regression adjusted for age and gender. Ordinal logistic regression was applied to examine the relationship between CDR global scores and BDNF values, adjusted by age and sex. Final models include only significant covariates, except BDNF which is always included.

Results

Demographic characteristics of the study population are shown in Table 1. Alzheimer subjects were significantly older ($p<0.001$) and less educated ($p<0.01$) than the control group. There were no significant differences between groups in terms of gender. The sample was primarily (94%) Caucasian. AD patients performed more poorly on the MMSE ($p<0.01$) and obtained higher CDR scores ($p<0.01$) than controls. Mean BDNF levels broken down by CDR global scores are presented in Table 2.

BDNF levels did not differ significantly according to dementia severity (via CDR global scores) (see Table 2). In adjusted and unadjusted models, BDNF scores did not significantly predict CDR sum of boxes or global scores, nor did they predict MMSE scores (see Table 3).

Discussion

The identification of biomarkers that have diagnostic, prognostic, and/or therapeutic implications in AD is of critical importance. While previous research suggested that BDNF levels might hold promise as a diagnostic marker of early AD, the current findings do not support this hypothesis.

There are noteworthy differences between the current study and previous work. In the Yasutake et al[9] study, the mean MMSE scores were 6.88 (sd = 6.78) and 6.75 (sd = 6.89) for the AD and VaD samples, respectively, whereas the mean MMSE score in the current sample was 20.5

(sd = 4.8) with the lowest score (10) being above the mean score of the Yasutake sample. Therefore, the Yasutake group consisted of late stage dementia patients, whereas current sample was comprised of early to moderate cases without any severe dementia cases available for analysis (i.e. CDR global score = 3). Additionally, the Laske et al[10] study utilized MMSE scores to split the cases into “early” and “late” AD rather than by using the CDR, which is the most commonly utilized instrument for staging dementia severity. The assay procedures utilized in the current study (multiplex immunoassay) were also different from the studies mentioned above (enzyme-linked immuno sorbent assay, ELISA), which may have led to differential results. However, it is unlikely that any difference between methodologies would lead to a stage-dependent impact to obscure a difference only in early stage AD. Additionally, a recent study by Leyhe et al[19] demonstrated that serum BDNF levels increased in patient's with Alzheimer's disease following 15-months treatment with donepezil, which may have impacted the current findings. However, the previous work does not offer any reason to hypothesize that the impact of drug utilization would be stage-dependent therefore it is unlikely that drug status altered BDNF levels only in those patients in the earliest stages of the disease (i.e. CDR = 0.5).

Taken together, the current study provides a more refined description of serum BDNF levels in early to moderate AD. There was a trend towards a possible decrease in BDNF levels in very early AD (CDR = 0.5); however, this difference did not reach statistical significance and completely disappeared once covariates were controlled for in the model. A recent study by Yu and colleagues[20] found significantly lower serum BDNF concentrations in patients with amnesic mild cognitive impairment (aMCI), a condition often thought to be a transition stage between normal cognition and Alzheimer's disease, further supporting the possibility of alterations in this marker early on during disease development. From the Yasutake study, it appears there is decline in BDNF levels once the disease has progressed to a severe level. The possibility of a decrease in BDNF in very early AD followed by normalization (compared to controls) during mild-to-moderate AD and subsequent decline in BDNF during late stage AD should be evaluated in a larger population with sufficient sample sizes across CDR stages of dementia severity. It is also possible that subgroups of early AD (and MCI) patients experience alterations in BDNF, which might explain the contradictory findings and future studies should look for such an endophenotype. It is likely that the most accurate approach to identifying biomarkers for the identification of AD (blood-based or otherwise) will be through an integrated approach that combines multiple markers reflective of various neurobiological pathways[1,21,22].

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Table 1

Baseline Demographic Characteristics

Number of subjects		AD 99	Control 99	p-value -----
Sex	Female	56 (56.6%)	60 (60.6%)	.056
	Male	43 (43.4%)	39 (39.4%)	
Age (years)	Mean (SD)	77.8 (8.17)	72.01 (8.56)	<0.01
	Range	54-100	55-93	
Race/Ethnicity (n,%)	Caucasian	94 (94.9%)	93 (93.9%)	0.76
	Non-Caucasian	5 (5.1%)	6 (6.1%)	
Education (years)	Mean (SD)	13.9 (2.7)	15.0 (2.7)	<0.01
	Range	6 - 19	8 - 20	
MMSE	Mean (SD)	20.5 (4.8)	29.3 (1.0)	<0.01
	Range	10 - 29	25 - 30	
CDR Sum of Boxes	Mean (SD)	6.9 (3.5)	0.1 (0.2)	<0.01
	Range	1.5 - 14.0	0.0 - 1.0	
BDNF	Mean (SD)	23.5 (7.4)	23.8 (6.3)	0.82
	Range	0.8 - 42.9	0.1 - 36.7	

Table 2

Mean BDNF level by CDR Global Score

CDR Global Score	Number of Subjects [*]	Mean (SD)
0	99	23.8 (6.3)
0.5	21	22.7 (5.4)
1	44	23.7 (7.8)
2	29	23.8 (8.6)

^{*} CDR scores missing on 5 AD subjects

Table 3

Relationship between BDNF and test score outcomes overall and stratified by case status.

Outcome		Population	Coefficient (SE)	P-Value
CDR SUM	Overall	Unadjusted	−0.003 (0.044)	0.95
		Adjusted ¹	0.053 (0.041)	0.20
	Cases	Unadjusted	0.009 (0.048)	0.85
		Adjusted ²	0.016 (0.048)	0.74
	Controls	Unadjusted ³	0.005 (0.003)	0.05
	Overall	Unadjusted	0.002 (0.020)	0.91
CDR Global ⁴		Adjusted ¹	−0.019 (0.021)	0.36
	Cases	Unadjusted	−0.011 (0.026)	0.66
		Adjusted ¹	−0.029 (0.027)	0.28
	Overall	Unadjusted	0.017 (0.058)	0.77
MMSE		Adjusted ¹	−0.049 (0.055)	0.38
	Cases	Unadjusted	0.018 (0.065)	0.78
		Adjusted ²	0.025 (0.069)	0.72
	Controls	Unadjusted ³	−0.010 (0.017)	0.57

Covariates include in final adjusted models =

¹ age only² age and sex³ no covariates reached statistic significance⁴ no models included for controls because CDR Global Score = 0 for all subjects.