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Increased plasma levels of brain-derived neurotrophic factor in patients with long-term bipolar disorder

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ABSTRACT

Recent data indicate that neurotrophins may play a role in the physiopathology of bipolar disorder (BD) and may be useful as biomarkers of the disease. The aim of this study was to evaluate the plasma concentrations of brain-derived neurotrophic factor (BDNF) in BD patients, and to correlate their levels with clinical parameters. BDNF was measured in plasma from 53 BD type I subjects (34 during mania and 19 during euthymia) and 38 healthy controls by enzyme-linked immuno-sorbent assay (ELISA). Patients were assessed by a structured clinical interview (Mini-plus), Young mania and Hamilton depression rating scales. Plasma BDNF levels were significantly increased in patients with mania ($P \leq 0.001$) and euthymia ($P \leq 0.001$) when compared with controls, but did not correlate with any clinical parameters. BDNF concentration was higher in BD patients with 10 or more years of disease. BDNF plasma levels were increased in BD patients, mainly in those with a longer course of disease. In line with previous studies, it is conceivable that BDNF may play a role in the pathophysiology of BD.
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Bipolar disorder (BD) is a severe, disabling chronic disease affecting the lives and function of millions worldwide [3]. The hallmark of BD is mania or hypomania, however the majority of these patients suffer from depressive episodes. Even under treatment, many of these individuals remain vulnerable for relapses, psychiatric and clinical co-morbidities, and cognitive impairment [3].

The neurobiology of bipolar disorder remains unclear. Much evidence has indicated that BD arises from a complex interaction of multiple susceptibility genes and environmental stressors possibly affecting intra-thecal plasticity. Neuro-plasticity is a vital process by which the brain perceives, adapts and responds to external and internal stimuli [26]. Neurotrophins are a family of proteins that regulate neuronal development by synaptic plasticity. Brain-derived neurotrophic factor (BDNF) is a neurotrophin widely expressed in the adult brain, playing a relevant role in the process of memory and learning. BDNF has been found to promote survival of all major cell types in the brain, such as hippocampal, cortical, cholinergic and aminergic neurons [19]. This has motivated the investigation of the role of BDNF in several neuropsychiatric disor-

Abbreviations: ANOVA, analysis of variance; BD, bipolar disorder; BDNF, brain-derived neurotrophic factor; DSM-IV, diagnostic and statistical manual of mental disorders, 4th edition; ELISA, enzyme-linked immuno-sorbent assay; GAD, generalized anxiety disorder; Ham-D, Hamilton depression rating scale; Mini-plus, Mini-international neuro-psychiatric interview; mL, milliliter; NGF, nerve growth factor; pg, pictogram; SD, standard deviation; SPSS, Statistical Package for the Social Sciences; *vs*, versus; χ^2-test, chi-square test; YMRS, Young mania rating scale.

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ders [7,8,20]. In line with this, the aim of the present study was to evaluate the plasma levels of BDNF in patients with BD in comparison with healthy subjects. Secondary analyses were performed in order to identify clinical and demographic factors associated with the variation of this neurotrophin levels.

Fifty-three BD type I patients and 38 healthy controls matched for age and gender were recruited for this study in a public hospital as in-patient or out-patient admission. The control group was recruited from the local population and they did not have any personal or family history of psychiatric disorders, suicide behavior, cognitive deficit or clinical diseases.

All procedures described in this study received approval from the local clinical research ethics committee and are in accordance with the Helsinki Declaration of 1975. Written informed consent was obtained from all patients and healthy subjects prior to conducting any study procedures.

All participants were over the legal age of 18 years old. Subjects with dementia and previous diagnosis of any neurodegenerative disorders, infectious or autoimmune diseases, or who had used cortico-steroids, non-steroidal anti-inflammatory drugs or antibiotics 4 weeks before the entry into the study were excluded. Psychiatric diagnosis was based on DSM-IV criteria [1] and was performed following a structured clinical interview (Mini-international neuro-psychiatric interview, Mini-plus) [22,2]. Healthy controls were assessed by Mini-plus to exclude any psychiatric condition. The severity of maniac and depressive symptoms was assessed by the Young mania rating scale (YMRS) [25] and the Hamilton depression rating scale, 17-item version (Ham-D) [9], respectively. Euthymia was defined by YMRS score lower than 12 and Ham-D score <7 points.

All blood samples were drawn at the moment of the clinical assessment. Five milliliters of blood was drawn from each subject by venipuncture into a vacuum tube containing heparin, and was immediately centrifuged twice at $3000 \times g$ for 10 min, and plasma was kept frozen at $-70\,^{\circ}C$ until assayed. Plasma concentration of BDNF was measured according to the procedure provided by the manufacturer using sandwich ELISA kits for BDNF (DuoSet, R&D Systems, Minneapolis, MN, USA). All samples were assayed in duplicate. The detection limits for these assays were 5 pg/mL. Values below the detection limits were assumed to be zero. Concentration is expressed as pg/mL.

Statistical analyses were performed using SPSS software version 15.0 (SPSS Inc., Chicago, IL, USA). Descriptive statistics were used to report socio-demographic and clinical characteristics of the sample. Association between dichotomous variables was assessed with χ^2-test or Fisher's exact test when appropriate, as indicated. All variables were tested for normality of distribution by means of the Kolmogorov–Smirnov test. Two groups (patients *vs*. controls) were compared with *t*-test or Mann–Whitney test in normally or non-normally distributed data, respectively. Three groups (patients with mania *vs*. patients in euthymia *vs*. controls) were compared with one-way ANOVA or Kruskal–Wallis test in normally or non-normally distributed data, respectively. Dunn's post-hoc test was performed to make comparisons among groups.

A correlation of Spearmann coefficients matrix was performed to examine the relationship of BDNF levels with age, length of illness, YMRS and Ham-D scores. All statistical tests were two-tailed and were performed using a significance level of $\alpha = 0.05$. Data are presented as means ± standard deviation (SD), median, or percentage, as indicated.

Demographic and social characteristics of all subjects are shown in Table 1. There was no statistically significant difference between BD patients and the control group regarding gender, age, marital status and number of children. As expected, difference between patients and controls was found when comparing their current work status: only 50.9% of BD patients were working, while the remaining were unemployed (17.0%) or retired (32.1%), in contrast with the control group in which 95.2% were employed.

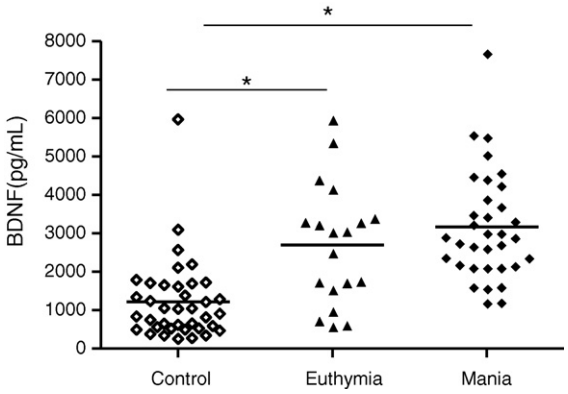

Fig. 1. Plasma BDNF levels in healthy subjects and BD patients in euthymia and in mania
$P \leq 0.001$ (Kruskal–Wallis with Dunn's post test).

At the time of the interview, 34 patients were in mania and 19 patients in euthymia. The clinical features of patients with BD are shown in Table 1. BD patients with mania and euthymia did not differ in many clinical variables, such as age of disease onset, number of hospitalizations, medications in use and psychiatric co-morbidities. BD patients with mania presented higher scores in YMRS in comparison with euthymic patients (28.5 ± 6.2 *vs*. 1.3 ± 2.5, $P \leq 0.001$). BD patients with mania also presented higher scores in Ham-D when compared to euthymic patients (3.8 ± 4.6 *vs*. 1.9 ± 1.8, $P = 0.01$).

Plasma levels of BDNF are shown in Fig. 1. BD patients had higher plasma concentrations of BDNF than healthy controls (2994.3 ± 1471.2 pg/mL *vs*. 1211.0 ± 1043.4 pg/mL, $P \leq 0.001$). BD patients with mania tended to have higher plasma levels than euthymic patients, but this difference did not reach statistical difference (3161.1 ± 1409.3 pg/mL *vs*. 2695.8 ± 1570.1 pg/mL, $P = 0.32$).

BDNF plasma levels did not correlate with age ($P = 0.22$), length of illness ($P = 0.23$), severity of manic ($P = 0.46$) or depressive ($P = 0.82$) symptoms. BDNF levels did not differ in BD patients categorized according to the presence of any psychiatric co-morbidity or the use of any mood stabilizing drug, *i.e*. antipsychotics ($P = 0.87$), lithium ($P = 0.18$), anticonvulsants ($P = 0.78$) or antidepressants ($P = 0.66$). Interestingly, patients suffering from BD for more than 10 years showed significantly higher levels of BDNF than patients with less than this disease course (3152.9 ± 1521.3 pg/mL *vs*. 2388.6 ± 1121.1 pg/mL, $P \leq 0.05$).

To date, this is one of the largest studies assessing BDNF levels in BD patients. Plasma concentrations of this neurotrophin were elevated in BD subjects when compared to that seen with healthy controls, regardless the mood state.

BD seems to be associated with plastic changes in the central nervous system (CNS) mediated by neurotrophins, mainly BDNF [19]. In line with the literature, our results demonstrated altered circulating levels of BDNF in BD. Most previous studies found decreased concentrations in the serum or plasma [4,10–12,14,23,5], however the current work is the first to report of increased levels of BDNF. One possible explanation for this contradiction could be that the present BD sample was composed mainly by patients with a longer disease duration, *i.e*. with more than 10 years duration of the illness (79.2%), inasmuch as the majority of the previous studies enrolled recently diagnosed subjects only [4,11,12,24]. The other 3 studies involving patients with a longer disease time had conflicting results with 2 of them demonstrating decreased BDNF levels [10,5], while the other showed unaltered concentrations [14]. These

Table 1
Demographic, social and clinical features of control subjects and BD patients.

Variables	Controls ($N = 38$)	BD patients		P
		Mania (N = 34)	Euthymia (N = 19)	
Gender (female %)	52.6	61.8	57.9	0.62*
Age (years)	42.9 ± 9.7	49.6 ± 14.2	44.5 ± 10.9	0.45†††
Ham-D	1.00 ± 1.5	3.8 ± 4.6	1.9 ± 1.8	0.01†††
YMRS	0	28.5 ± 6.2	1.3 ± 2.5	<0.001†††
Age of first mood episode (years)	–	30.1 ± 11.5	21.3 ± 7.4	0.12†
Age of first mania episode (years)	–	34.9 ± 11.4	24.9 ± 7.8	0.13†
Length of disease (years)	–	19.5 ± 13.9	20.2 ± 10.1	0.49†
Number of hospitalization	–	3.6 ± 3.5	5.4 ± 3.2	0.12†
Medication in use (frequency, %)				
Lithium	–	41.2%	68.4%	0.06*
Anticonvulsants	–	67.6%	68.4%	0.95*
Antipsychotics	–	67.6%	63.2%	0.74*
Antidepressants	–	2.9%	15.8%	0.13*
Alcohol dependency (frequency, %)				
Current	–	5.9%	5.3%	1.00**
Past	–	26.5%	21.1%	0.74**
Tobacco dependency (frequency, %)				
Current	–	26.5%	47.4%	0.14*
Past	–	26.5%	47.4%	0.14*
Substance dependency (neither alcohol nor tobacco) (frequency, %)				
Current	–	5.9%	5.3%	1.00**
Past	–	5.9%	5.3%	1.00**
Psychosis in life (frequency, %)	–	50.0%	63.2%	0.40**
Generalized anxiety disorder	–	20.6%	21.1%	1.00**

†† t-test.
* χ^2-test.
** Fisher exact test.
† Mann–Whitney's test.
††† Kruskal–Wallis test.

results, therefore, strongly suggest that BDNF levels vary during the course of the disease.

This assumption is in line with evidence from other neuropsychiatric disorders. For instance, elevated serum levels of BDNF were described in long-term schizophrenic patients [7,20]. Furthermore, Schulte-Herbrüggen et al. [21], in an experimental model of Alzheimer's disease (AD), showed that there is an increase in the BDNF concentration mainly in the frontal and occipital cortices, striatum and hippocampus, with the evolution of the disorder. Considering that circulating levels of BDNF may reflect cerebral abundance of BDNF, it is possible that high concentration BDNF in the long course of BD may represent a reaction to the cerebral damage that occurred in the early years of the disease when neuro-degenerative processes seem to be more intense and BDNF levels would be decreased [13].

An alternative hypothesis would involve the effect of treatment. Increased BDNF levels in patients with long-course disease might be explained by the cumulative effect of the prolonged exposition to drugs. Indeed, all the primary antidepressant modalities, as well as long-term antipsychotic management could interfere with BDNF levels [6,18,15], and increased concentrations have actually been found following treatment with antidepressants or mood stabilizers in BD and other mood depressive disorders [4,24,23,16,17]. BDNF levels also tend to increase in the brain of rats chronically treated with mood stabilizers or anticonvulsants (lithium or valproic acid) [6]. It is pertinent to mention that most studies in BD involved medicated patients and they did not control a possible confounding effect of drug treatment on BDNF levels: only one study involved drug-naïve BD patients [11]. The limitations from previous studies, as well as the present report, stems from the fact that BD participants used mood stabilizing agents, antidepressants and antipsychotics, and it is not possible to discard the effects of drugs on circulating levels of neurotrophins. Due to the high probability of rapid decompensation of the disease that is otherwise successfully stabilized by chronic medication, it is neither recommended, nor ethically acceptable to terminate drugs in order to rule out the impact of psychotropics on BDNF levels. Although prolonged use of medications may yield metabolic syndrome in patients that could also alter neurotrophin concentrations, it is impossible to discontinue drugs for several years or so to monitor psychotropic-free BDNF plasma abundance on the long term (*e.g.* a decade or more). However, given the fact that BDNF plasma levels did not correlate with any medical treatment in this study, it is reasonable to hypothesize that our findings represent real BD-specific BDNF pattern in the blood. Nevertheless, due to the cross-sectional design of the present study, it is not possible to assure that the observed difference indicates direct causality. Other limitation of the present study is the sample size.

Authors here report altered plasma BDNF levels in BD patients, notably in those with a disease longer than 10 years. This is consistent with the emerging literature that points to the abnormality of plastic changes mediated by neurotrophins in the pathophysiology of neuro-psychiatric disorders, however further studies are required to evaluate the putative bi-phasic bio-marker pattern, *i.e.* the shift from initially decreased BDNF concentrations to elevated levels after a decade of disease progression, as confirmed in this communication.

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