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Research report

Abnormality in serum levels of mature brain-derived neurotrophic factor (BDNF) and its precursor proBDNF in mood-stabilized patients with bipolar disorder: A study of two independent cohorts

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ABSTRACT

Background: Early detection and diagnosis of bipolar disorder can be difficult. Tools are needed to help clinicians detect bipolar disorder earlier, which would ameliorate the prognosis.
Methods: ELISA kits that distinguish between mature brain derived neurotrophic factor (BDNF) and proBDNF, we compared serum levels of mature BDNF, proBDNF, and matrix metalloproteinase-9 (MMP-9) in two independent cohorts (Sahlgrenska cohort and Karolinska cohort) of mood-stabilized bipolar patients and healthy controls. The total sample size in both cohorts consisted of 263 (48+215) bipolar patients and 155 (43+112) healthy controls.
Results: Levels of mature BDNF and the ratio mature BDNF/proBDNF were significantly higher in patients than in controls. Serum levels of proBDNF were significantly lower in patients compared to controls. Serum levels of MMP-9 did not differ between the groups but MMP-9 correlated positively and significantly with mature BDNF.

Mature BDNF, proBDNF, the ratio of mature BDNF/proBDNF and interactions with MMP-9 explained the diagnostic dichotomy in both cohorts with high significance, using multivariate logistic ANCOVA (gender, age, and BMI were covaried out). The model explained 41% of the diagnostic variance in the Sahlgrenska cohort ($p < 0.0001$) and 15% in the Karolinska cohort ($p < 0.0001$). In both cohorts, the equations provided good power for diagnostic classification. The diagnostic sensitivity was 89% in the Sahlgrenska and 74% in the Karolinska cohort, and specificity 77% and 64%, respectively.
Limitation: The study is cross-sectional with no longitudinal follow up. The cohorts are relatively small with no medication-free patients. There are no "ill patient controls".
Conclusion: Abnormalities in the conversion of proBDNF to mature BDNF may be associated with pathogenesis of bipolar disorder. Clinical use of these biomarkers may provide opportunities for earlier detection and correct treatment.

1. Introduction

Bipolar disorder is a debilitating mental illness with a high mortality rate (Angst et al., 2002; Belmaker, 2004). About 1–2% of the world population suffers from bipolar disorder, affecting males and females equally. Despite syndrome remission, the premorbid functional level rarely fully recovers (Goldstein et al., 2009; Tohen et al., 2000). Bipolar disorder has been shown to comprise neurodegenerative features in which relapses are toxic (Ekman et al., 2010), underlining the importance of early detection in order to prevent an otherwise negative prognosis.

The causal factors of psychiatric disorders are multifactorial, and most diagnostic phenotypes have multiple underlying etiologies with similar clinical expressions. The challenge is to define a set of biomarkers that, despite different underlying mechanisms, can be linked to a clinical phenotype, and which would allow early detection.

Brain-derived neurotrophic factor (BDNF) is a protein synthesized from a precursor, preproBDNF, which is converted to proBDNF. ProBDNF is cleaved to generate mature BDNF by extracellular proteases, and BDNF crosses the blood–brain barrier (Pan et al., 1998; Schmidt and Duman, 2010). One such extracellular

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protease is matrix metalloproteinase-9 (MMP-9), but there are others, *e.g.* plasmin (Park and Poo, 2013). BDNF has been proposed to be a state-related biomarker of mood disorders (Fernandes et al., 2011; Hashimoto, 2010; Lin, 2009). BDNF is widespread in the brain and abundant in the hippocampus and cerebral cortex. BDNF plays roles in mood, emotion and cognition (Ernfors et al., 1990). BDNF has also been shown to be correlated to therapeutic effects of antidepressants (Altar, 1999; Duman et al., 1997; Hashimoto, 2010, 2013; Hashimoto et al., 2004; Martinowich et al., 2007; Nestler et al., 2002).

Accumulating evidence suggests a key role of BDNF in the pathogenesis of bipolar disorder (Hashimoto, 2010). It has been reported that blood levels of BDNF were decreased in patients with bipolar disorder during manic (Cunha et al., 2006; de Oliveira et al., 2009; Machado-Vieira et al., 2007; Palomino et al., 2006), and depressed phases (Cunha et al., 2006; de Oliveira et al., 2009; Yoshimura et al., 2006). One study also found decreased levels of BDNF during euthymia (Monteleone et al., 2008). However, these findings were not replicated in other reports (Dias et al., 2009; Huang et al., 2012; Kauer-Sant'Anna et al., 2009; Mackin et al., 2007). Meta-analyses (Fernandes et al., 2011; Huang et al., 2012; Lin, 2009) have concluded serum levels of BDNF to be significantly lower in patients with bipolar disorder, and BDNF levels were normalized after recovery from depression (Hashimoto, 2010).

One likely reason for the divergent results is that earlier ELISA kits were unable to distinguish between mature BDNF and proBDNF (Yoshida et al., 2012a). Formerly, proBDNF was argued to be biologically inactive, but subsequent studies have shown that proBDNF and mature BDNF have opposite effects via $p75^{NTR}$- and TrkB-receptors. Both play an important role in different physiological functions (Dieni et al., 2012; Hashimoto, 2007, 2010, 2013; Pang and Woo, 2005), such as synaptic plasticity, neuronal survival, and neuronal differentiation (Poo, 2001). Supporting the notion that it is important to distinguish between proBDNF and mature BDNF, Yoshida and collaborators (Yoshida et al., 2012b) reported that serum levels of mature BDNF, but not proBDNF, were significantly lower in patients with major depressive disorder (MDD) than those of healthy controls.

The aims of this study were (i) to test the hypothesis that the BDNF synthetic pathway is altered in bipolar disorder compared to normal controls, and (ii) to elucidate whether BDNF-related independent variables are useful for clinical diagnostic predictions. To these ends, we studied subcomponents in the BDNF synthetic pathway (proBDNF, mature BDNF, and MMP-9) in two independently collected case-control cohorts of bipolar patients and healthy controls.

2. Methods and materials

2.1. Participants in the Sahlgrenska cohort

Subjects were recruited primarily from an outpatient unit specialized in bipolar patients. The catchment area for the unit is socioeconomically diverse and multinational. Forty-eight mood-stabilized Caucasian patients with bipolar disorder and 43 Caucasian healthy controls were enrolled. All patients had a prior clinical diagnosis meeting the DSM-IV criteria for bipolar disorder. Healthy controls were recruited by advertisement in a newspaper. Exclusion criteria for subjects in both groups included any current or past history of metabolic disease and/or active substance abuse or dependence. Patients and controls were not matched.

Prior to commencement of the study and signing the written consent, all subjects were provided with verbal and written information about the study and about potential risks and benefits of study participation. The Regional Research Ethics Board in Gothenburg approved the study (172-08).

Serum samples were collected from fasting subjects between 9:00 and 12:00 am. The samples were centrifuged on site and stored at −20 °C until delivered by courier mail, frozen in 2 batches on dry ice, to Chiba University, Japan, for analysis.

2.2. Participants in the Karolinska cohort

The study population was recruited from the St. Göran Bipolar Project, which provides assessment, treatment, and follow-up of patients with bipolar disorder within the Northern Stockholm Mental Health Service. The project also serves as a basis for research in bipolar disorder. The methodology has previously been outlined in detail (Ekman et al., 2010; Rydén et al., 2009).

A total of 215 patients with bipolar disorder and 112 healthy controls were included. All patients had a prior clinical diagnosis meeting the DSM-IV criteria for bipolar disorder. Healthy controls were selected randomly from the national population register by Statistics Sweden (www.scb.se). These control subjects were living in the same catchment area as the patients. Exclusion criteria for controls were: (1) any on-going psychiatric or neurological disorder; (2) current treatment with any psychotropic drug; (3) past history of bipolar disorder, schizophrenia, recurrent depression or other psychiatric disorder leading to extended sick-leave; and (4) a first-degree relative with schizophrenia or bipolar disorder. Patients and controls were not matched (except for catchment area).

Prior to commencement of the study and signing the written consent, all subjects were provided with verbal and written information about the study and about potential risks and benefits of study participation. The Regional Research Ethics Board in Stockholm approved the study (2005-554-31/3).

Serum samples were collected from fasting subjects between 8:00 and 9:00 am. The samples were centrifuged on site and stored at −80 °C until delivered by courier mail, frozen on dry ice, to Chiba University, Japan, for analysis.

2.3. Assessment of clinical variables in the Sahlgrenska cohort

For patients, their diagnosis of bipolar disorder was confirmed by a thorough interview and clinical assessment of a psychiatrist (KS). To validate the diagnosis we used the structured psychiatric interview M.I.N.I., version 6 (Sheehan et al., 1998) in an authorized Swedish translation. Table 1 shows the subdiagnostic composition.

Age, height, weight, sagittal abdominal diameter, and waist circumference was measured, and the BMI was calculated. Age, age at first diagnosis, the latency before diagnosis, years with diagnosis, number of suicide attempts, and numbers of depressive, manic and mixed episodes were noted. For dimensional assessments, the Montgomery–Åsberg depression rating scale (MADRS) (Montgomery and Åsberg, 1979) and Young mania rating scale (YMRS) (Young et al., 1978) were used to assess mood states, and mini-mental state examination (MMSE) (Folstein et al., 1975) for cognitive impairment. Disability was assessed by Global Assessment of Functioning (GAF) (Hall, 1995). To assess addiction alcohol use disorder test (AUDIT) (Conigrave et al., 1995), and drug use disorder test (DUDIT) (Cassidy

Table 1
Diagnoses of bipolar patients and healthy controls in the 2 sets of data.

	Sahlgrenska set		Karolinska set	
	Males	Females	Males	Females
Bipolar I, psychotic	7	7	28	52
Bipolar I, non-psychotic	0	2	11	10
Bipolar II	4	4	31	59
Bipolar NOS	4	20	12	12
Healthy controls	19	24	48	64

Table 2
Distributions of present and past pharmacotherapy of bipolar patients and healthy controls in two independent sets

Sahlgrenska set	***Diagnosis:***			
	Bipolar I	**Bipolar II**	**Bipolar NOS**	**Healthy controls**
Pharmacotherapy:				
Antidepressants presently	7	3	10	0
Antipsychotics presently	11	4	15	0
Lithium presently	11	6	11	0
Antiepileptics presently	9	2	12	0
Antidepressants earlier	12	6	17	0
Antipsychotics earlier	11	2	13	0
Lithium earlier	5	0	3	0
Antiepileptics earlier	6	1	4	0
Karolinska set	***Diagnosis:***			
	Bipolar I	**Bipolar II**	**Bipolar NOS**	**Healthy controls**
Pharmacotherapy:				
Antidepressants presently	36	48	9	0
Antipsychotics presently	33	11	7	0
Lithium presently	70	44	13	0
Antiepileptics presently	31	38	6	0
Antidepressants earlier	76	82	20	8
Antipsychotics earlier	75	25	9	0
Lithium earlier	78	41	12	0
Antiepileptics earlier	38	36	9	0

et al., 2008) were used. When needed, supplementary information was collected from medical records. Healthy controls were assessed by the AUDIT, DUDIT, GAF, YMRS, MADRS and MMSE. All clinical measurements are listed in Table 3, split for sex.

2.4. Assessment of clinical variables in the Karolinska cohort

Patients were assessed by a psychiatrist or resident in psychiatry using the affective disorders evaluation (ADE), which is a standardized protocol adapted from the systematic treatment enhancement program of bipolar disorder (STEP-BD) (Sachs et al., 2003). The ADE guides the interviewer through a systematic assessment of the patient's current and past mental state, and provides a diagnosis according to DSM-IV criteria. Table 1 shows the subdiagnostic composition.

The number of lifetime affective episodes and their characteristics were documented. Other modules assess alcohol and drug misuse, violent behavior, childhood history, family history, treatment history, reproductive history, and somatic illnesses. Interpersonal violence is defined as a violent act or serious physical threat to another person. Suicide attempt is defined as a deliberate and serious self-injury, including intoxication with medication.

The final diagnosis was established using LEAD (Longitudinal observation by Experts using All Data) (Spitzer, 1983) and confirmed by a consensus panel of 2–4 experienced clinicians. Inclusion criteria for this sub-study were diagnoses of bipolar I or II disorders. Disease severity was assessed using the clinician rated global assessment of function (GAF) and clinical global impression (CGI) scales (Guy, 1976; Luborsky, 1962). For dimensional assessments, the MADRS and YMRS were used to assess mood states, and an extensive neuropsychological test battery was used to assess cognitive impairment. To assess addiction, AUDIT and DUDIT were used. Healthy controls were assessed by the AUDIT, DUDIT, and GAF. All clinical measurements are listed in Table 4, split for sex.

2.5. Assessment of present and past pharmacotherapy

Table 2 shows distributions of present and past psychiatric drug treatment in both the Sahlgrenska and the Karolinska cohorts, split for sub diagnoses. Treatments are presented in a dichotomous way (Yes|No) for antidepressants, antipsychotics, lithium, and antiepileptics, both “presently” and “earlier”. In a Supplemental table (available on the web) are given the generic names and daily dosages of the current psychopharmacological treatment for each patient in the Sahlgrenska sample.

2.6. Measurement of serum levels of proBDNF, mature BDNF, and MMP-9

Serum levels of proBDNF, mature BDNF, and MMP-9 were measured in duplicates using human proBDNF ELISA kit, mature BDNF kit (Adipo Bioscience, Santa Clara CA, USA), and the human MMP-9 ELISA Kit (R&D Systems, Minneapolis MN, USA), respectively, following manufacturers' instructions. To minimize assay variance, serum levels of proBDNF, mature BDNF and MMP-9 from all subjects in the Sahlgrenska cohort were measured on the same day. The same procedures were followed for the Karolinska cohort. A 50-fold dilution of serum was used to measure mature BDNF and MMP-9. The optical density of each well was measured using an automated microplate reader (Emax, Molecular Devices, Sunnyvale CA, USA).

Tables 3 and 4 show the results of the analysis of serum levels in the two cohorts.

2.7. Statistical analyses

The JMP 10.0.1 package from SAS, Inc., was used to analyze data. All non-dichotomous variables were inspected for skewness, and $_{10}$log transformations were applied when needed in order to normalize distributions. For ratios, the arctan transform was used, presenting angular radians in trigonometric space in order to avoid outliers. Multivariate nominal logistic fits (effect likelihood ratio tests) were applied with diagnostic categories as the predicted variables, and the serum measures and their interactions as the independent predictor variables, covarying out the influence of sex, age and body composition (BMI). Linear multiple regression models were used to investigate variation components in the serum variables measured. GraphPad Prism was used to generate graphs.

Table 3
Description and comparison of measured variables in the Sahlgrenska training cohort.

	Total	Patients				Healthy controls				*All patients vs. all controls*[§]	
	n	*Males*	*n*	*Females*	*n*	*Males*	*n*	*Females*	*n*	*t*	*p*
Arithmetic means (± SD)											
mature BDNF [ng/mL]	90	25.39 ± 9.61	15	22.86 ± 6.61	32	18.99 ± 6.55	19	15.45 ± 4.97	24	−4.42	****
proBDNF [ng/mL]	79	32.75 ± 22.44	13	23.15 ± 6.36	28	26.42 ± 9.09	17	55.65 ± 66.78	21	2.00	*
Ratio matBDNF/proBDNF	79	1.11 ± 0.72	13	1.05 ± 0.40	28	0.77 ± 0.45	17	0.57 ± 0.40	21	−4.04	****
MMP-9 [ng/mL]	90	779.6 ± 378.0	15	648.7 ± 370.5	32	559.6 ± 247.4	19	572.7 ± 382.9	24	−1.54	ns
Age [yrs]	91	36.7 ± 8.0	15	40.6 ± 11.7	33	33.8 ± 15.9	19	29.6 ± 11.2	24	−3.79	***
Height [cm]	91	181.4 ± 7.48	15	167.7 ± 7.25	33	180.1 ± 4.71	19	168.2 ± 6.83	24	0.74	ns
Weight [kg]	91	92.4 ± 22.0	15	77.2 ± 18.6	33	79.0 ± 9.14	19	63.6 ± 8.87	24	−3.20	**
Sagittal diameter [cm]	91	19.6 ± 3.8	15	19.7 ± 3.5	33	17.6 ± 2.8	19	15.4 ± 1.6	24	−5.13	****
Waist circumference [cm]	91	98.1 ± 16.7	15	90.3 ± 16.0	33	86.6 ± 10.0	19	72.2 ± 7.4	24	−4.76	****
BMI	91	27.8 ± 5.0	15	27.4 ± 6.1	33	24.4 ± 3.2	19	22.5 ± 3.0	24	−4.24	****
Age first symptoms	46	19.4 ± 7.4	14	17.5 ± 5.8	32						
Age diagnosis	45	29.8 ± 7.0	14	32.1 ± 10.8	31						
Diagnostic latency	45	10.4 ± 7.1	14	14.5 ± 10.4	31						
Yrs with diagnosis	45	7.1 ± 6.7	14	8.2 ± 10.1	31						
Suicide attempts2;[#]	46	0.0	14	1.75 ± 2.99	32						
Depressive episodes2;[#]	38	10.0 ± 7.9	13	14.0 ± 17.1	25						
Manic episodes2;[#]	34	6.4 ± 6.8	12	8.6 ± 8.5	22						
Mixed episodes2;[#]	26	0.25 ± 0.46	8	0.06 ± 0.24	18						
Medians (range)											
Age first symptoms	46	19 (6–37)	14	15.5 (7–31)	32						
Age when diagnosis	45	31.5 (20–41)	14	31.0 (18–55)	31						
Diagnostic latency	45	10.5 (0–20)	14	13.0 (0–32)	31						
Yrs with diagnosis	45	5 (0–22)	14	4 (0–38)	31						
Suicide attempts2;[#]	46	0	14	0.5 (0–10)	32						
Depressive episodes2;[#]	38	8 (1–30)	13	6 (1–60)	25						
Manic episodes2;[#]	34	4 (1–20)	12	5.5 (0–32)	22						
Mixed episodes2;[#]	26	0 (0–1)	8	0 (0–1)	18						
Fractions					χ^2						
Psychosis		57% (8/14)		56% (18/32)	ns						
Mood congruence		63% (5/8)		78% (14/18)	ns						
AD presently		29% (4/14)		50% (16/32)	ns						
AP presently		71% (10/14)		63% (20/32)	ns						
Li presently		93% (13/14)		47% (15/32)	**						
AE presently		36% (5/14)		56% (18/32)	ns						
Arithmetic means (± SD)											
AUDIT	90	5.2 ± 4.4	15	3.2 ± 2.9	32	6.1 ± 3.9	19	4.8 ± 2.6	24	2.18	*
DUDIT	90	2.3 ± 4.3	15	1.9 ± 3.8	32	0.3 ± 0.7	19	0.8 ± 1.5	24	−2.40	*
GAF	89	69.7 ± 11.2	14	66.8 ± 15.6	32	89.2 ± 4.0	19	88.0 ± 3.2	24	9.55	****
YMRS	89	1.9 ± 3.3	14	0.3 ± 0.9	32	0.2 ± 0.6	19	0.2 ± 0.7	24	−1.82	ns
MADRS	87	7.3 ± 6.7	14	10.5 ± 8.6	31	1.9 ± 5.0	19	0.7 ± 6.6	23	−6.53	****
MMSE	88	28.2 ± 2.34	13	28.5 ± 1.4	32	29.7 ± 0.6	19	29.6 ± 0.6	24	11.22	****

[§] Comparisons based on log transformed values.
[#] Ordinal scale.

3. Results

Serum levels of mature BDNF were significantly *higher* in patients than in controls, in both cohorts ($p < 0.0001$ and $p < 0.001$), as shown in Tables 3 and 4, split for sex. Serum levels of proBDNF were significantly *lower* in patients in both cohorts ($p < 0.05$ and $p < 0.0001$). The ratio mature BDNF/proBDNF was significantly *higher* in patients in both cohorts ($p < 0.0001$ and $p < 0.0001$). There was no significant difference in MMP-9 in either cohort. Fig. 1 illustrates these differences graphically, here not split for sex.

Categorical medication status among the Sahlgrenska patients was analyzed as 4 independent variables (Yes|No for antidepressants, antipsychotics, lithium, and antiepileptics) and used as independent variables in ANCOVAs, covarying out sex, age, and BMI. Dependent variables were the serum measurements, in turns. Linear multiple regressions are shown in Table 5, demonstrating no significant overall *F* values, and no significant medication effects. The one exception was that present medication with antiepileptic drugs was linked with low MMP-9 values, but only at trend level.

Table 6 shows results from multivariate logistic analyses used to discriminate the diagnostic dichotomy (Bipolar|Control). Significant influences from sex, age, and BMI were covaried out. The results were similar in both cohorts. ProBDNF and the ratio mature BDNF/proBDNF were significant predictors in both cohorts. Mature BDNF was significant in the Sahlgrenska cohort, but only at a trend level in the Karolinska cohort. There were no significant statistical interactions between mature BDNF and proBDNF. MMP-9 was a non-significant predictor, with no detectable interactions with the BDNFs.

This discriminatory model explained 41% of the diagnostic variation ($p < 0.0001$) in the Sahlgrenska cohort and 15% in the Karolinska cohort ($p < 0.0001$). In both cohorts, the equations provided good power for diagnostic classification. Sensitivity was 89% in the Sahlgrenska cohort and 74% in the Karolinska cohort, and specificity was 77% and 64%, respectively.

ProBDNF correlated negatively with mature BDNF in the whole Sahlgrenska cohort ($r = -0.28$, $p = 0.014$; Spearman $\rho = -0.26$). This correlation strengthened when covarying out diagnosis, sex, age, and BMI ($p < 0.0020$). MMP-9 correlated positively with

Table 4
Description and comparison of measured variables in the Karolinska replication cohort.

	Total	**Patients**				**Healthy controls**				*All patients vs. all controls*[§]	
	n	***Males***	***n***	***Females***	***n***	***Males***	***n***	***Females***	***n***	***t***	***p***
Arithmetic means (± SD)											
mature BDNF [ng/mL]	327	30.6 ± 7.3	82	31.5 ± 7.4	133	30.3 ± 7.2	48	27.2 ± 7.0	64	−3.24	***
proBDNF [ng/mL]	327	36.3 ± 62.7	82	58.0 ± 97.2	133	47.5 ± 58.2	48	161.2 ± 400.4	64	4.81	****
Ratio matBDNF/proBDNF	327	1.57 ± 1.10	82	1.37 ± 1.16	133	0.97 ± 0.56	48	0.70 ± 0.52	64	−6.10	****
MMP-9 [ng/mL]	327	533.2 ± 242.2	82	523.7 ± 223.3	133	501.7 ± 192.7	48	559.1 ± 271.3	64	0.00	ns
Age [yrs]	327	41.7 ± 12.8	82	37.3 ± 12.9	133	40.2 ± 14.4	48	36.3 ± 12.9	64	−0.73	ns
Height [cm]	322	181.0 ± 6.5	82	167.0 ± 5.9	133	183.0 ± 5.7	47	167.4 ± 6.3	60	1.76	ns
Weight [kg]	322	85.3 ± 12.2	82	68.8 ± 13.2	133	82.1 ± 11.1	47	66.2 ± 11.5	60	−1.10	ns
BMI	322	26.0 ± 3.2	82	24.7 ± 4.8	133	24.5 ± 2.8	47	23.6 ± 3.9	60	−2.54	*
Age first symptoms	212	21.2 ± 12.0	82	18.6 ± 10.4	130						
Suicide attempts2;[#]	212	0.9 ± 2.6	82	1.9 ± 5.2	130						
Depressive episodes2;[#]	212	19.1 ± 24.8	81	18.8 ± 25.4	131						
Manic episodes2;[#]	215	1.4 ± 2.1	82	1.5 ± 2.5	133						
Hypomanic episodes2;[#]	215	6.1 ± 11.9	82	7.7 ± 15.4	133						
Mixed episodes2;[#]	213	1.6 ± 5.5	82	1.1 ± 4.2	131						
Medians (range)											
Age first symptoms	213	19 (3-19)	82	16 (2-64)	131						
Suicide attempts2;[#]	212	0 (0-20)	82	0 (0-30)	130						
Depressive episodes2;[#]	212	10 (0-160)	81	9 (1-150)	131						
Manic episodes2;[#]	215	1 (0-10)	82	0 (0-15)	133						
Hypomanic episodes2;[#]	215	2 (0-80)	82	2 (0-99)	133						
Mixed episodes2;[#]	213	0 (0-30)	82	0 (0-30)	131						
Fractions						χ^2					
Psychosis		43.9% (36/46)	82	47.4% (63/70)	133	ns					
AD presently		35.4% (29/53)	82	48.1% (64/69)	133	ns					
AP presently		30.5% (25/57)	82	19.5% (26/107)	133	ns					
Li presently		63.4% (52/30)	82	56.4% (75/58)	133	ns					
AE presently		32.9% (27/55)	82	36.1% (48/85)	133	ns					
Arithmetic means (± SD)											
$GAF_{function}$	215	68.3 ± 11.9	82	66.3 ± 9.5	133						
$GAF_{symptom}$	215	67.2 ± 13.5	82	66.5 ± 9.5	133						
YMRS	172	1.3 ± 2.7	69	1.1 ± 2.0	103						
MADRS	327	4.2 ± 6.2	69	2.8 ± 4.5	103						

[§] Comparisons based on log transformed values.
[#] Ordinal scale.

mature BDNF ($r=0.37$, $n=90$, $p=0.0003$), but not with proBDNF ($r=-0.01$, $n=79$, n.s.). Fig. 1 shows the slopes of the two regression lines compared in the Sahlgrenska cohort. There were no significant intercorrelations between these variables in the Karolinska cohort.

For the Sahlgrenska cohort only, Table 7, Column A shows global assessment of function (GAF) scores to be highly discriminatory for the Bipolar|Control dependent variable, which is to be expected (variance explained 54%, sensitivity 88%, specificity 78%, $\chi^2=32.26$). In Column B, sex, age, and BMI have been added (variance explained 67%, sensitivity 95%, specificity 85%, $\chi^2=54.26$). Column C shows that the predictive power increased even more by adding mature BDNF, proBDNF, their ratio, and their interactive product (variance now explained was 85%, sensitivity 100%, specificity 95%, $\chi^2=65.65$). The strongest predictor was mature BDNF. Similarly highly significant results, although somewhat weaker, were obtained when mini mental state examination (MMSE) scores were used instead of GAF (results not shown). This subanalysis could only be done on the Sahlgrenska cohort, since GAF data had not been routinely collected in the Karolinska healthy controls.

Subdiagnostic categorical divisions (between bipolar I, bipolar II, and bipolar NOS) and the controls were compared with the plasma variables in focus (proBDNF, mature BDNF, their ratio, and MMP-9), using the All Pairs Tukey–Kramer test (a post-hoc conservative test which protects significance tests of all combinations of pairs) from the JMP package. There were no significant or even trend differences between any of the three possible bipolar subcategories (all $p>0.20$).

Current MDRS and YMRS scores ($_{10}$log transformed after adding unity) were compared between the above categorical divisions. The 3 bipolar subgroups all had significantly higher MADRS score than controls, but they did not differ significantly between themselves. As for the YMRS score, no significant differences were seen even *versus* the control group. The MDRS and YMRS scores and the subdiagnostic 4-pronged category were then compared (as independent variables) in turns with all plasma data (as dependent variables). No significant or trend correlation was discovered.

4. Discussion

We examined two independently collected cohorts of mood-stabilized bipolar patients and healthy controls and found that serum levels of mature BDNF were significant higher in patients compared to controls, in both cohorts. Moreover, the ratio of mature BDNF/proBDNF was significantly higher in patients than controls. In logistic ANCOVAs, the consistently significant independent variables predicting the diagnostic dichotomy were proBDNF and the ratio mature BDNF/proBDNF. Using the serum-only information for diagnostic predictions, it was possible to classify bipolar disorder patients *versus* healthy controls with

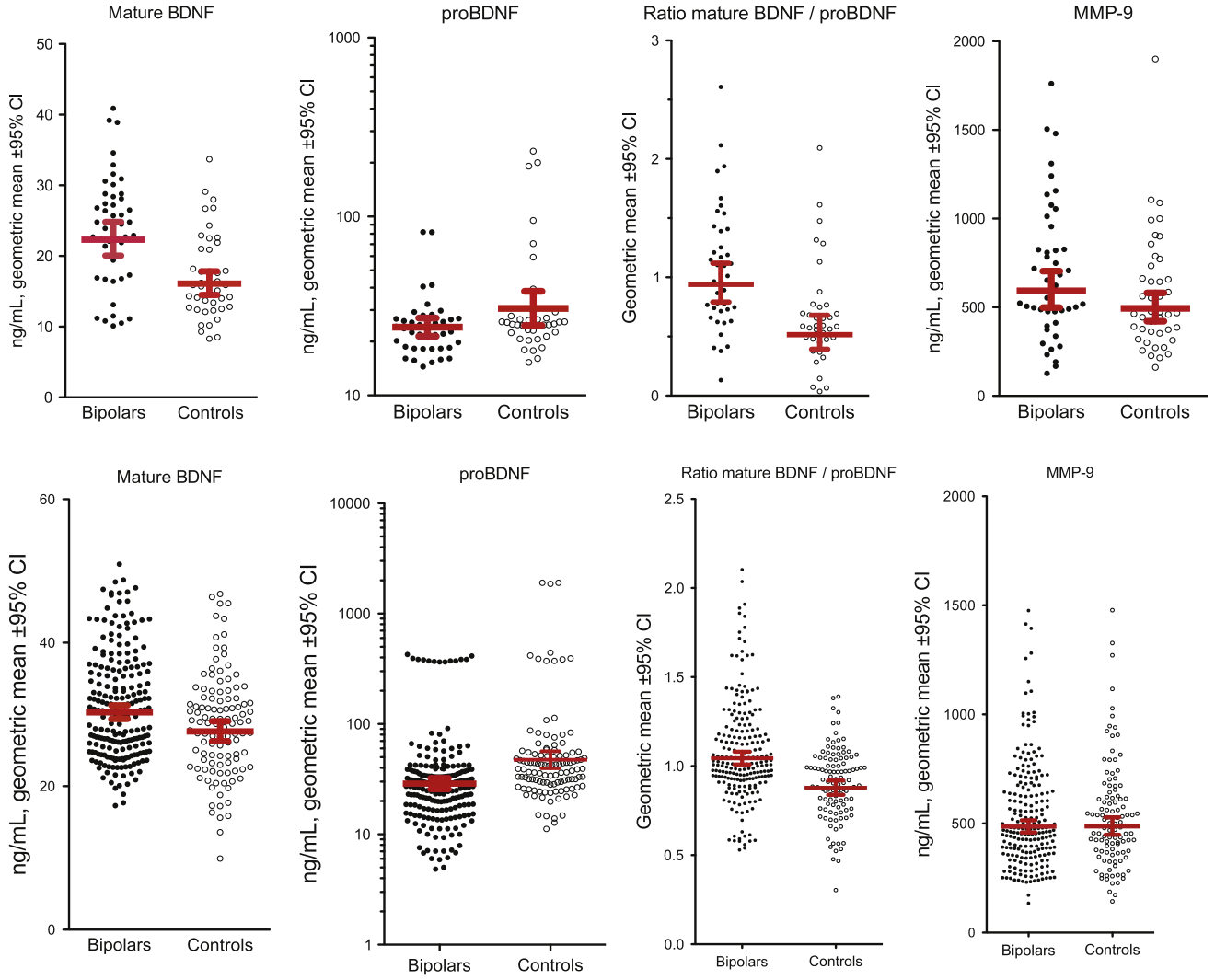

Fig. 1. Distributions and compared geometric means of serum measures.

Table 5
Linear regressions in Sahlgrenska bipolar patients, predicting serum values from clinical information and current medication. Sahlgrenska training cohort.

	Predicted variables:											
	log proBDNF			**log mature BDNF**			**arctan(log proBDNF/log matBDNF)**			**log MMP-9**		
	β	***t***	***p***	***β***	***t***	***p***	***β***	***t***	***p***	***β***	***t***	***p***
Predictor variables:												
Intercept	1.54	4.04	**0.0003**	1.10	4.60	**0.0001**	0.70	3.99	**0.0003**	2.42	5.60	**0.018**
Sex	−0.03	−0.69	> 0.10	−0.02	−0.83	> 0.10	−0.03	−0.16	> 0.10	−0.03	−0.69	> 0.10
logAge	−0.02	−0.07	> 0.10	0.25	1.61	> 0.10	0.06	0.49	> 0.10	0.16	0.55	> 0.10
BMI	−0.00	−0.78	> 0.10	0.00	−1.28	> 0.10	0.00	0.03	> 0.10	0.00	0.59	> 0.10
Antidepressant tx presently	−0.01	−0.22	> 0.10	0.01	0.35	> 0.10	0.00	0.27	> 0.10	0.01	0.24	> 0.10
Antipsychotic tx presently	−0.01	−0.30	> 0.10	−0.01	−0.33	> 0.10	0.00	−0.20	> 0.10	0.01	0.28	> 0.10
Lithium tx presently	0.02	0.53	> 0.10	0.03	1.27	> 0.10	0.00	−0.06	> 0.10	0.06	1.55	> 0.10
Antiepileptics tx presently	−0.03	−1.00	> 0.10	−0.03	−1.61	> 0.10	0.00	0.13	> 0.10	−0.07	−1.95	0.060
Overall ANOVA	F=0.68		> 0.10	F=1.34		> 0.10	F=0.06		> 0.10	F=1.51		> 0.10
df	7,38			7,46			7,38			7,46		
n	46			54			46			54		
R^2	0.112			0.170			0.012			0.187		

sensitivities of 89% for the Sahlgrenska cohort and 74% for the Karolinska cohort, and specificities of 77% and 64%, respectively; all highly significant. Significant influences from sex and BMI (as well as a non-significant age influence) were covaried out. Our results indicate that BDNF measurements have a potential for usage as clinical biomarkers by differentiating bipolar patients

Table 6
Nominal logistic fits predicting diagnosis with serum measures as predictors, covarying out sex, age, and BMI. Sahlgrenska training cohort and Karolinska replication cohort compared.

	Predicted variable: all bipolars vs. controls			
	Sahlgrenska cohort		Karolinska cohort	
	χ^2	***p***	χ^2	***p***
Predictor variables:				
Sex	7.65	**0.0057**	4.34	**0.037**
log Age	0.62	> 0.10	0.49	> 0.10
BMI	9.66	**0.0019**	5.52	**0.019**
log mature BDNF	6.19	**0.013**	3.31	0.069
log proBDNF	4.10	**0.043**	8.46	**0.0036**
arctan(matBDNF/proBDNF)	4.28	**0.039**	14.80	**0.0001**
log matBDNF × log proBDNF	1.10	> 0.10	2.53	> 0.10
log MMP-9	0.01	> 0.10	0.18	> 0.10
log MMP-9 × log matBDNF	2.07	> 0.10	0.23	> 0.10
log MMP-9 × log proBDNF	0.71	> 0.10	2.86	0.091
Whole model test	44.34	< **0.0001**	61.64	< **0.0001**
df	10		10	
n	79		321	
R^2	0.405		0.151	
Classification sensitivity	89%		74%	
Classification specificity	77%		64%	
Classification matrix, df = 1, χ^2	31.27	< **0.0000**	30.84	< **0.0000**
ROC AUC	0.88		0.75	

Table 7
Nominal logistic fits predicting diagnosis with serum measures as predictors, covarying out GAF scores, sex, age, and BMI. Sahlgrenska cohort only.

Effect likelihood ratio tests	*Predicted variable: all bipolars vs. controls*					
	A		B		C	
Sahlgrenska cohort	χ^2	*p*	χ^2	*p*	χ^2	*p*
Predictor variables:						
GAF	66.81	< **0.0001**	58.20	< **0.0001**	46.28	< **0.0001**
Sex			2.72	0.099	8.53	**0.0035**
log Age			8.98	**0.0027**	6.87	**0.0088**
BMI			1.48	> 0.10	0.03	> 0.10
log mature BDNF					7.16	**0.0075**
log proBDNF					5.65	**0.017**
arctan(matBDNF/proBDNF)					5.83	**0.016**
log matBDNF × log proBDNF					2.15	> 0.10
Whole model test	66.81	< **0.0001**	82.35	< **0.0001**	90.88	< **0.0001**
df	1		4		8	
n	89		89		77	
R^2	0.542		0.668		0.852	
Classification sensitivity	88%		95%		100%	
Classification specificity	78%		85%		95%	
Classification matrix, df = 1, χ^2	32.26	< **0.0000**	54.26	< **0.0000**	65.65	< **0.0000**
ROC AUC	0.92		0.97		0.99	

from healthy control individuals. Future studies should explore whether this usefulness extends to differentiating bipolar disorder from MDD and schizophrenia.

Our study is the first to investigate proBDNF and mature BDNF in bipolar disorder. BDNF in mood disorders has primarily been studied in unipolar depression. BDNF has been argued to be state-related, but previous results have been divergent. One reason is that earlier commercially available human BDNF ELISA kits were unable to distinguish between proBDNF and mature BDNF (Yoshida et al., 2012a). Consequently, earlier studies have reported combined levels of proBDNF and mature BDNF.

Our results are cross-sectional and can therefore neither support nor contradict whether BDNF levels are mainly related to state or trait. An abnormal conversion of proBDNF→mature BDNF, leading to increased levels of mature BDNF and reduced levels of proBDNF, may nevertheless play a role in the pathophysiology of bipolar disorder. There is a large preclinical literature on links between BDNF and neurogenesis within the brain, being relevant for human mood disorders (Bath et al., 2012; Bekinschtein et al., 2011; Marlatt et al., 2012; Peng et al., 2008; Rossi et al., 2006), but we cannot at this stage predict which BDNF component would be pivotal. Somewhat unexpectedly, we found no difference in levels between the three subdiagnostic categories. Current depressive symptoms (MADRS scores) or (hypo)manic symptoms (YMRS scores) had no influence. This would offer some indirect evidence favouring differences in plasma BDNF levels as to represent trait phenomena.

We found no difference in serum MMP-9 levels between patients with bipolar disorder and healthy controls, consistent with previous reports that serum MMP-9 levels were not altered in patients with major MDD (Yoshida et al., 2012b). A positive correlation has been reported between serum MMP-9 levels and the severity of depression in MDD patients, although the role of MMP-9 in the pathophysiology of MDD is currently unknown (Yoshida et al., 2012b). We did not find any correlation with diagnosis—except an interaction at trend level between MMP-9 and proBDNF (but only in the Karolinska cohort). Further studies are necessary to examine the role of MMP-9 in the pathophysiology of bipolar disorder. Nevertheless, MMP-9 has been shown to play a role in synaptic plasticity of the brain as well as in mood disorders (Ethell and Ethell, 2007; Hashimoto, 2013; Yoshida et al., 2012b).

The main strength of this study is that significant findings have been replicated in two independent cohorts. A limitation is that all patients in the two cohorts were on psychoactive medication. Previous studies show that blood BDNF (sum of proBDNF and mature BDNF) levels were significantly increased after the pharmacological treatment of manic state (Fernandes et al., 2011; Hashimoto, 2010; Lin, 2009), indicating that the medication might "restitute" serum levels of proBDNF+mature BDNF. Yet, in the analyzable Sahlgrenska cohort, we found no correlation between medication with any drug (antidepressants, antipsychotics, lithium, and antiepileptics), and any measured serum variable. Thus, the dynamics of pharmacological influence is not well understood, and there is a need to analyze medication-free patients, even though they may be hard to find.

5. Conclusion

Using serum-only information on proBDNF and mature BDNF to predict diagnosis, it was possible to correctly classify bipolar disorder patients *versus* healthy controls with sensitivities of 89% for the Sahlgrenska cohort and 74% for the Karolinska cohort, and with specificities of 77% and 64%, respectively, all highly significant. Adding a clinical assessment scale strengthened both sensitivity and specificity to over 90%, which would be strong enough to work as a clinical biomarker predicting the diagnostic dichotomy.

6. Limitations

Further longitudinal studies will be needed – measuring serum levels of proBDNF, mature BDNF, the ratio mature BDNF/proBDNF, and MMP-9 – using larger cohorts, if possible with medication-free patients, and "ill patient controls" with MDD and schizophrenia. A next step would then be to investigate if this type of algorithm could be used as a clinical aid to differentiate between a bipolar depressive disorder and, *e.g.*, MDD.

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Data access and responsibility

H.Å. had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Conflict of interest
None.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.jad.2014.01.009.

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