



ORIGINAL ARTICLE

Serum BDNF levels in unaffected first-degree relatives of patients with bipolar disorder

Fabiano G. Nery,^{1,2} Alexandre D. Gigante,¹ Jose A. Amaral,¹ Francy B. Fernandes,¹ Mariangeles Berutti,¹ Karla M. Almeida,¹ Laura Stertz,³ Giovana Bristot,^{3,4} Flavio Kapczinski,^{3,5} Beny Lafer¹

¹Programa de Transtorno Bipolar, Departamento de Psiquiatria, Faculdade de Medicina, Universidade de São Paulo (USP), São Paulo, SP, Brazil. ²Department of Psychiatry and Behavioral Neuroscience, University of Cincinnati College of Medicine, Cincinnati, OH, USA.

³Laboratório de Psiquiatria Molecular, Centro de Pesquisas Experimentais, Hospital de Clínicas de Porto Alegre (HCPA), Instituto Nacional de Ciência e Tecnologia – Medicina Translacional (INCT-TM), Porto Alegre, RS, Brazil. ⁴Programa de Pós-Graduação em Ciências Biológicas: Bioquímica, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil. ⁵Programa de Pós-Graduação em Medicina, Psiquiatria, UFRGS, Porto Alegre, RS, Brazil.

Objective: Unaffected relatives of bipolar disorder (BD) patients have been investigated for the identification of endophenotypes in an attempt to further elucidate the pathophysiology of the disease. Brain-derived neurotrophic factor (BDNF) is considered to be implicated in the pathophysiology of BD, but its role as an endophenotype has been poorly studied. We investigated abnormal serum BDNF levels in BD patients, in their unaffected relatives, and in healthy controls.

Methods: BDNF levels were obtained from 25 DSM-IV bipolar I disorder patients, 23 unaffected relatives, and 27 healthy controls. All BD patients were in remission. The unaffected subjects were first-degree relatives of the proband who had no lifetime DSM-IV diagnosis of axis I disorder. BDNF serum levels were determined by sandwich ELISA using monoclonal BDNF-specific antibodies.

Results: There were no statistical differences in BDNF levels among BD patients, relatives, and healthy controls.

Conclusion: Serum BDNF levels may not indicate high genetic risk for BD, possibly acting as state markers rather than trait markers of the disease.

Keywords: Bipolar disorder; endophenotypes; cerebral cortex; hippocampus; brain-derived neurotrophic factor

Introduction

Bipolar disorder (BD) is a highly heritable condition.¹⁻³ Although numerous investigations have tried to identify specific genes associated with vulnerability to this disease, this task has been hampered, among other factors, by the phenotypic heterogeneity of BD.⁴ A possible solution to this problem would be the identification of endophenotypes, which are genetically mediated biomarkers involved in the pathophysiology of BD and also detected in first-degree relatives.^{5,6}

It has been hypothesized that decreased neurotrophin expression, including brain-derived neurotrophic factor (BDNF), plays a role in the pathophysiology of BD and contributes to neuroplastic changes observed in the brains of patients with mood disorders.⁷⁻⁹ BDNF is a protein produced by neurons and platelets involved in several brain functions, including neuronal survival, synaptic plasticity, axonal and dendritic growth, and brain connectivity.¹⁰ BD patients have

low serum BDNF levels compared with healthy controls (HC)¹¹⁻¹³ and these levels correlate negatively with the severity of depressive and manic symptoms.^{11,12} Additionally, BDNF seems to be regulated by epigenetic mechanisms in BD¹⁴ and there is evidence in favor of BDNF as a potential clinical biomarker for this disorder.¹⁵ However, few studies have investigated whether serum BDNF levels could indicate high genetic risk for BD.¹⁶

Thus, the objective of this study was to investigate the differences in serum BDNF levels among BD patients, their unaffected relatives, and HC. The investigation of this biochemical alteration in individuals at high genetic risk for BD may potentially identify an endophenotype of this highly disabling disease.

Methods

Patients

Patients were recruited from the outpatient clinic of the Institute of Psychiatry of the School of Medicine, Universidade de São Paulo, Brazil, or were selected from the community through advertising in the media. The sample consisted of 25 BD patients, 23 unaffected relatives, and

Correspondence: Alexandre Duarte Gigante, Departamento de Psiquiatria, Faculdade de Medicina, Universidade de São Paulo, Rua Dr. Ovidio Pires de Campos, 785, CEP 05403-010, São Paulo, SP, Brazil.

E-mail: adgigante@gmail.com

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27 HC. All of the patients signed a written informed consent for their participation in the study.

Patients were diagnosed with BD type I, according to DSM-IV criteria, were older than 18 years, and were in remission. They had to have at least one first-degree relative older than 18 years willing to participate in the study. Remission was defined as failure to meet the criteria for any mood episode in the last 2 months and Hamilton Depression Rating Scale (HDRS) and Young Mania Rating Scale (YMRS) scores below 8 on the day of participation.¹⁷ Patients were allowed to continue taking their current medication.

The unaffected relatives group consisted of proband's first-degree relatives older than 18 years and without a lifetime axis I disorder. Inclusion criteria for HC were age older than 18 years, no lifetime DSM-IV axis I disorder, and no lifetime DSM-IV axis I disorder among first-degree relatives. Subjects were excluded if they were pregnant, if they had a severe and/or decompensated medical condition with central nervous system (CNS) involvement, such as diabetes mellitus, hypertension, or hypothyroidism, and if they had neurological disorders, such as epilepsy or stroke. The presence of active alcohol/drug use disorders in the last 12 months was an additional exclusion criteria for BD patients. All the procedures were carried out according to the Declaration of Helsinki and the study protocol was approved by the Ethics Committee of the School of Medicine at Universidade de São Paulo.

Psychiatric assessments

The Structured Clinical Interview for DSM-IV diagnosis (SCID), versions for both patients and non-patients, was used to confirm the diagnosis of BD type I in patients and to rule out the diagnosis of psychiatric disorders in unaffected relatives and HC.¹⁸ The 17-item HDRS¹⁹ and the YMRS²⁰ were used to evaluate the presence of depressive and manic symptoms, respectively. Board-certified psychiatrists (FGN, JAA) with extensive research experience in mood disorders applied the SCID, HDRS, and YMRS to all subjects.

Sampling and BDNF analysis

Blood for measurement of BDNF levels was collected at the same time (between 10 a.m. and noon). Serum samples were separated and frozen at 80 °C until analysis. Serum BDNF levels were determined by sandwich-ELISA using BDNF-specific monoclonal antibodies, as described previously²¹ (R&D Systems, Minneapolis, MN, United States). Briefly, microtiter (96-well flat-bottom) plates were coated overnight at room temperature with monoclonal anti-BDNF antibody (4 µg/mL in phosphate buffer solution (PBS)) (Laborclin, Paraná, Brazil). Thereafter, plates were washed three times (PBS, pH 7.4, with 0.05% Tween 20 – Nuclear, São Paulo, Brazil) and blocked for 1 h at room temperature with PBS containing 5% nonfat milk powder. After being washed again, plates were coated for 2 h at room temperature with the samples diluted 1:200 (PBS with 1% bovine serum albumin – Sigma-Aldrich, St. Louis, MO, United States), and the standard curve ranged from 7.8 to

500 pg/mL of BDNF. Plates were washed again, 0.2 µg/mL of a biotinylated anti-BDNF antibody in PBS was added, and they were incubated again for 2 h at room temperature. After washing, incubation was carried out with streptavidin-peroxidase conjugate (diluted 1:200 in sample diluents) for 20 min at room temperature. Plates were then washed and incubated with the substrate for 20 min at room temperature. Finally, a stop solution (H₂SO₄, 1 M – Nuclear, São Paulo, Brazil) was added and the BDNF levels were determined by absorbance at 450 nm with correction at 540 nm. The standard curve indicated a direct relationship between optical density and BDNF concentration.

Statistical analysis

Chi-square tests were used for cross-tabulated qualitative data to compare clinical and demographic variables. An analysis of variance (ANOVA) was performed for ordinal and interval scale data to compare BD patients, unaffected relatives, and HC. With respect to serum BDNF levels, the effect of status (BD patients vs. relatives vs. HC) was assessed using ANOVA and the analysis was repeated with age and gender as covariates, as these variables have potential effects on BDNF levels.^{22,23} Statistical analyses were made using SPSS version 14.0. A $p < 0.05$ was considered statistically significant.

Results

Demographic and clinical information about BD patients, unaffected relatives, and HC is displayed in Table 1. Patients were fully euthymic, with an HDRS mean score of 2.9 ± 2.6 and a YMRS score of 1.2 ± 2.1 . Three relatives and one HC did not provide blood for BDNF analysis. Among the available 71 subjects, mean serum BDNF levels were 30.1 ± 15.9 ng/mL for patients, 28.6 ± 9.0 ng/mL for unaffected relatives, and 25.0 ± 5.2 mg/dL for HC (Figure 1). There were no statistically significant differences in serum BDNF levels ($F_{2,68} = 1.45$, $p = 0.24$) among BD patients, unaffected relatives, and HC. These results did not change after covarying for age and gender ($F_{1,65} = 1.55$, $p = 0.22$) or after excluding one outlier in the patient group (mean BDNF levels in the BD group without outlier: 24.9 ± 5.2 ng/mL, ANOVA: $F_{2,67} = 1.66$, $p = 0.2$).

Discussion

We found that the differences in serum BDNF levels were not statistically significant among BD patients, unaffected relatives, and HC. Confounding variables, such as age and gender, did not have any significant effects on BDNF levels.

Our results are consistent with a previous study of peripheral BDNF levels in twin pairs, one of which was affected by mood disorder (187 with major depressive disorder, 19 with BD) and the other of which was unaffected.¹⁶ This study found no differences in whole blood BDNF levels between patients and their unaffected co-twins. However, no separate analysis for the subgroup of BD twin pairs was provided. To our knowledge, this is the first study to

Table 1 Demographic and clinical characteristics of BD patients, unaffected relatives, and HC

	BD patients (n=25)	Unaffected relatives (n=23)	HC (n=27)	p-value
Age (years)	35.7±8.9	31.6±6.7	31.2±9.5	0.07
Gender (male)	8 (32)	9 (39.1)	11 (40.7)	0.79
Right-handed	21 (84)	22 (95.7)	26 (96.3)	0.12
Family history of BD	7 (28)	23 (100)	-	-
Relative participating in the study	19 (76)	18 (78)	-	-
Time in remission (months)	6.6±4.0	-	-	-
HDRS	2.9±2.6	-	-	-
YMRS	1.2±2.1	-	-	-
Age at disease onset (years)	22.1±8.5	-	-	-
Length of illness (years)	13.6±8.1	-	-	-
Number of mood episodes*	6.0±4.0	-	-	-
Current medication				
Lithium	12 (48)	-	-	-
Anticonvulsants	10 (40)	-	-	-
Atypical antipsychotics	13 (52)	-	-	-
Antidepressants	5 (20)	-	-	-
Sedative-hypnotics	2 (8)	-	-	-

Data presented as mean ± standard deviation or n (%).

BD = bipolar disorder; HC = healthy controls; HDRS = Hamilton Depression Rating Scale 17 items; YMRS = Young Mania Rating Scale

* Information about number of episodes was reliably available for 14 patients. For the remaining 11 patients, number of episodes was unreliable or too many to count.

specifically report peripheral BDNF levels in subjects with genetic predisposition to BD.

Our findings are also in line with most,^{11,24-26} but not all,¹³ studies on serum BDNF levels in euthymic BD patients. A recent meta-analysis reported decreased BDNF levels during mood episodes in BD patients but normal levels during euthymia.²⁷ Taken together, our results add further evidence to the literature, corroborating that serum BDNF levels are state markers rather than trait markers of the disease.

There were some limitations in our study. All patients were medicated, and the potential confounding effects of psychotropic medications on BDNF levels²⁸ should not be ruled out, as they might explain the inconsistency between

our findings and those from some previous studies. However, the comparisons between relatives and HC were not confounded by the effects of medication. Also, peripheral BDNF levels may not reflect CNS levels of BDNF in the same subjects. BDNF expression in the CNS of BD patients or unaffected relatives may be abnormal, putting them at risk for disease, while their peripheral BDNF levels may either be normal or only be affected during the clinical expression of a mood episode. Nevertheless, BDNF crosses the blood brain barrier, and peripheral BDNF levels have been highly correlated with CNS levels of BDNF in animal studies.²⁹⁻³¹ This is a poorly understood area, and future studies should investigate cross-sectional or prospective associations between peripheral BDNF levels and CNS biomarkers. Finally, the sample sizes in our comparison groups were relatively small. The heritability of BD likely involves a multifactorial transmission pattern, and hundreds or thousands of genes of small effect may act together, eventually predisposing individuals to BD.^{4,32} Thus, when we recruited first-degree relatives of BD patients, we may have included individuals who share only some of the putative BD genes, and therefore, the effect of these few genes on BDNF levels may be so small that it is hardly detected by biochemical studies with a small sample size. Accordingly, studies with large samples are more likely to detect subtle but relevant abnormalities.

Some strengths of our study should be highlighted. Our sample was clinically well-characterized and reasonably well-matched and we used stringent criteria for euthymia, thereby minimizing the influence of mood disorder symptoms on our findings. Moreover, the unaffected relatives of BD patients were carefully examined for the presence of previous psychopathology to rule out the possible confounding effects of another axis I disorder. Besides, to the best of our knowledge, this is the first report of serum BDNF in individuals at high genetic risk for BD type I. These findings may help elucidate the neurobiological mechanisms of BD vulnerability and the brain changes possibly associated with disturbed mechanisms of neuroplasticity.

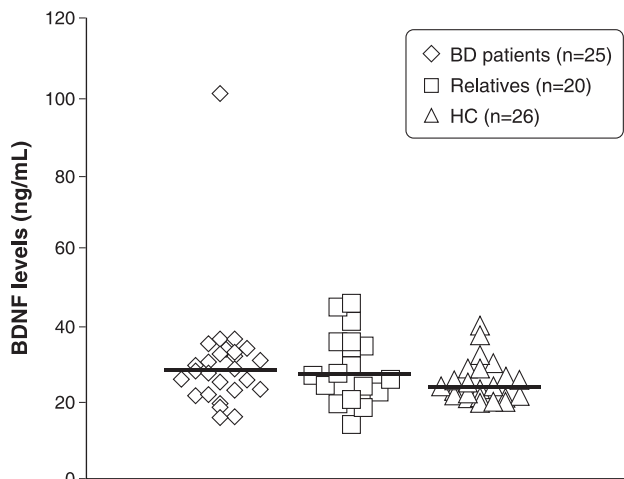


Figure 1 Serum BDNF levels in BD patients, unaffected relatives, and HC. Analysis of variance ($F_{2,68} = 1.45$, $p = 0.24$). These results did not change after covarying for age and gender ($F_{1,65} = 1.55$, $p = 0.22$) or after excluding one outlier in the patient group ($F_{2,67} = 1.66$, $p = 0.2$). BD = bipolar disorder; BDNF = brain-derived neurotrophic factor; HC = healthy controls.

Normal serum BDNF levels were found among euthymic BD patients, unaffected relatives, and HC. These results add to current evidence that peripheral BDNF levels are state markers of disease expression.

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Disclosure

FGN holds a temporary position as a medical advisor at Eli Lilly. FK has received grants/research support from AstraZeneca, Eli Lilly, Janssen-Cilag, and Servier; has also been a member of the Speakers' Bureau at AstraZeneca, Eli Lilly, Janssen-Cilag, and Servier; and has served as a consultant for Servier. The remaining authors report no conflicts of interest.

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