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A positive correlation between serum levels of mature brain-derived neurotrophic factor and negative symptoms in schizophrenia

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ABSTRACT

A meta-analysis study reported serum brain-derived neurotrophic factor (BDNF) levels as a potential biomarker for schizophrenia. However, at the time, commercially available human ELISA kits were unable to distinguish between pro-BDNF (precursor BDNF) and mature BDNF, because of limited antibody specificity. Here, we used new ELISA kits, to examine serum levels of mature BDNF and matrix metalloproteinase-9 (MMP-9), which converts pro-BDNF to mature BDNF in schizophrenia. Sixty-three patients with chronic schizophrenia and 52 age- and sex-matched healthy controls were enrolled. Patients were evaluated using the Brief Psychiatry Rating Scale, the Scale for the Assessment of Negative Symptoms (SANS) and neuropsychological tests. Neither serum mature BDNF nor MMP-9 levels differed between patients and controls. In male subgroups, serum MMP-9 levels of smoking patients were higher than those of non-smoking patients, but this was not observed in male controls or the female subgroup. In patients, serum mature BDNF levels were associated with SANS total scores and the Information subtest scores of the Wechsler Adult Intelligence Scale Revised (WAIS-R), while serum MMP-9 levels were associated with smoking and category fluency scores. These findings suggest that neither mature BDNF nor MMP-9 is a suitable biomarker for schizophrenia, although further studies using large samples are needed.

1. Introduction

Accumulating evidence implicates brain-derived neurotrophic factor (BDNF) in the pathophysiology of schizophrenia (Autry and Monteggia, 2012; Favalli et al., 2012; Martinotti et al., 2012; Nurjono et al., 2012). A meta-analysis suggested reduced blood BDNF levels in patients with schizophrenia, regardless of medication exposure and gender, and an association between reduced BDNF levels in schizophrenia and increasing age (Green et al., 2011). A number of studies have also reported reduced blood BDNF levels in patients with schizophrenia (Chen da et al., 2009; Fernandes et al., 2010; Pillai et al., 2010; Rizos et al., 2010a; Rizos et al., 2010b; Lee et al., 2011; Rizos et al., 2011; Yang et al., 2011; Zhang et al., 2012a; Zhang et al., 2012b; Zhang et al., 2012c). In contrast, several case-control studies have found increased peripheral blood BDNF levels in patients with schizophrenia (Reis et al., 2008; Domenici et al., 2010). We previously reported that serum levels of BDNF in schizophrenics were indistinguishable from those of healthy controls (Shimizu et al., 2003; Niitsu et al., 2011), a finding replicated by other studies (Huang and Lee, 2006; Mackin et al., 2007; Goto et al., 2011). As yet, there is no plausible explanation for this heterogeneity of findings and thus the role of BDNF in schizophrenia pathophysiology remains unclear.

Mature BDNF is synthesized as a precursor protein, pre-pro-BDNF, in the endoplasmic reticulum. Following cleavage of the signal peptide, pro-BDNF is converted to mature BDNF, by extracellular proteases, such as matrix metalloproteinase-9 (MMP-9) and plasmin (Lu, 2003; Hwang et al., 2005; Lu et al., 2005; Ethell and Ethell, 2007; Hashimoto, 2007, 2010, 2013). It was initially thought that only secreted mature BDNF was biologically active, and that pro-BDNF, which localizes intracellularly, served as an inactive precursor. However, new evidence shows that pro-BDNF and mature BDNF elicit opposing effects via the p75NTR and TrkB

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receptors, respectively, and that both pro- and mature BDNF play important roles in several physiological functions (Lu, 2003; Lu et al., 2005; Hashimoto, 2007, 2010, 2013). Considering the physiological importance of both proteins, it would be informative to measure individual levels of pro-BDNF and mature BDNF in human body fluids (Hashimoto, 2010, 2012, 2013). A previous study reported increased serum levels of mature- and pro-BDNF, and decreased serum levels of truncated BDNF in patients with schizophrenia, as measured by western-blotting (Carlino et al., 2011). Although BDNF levels in human blood can be measured using newer commercially available human BDNF, enzyme-linked immunosorbent assay (ELISA) kits, earlier versions of these kits were unable to distinguish between pro-BDNF and mature BDNF due to the limited specificity of the BDNF antibody (Yoshida et al., 2012a; Yoshida et al., 2012b). It is highly possible that the limited specificity of these ELISA kits has contributed to the heterogeneity of results in previous studies examining blood BDNF levels in schizophrenics.

MMP-9 plays a key role in synaptic plasticity of the brain, and acts by converting pro-BDNF to mature BDNF (Hwang et al., 2005; Ethell and Ethell, 2007). A study using MMP-9 knock-out mice demonstrated that MMP-9 plays a role in the development of pentylenetetrazole-induced kindling, by converting pro-BDNF to mature BDNF in the hippocampus (Mizoguchi et al., 2009). Another study suggested that serum levels of MMP-9 increased in patients with major depressive disorder and schizophrenia (Domenici et al., 2010). Therefore, it is plausible that serum levels of both mature BDNF and MMP-9 could play roles in the pathophysiology of schizophrenia.

Considering the evidence presented above, we hypothesized that in patients with schizophrenia, serum levels of mature BDNF and MMP-9 would be higher than those of the healthy controls. In this study, we examined serum levels of mature BDNF and MMP-9 in patients with chronic schizophrenia, and their association with demographic and clinical variables, including cognition.

2. Methods

2.1. Study design

The ethics committee of Chiba University Graduate School of Medicine approved the present study. All subjects provided written informed consent for participation in the study, after the procedure had been fully explained. This study is an exploratory, cross-sectional, and case-control design.

2.2. Participants

Sixty-three Japanese patients with schizophrenia (DSM-IV) were recruited from the outpatient departments of Chiba University Hospital and its affiliated hospitals, in Chiba, Japan. Fifty-two age- and sex-matched healthy Japanese subjects were recruited as healthy controls. Entry criteria of participants are described in detail elsewhere, and this study used the same sample as our previous study (Niitsu et al., 2011).

2.3. Clinical assessments

Clinical symptoms were assessed using the Brief Psychiatry Rating Scale (BPRS) and the Scale for the Assessment of Negative Symptoms (SANS). Drug-induced extrapyramidal symptoms were evaluated using the Drug Induced Extrapyramidal Symptoms Scale (DIEPSS). Intelligence quotient (IQ) scores were estimated using the short version of the Japanese Wechsler Adult Intelligence Scale Revised (WAIS-R), which consisted of the Information, Digit Span, and Picture Completion subtests. Age at onset, duration of illness, duration of untreated psychosis and smoking status were evaluated.

2.4. Cognitive assessments

Cognitive assessments of participants were performed by neuropsychological tests. Details of cognitive assessments and results are available elsewhere (Niitsu et al., 2011). Briefly, participants were assessed using the Verbal Fluency Test (letter, category) (Sumiyoshi et al., 2005), the Wisconsin Card Sorting Test (WCST, Keio version) (the number of achieved categories and perseverative errors) (Igarashi et al., 2002; Hori et al., 2006), the Trail Making Test (Part A and Part B), and the Stroop Test (Part D, a list of 24 colored dots; Part C, 24 words naming a color, written in an incongruent color) (Carter et al., 1995; Chan et al., 2004).

2.5. Measurement of mature BDNF and MMP-9 levels from serum

Serum samples of participants were collected between 10:00 and 13:00 h and stored at −80 °C until assayed. Levels of mature BDNF and MMP-9 were measured using a human BDNF ELISA Kit (Adipo Bioscience, Santa Clara, CA, USA) and a human MMP-9 ELISA Kit (R&D Systems, Minneapolis, MN, USA), respectively. To minimize assay variance, serum levels of mature BDNF and MMP-9 from each subject were measured on the same day. All experiments were performed in duplicate. Protocols were performed according to the manufacturer's instructions. The optical density of each well was measured using an automated microplate reader (Emax; Molecular Devices, Sunnyvale, CA, USA).

2.6. Statistical analysis

For the comparisons between groups, the Chi-squared test was employed for categorical variables, and Student's *t*-test for continuous variables. Two-way analysis of variance (ANOVA) was employed to examine the effects of diagnosis and gender on serum levels of mature BDNF and MMP-9. Effects of smoking status stratified by gender were also examined. Bonferroni correction was used for post hoc tests. Associations between serum levels of mature BDNF and MMP-9, and clinical and cognitive variables were tested for, using Pearson's correlation coefficients and stepwise multiple regression analysis. Since serum levels of MMP-9 did not show normal distribution, the logarithm transformation was used for this variable. Statistical analyses were performed in two-sided tests using SPSS, version 18.0 J software (IBM, Tokyo, Japan). The statistical significance was set at $P<0.05$ with power $(1-\beta)=0.80$. ANOVAs with a total of 115 samples and 52 male samples would have enabled us to detect the following effect sizes: $f=0.31$ (medium-to-large) and 0.48 (large).

3. Results

3.1. Demographic data and clinical variables

Characteristics of the participants are shown in Table 1. Gender, age, education and smoking status did not differ between patients and healthy controls. The proportions of smokers between patients and controls differed in the male (Fisher's exact test, $P=0.04$) but not female subgroup ($P>0.05$) (Tables 2 and 3). The correlations of cognitive data with serum levels of mature BDNF

Table 1
Sample characteristics.

	Controls ($n=52$)	Patients ($n=63$)	*P*
Gender (Male/female)	25/27	26/37	NS[a]
Age (years)	34.9 (7.3)	35.9 (8.2)	NS
Education duration (years)	14.7 (2.7)	13.8 (2.3)	NS
Smoking status (No/yes)	43/9	45/18	NS[a]
Estimated IQ	110.2 (12.0)	102.4 (13.9)	<0.01
Age at onset of illness (years)	–	26.8 (7.0)	–
Duration of illness (years)	–	9.1 (7.3)	–
DUP (months)	–	8.1 (13.4)	–
BPRS	–	25.5 (7.5)	–
SANS	–	70.4 (11.8)	–
DIEPSS	–	2.7 (2.7)	–
Antipsychotic dose (mg/day) [#]	–	323.9 (184.2)	–
Mature BDNF (ng/ml)	28. 10 (7.18)	29.79 (6.09)	NS
MMP-9 (ng/ml)	672.49 (378.36)	700.92 (330.81)	NS

Values represent mean (S.D.). NS, not significant.
Abbreviations: DUP, Duration of Untreated Psychosis; BPRS, Brief Psychiatric Rating Scale; SANS, Scale for the Assessment of Negative Symptoms; and DIEPSS, Drug Induced Extra-Pyramidal Symptoms Scale.
[a] χ^2 test. Other *p*-values are calculated by Student's *t*-test.
[#] Chlorpromazine equivalent dose ($n=60$).

Table 2
Serum levels of mature BDNF (ng/ml).

	Controls ($n=52$)		Patients ($n=63$)		Diagnosis		Gender		Diagnosis × gender	
		n		*n*	*F*	*P*	*F*	*P*	*F*	*P*
Gender					2.09	NS	1.17	NS	0.03	NS
Male	28.70 (7.97)	25	30.70 (6.61)	26						
Female	27.54 (6.46)	27	29.16 (5.71)	37						
							Smoking		**Diagnosis × smoking**	
							F	*P*	*F*	*P*
Total					3.48	NS	0.25	NS	1.66	NS
Nonsmokers	28.57 (7.58)	43	29.45 (4.89)	45						
Smokers	25.85 (4.48)	9	30.66 (8.51)	18						
Male ($n=51$)					1.80	NS	0.16	NS	1.45	NS
Nonsmokers	29.44 (8.67)	20	29.77 (4.62)	13						
Smokers	25.70 (3.11)	5	31.64 (8.23)	13						
Female ($n=64$)					0.65	NS	0.46	NS	0.02	NS
Nonsmokers	27.80 (6.58)	23	29.32 (5.06)	32						
Smokers	26.04 (6.37)	4	28.10 (9.67)	5						

Values represent mean (S.D.). NS, not significant. Statistical values are calculated by two-way ANOVA.
Abbreviation: BDNF, Brain-derived neurotrophic factor.

Table 3
Serum levels of MMP-9 (ng/ml).

	Controls ($n=52$)		Patients ($n=63$)		Diagnosis		Gender		Diagnosis × gender	
		n		*n*	*F*	*P*	*F*	*P*	*F*	*P*
Gender					0.37	NS	0.16	NS	0.01	NS
Male	676.5 (451.1)	25	706.3 (369.8)	26						
Female	668.8 (304.9)	27	697.2 (305.7)	37						
							Smoking		**Diagnosis × smoking**	
					F	*P*	*F*	*P*	*F*	*P*
Total ($n=115$)					1.33	NS	2.54	NS	2.14	NS
Nonsmokers	675.5 (400.1)	43	637.2 (305.5)	45						
Smokers	658.2 (267.9)	9	860.3 (346.3)	18						
Male ($n=51$)					0.23	NS	2.39	NS	4.60	0.037 [a]
Nonsmokers	688.8 (479.3)	20	527.9 (290.3)	13						
Smokers	627.5 (354.7)	5	884.6 (362.9)	13						
Female ($n=64$)					0.18	NS	0.92	NS	0.001	NS
Nonsmokers	664.0 (327.0)	23	681.5 (304.6)	32						
Smokers	696.4 (142.2)	4	797.3 (328.5)	5						

Values represent mean (S.D.). NS, not significant. Statistical values are calculated by two-way ANOVA with log-transformed serum levels of MMP-9.
Abbreviation: MMP-9, Matrix Metalloproteinase-9.
[a] Post hoc test revealed a significant difference between smokers and non-smokers in male patients with schizophrenia ($P=0.004$).

and MMP-9 are shown in Table 4. Mean estimated IQ of patients with schizophrenia was significantly lower than that of the healthy controls ($P<0.01$). Furthermore, patients with schizophrenia showed significantly lower scores in all neuropsychological tests ($P<0.05$) (Niitsu et al., 2011).

3.2. Serum mature BDNF levels and MMP-9

Serum levels of mature BDNF did not differ between the diagnosis groups (Table 1). Two-way ANOVA on serum levels of mature BDNF showed no significant effects for diagnosis and gender, or for diagnosis and smoking status (Table 2). After stratification by gender, two-way ANOVA on serum levels of mature BDNF showed no significant effects for diagnosis and smoking status, and their interaction (Table 2). In patients with schizophrenia, serum levels of mature BDNF showed no significant differences among the four types of antipsychotic medications: risperidone (31.20 ± 6.81 [mean $\pm$ S.D., ng/mL], $n=25$); olanzapine (28.34 ± 5.76, $n=18$); aripiprazole (30.48 ± 5.46, $n=9$); and quetiapine (27.25 ± 5.43, $n=8$); ($P=0.296$).

Serum levels of MMP-9 did not differ between the diagnosis groups (Table 1). Two-way ANOVA on serum levels of MMP-9 showed no significant effects for diagnosis and gender, or diagnosis and smoking status (Table 3). However, in the male subgroup, after stratification by gender, a significant interaction effect for diagnosis and smoking status was observed in MMP-9 serum levels ($P=0.037$). This effect was not observed in the female subgroup (Table 3). Simple effects analysis revealed that serum levels of MMP-9 in male smoking patients were significantly higher than those in male non-smoking patients ($P=0.004$). In patients with schizophrenia, serum levels of MMP-9 showed no significant differences among the four types of antipsychotic medication:

Table 4
Cognitive data and their correlation coefficients with serum levels of mature BDNF and MMP-9.

	Cognitive data		Correlation coefficients			
	Controls ($n=52$)	Patients ($n=63$)	Mature BDNF		MMP-9 [a]	
			Controls	Patients	Controls	Patients
Estimated IQ	110.2 (12.0)	102.4 (13.9)	−0.167	0.245	−0.108	-0.131
Information	11.1 (2.6)	10.1 (2.7)	−0.245	0.271*	−0.081	−0.123
Digit span	11.7 (2.9)	10.6 (2.9)	−0.029	0.199	−0.087	−0.138
Picture completion	11.0 (1.9)	10.5 (2.2)	0.033	−0.034	0.054	0.066
Letter fluency test (words)	35.2 (9.0)	28.0 (8.9)	−0.102	−0.021	−0.096	−0.170
Category fluency test (words)	49.1 (6.8)	39.9 (6.9)	−0.163	−0.017	−0.017	−0.323**
WCST, accomplished categories (n) [b]	4.9 (1.5)	3.3 (2.2)	−0.154	0.157	0.071	0.080
WCST, perseverative errors (n) [b]	0.9 (1.8)	4.5 (6.7)	0.149	−0.064	−0.120	−0.079
Trail making test A (s)	27.2 (7.7)	33.8 (10.1)	0.081	0.254*	0.141	−0.090
Trail making test B (s)	52.9 (16.0)	80.5 (27.1)	0.001	−0.034	0.083	0.071
Stroop Test part D (s)	12.7 (2.5)	14.2 (2.6)	0.259	0.146	0.200	0.110
Stroop test part C (s)	18.6 (5.3)	22.7 (5.9)	0.078	0.157	0.157	0.182

Cognitive data represent mean (S.D.).
Abbreviations: BDNF, Brain-derived neurotrophic factor; MMP-9, Matrix Metalloproteinase-9; WCST, Wisconsin Card Sorting Test.
[a] Log-transformed serum levels of MMP-9 are used.
[b] Spearman's correlation coefficients are calculated for WCST. The other statistical values represent Pearson's correlation coefficients.
* $P<0.05$.
** $P<0.01$.

risperidone (747.1 ± 318.8 [mean ± S.D., ng/mL], $n=25$); olanzapine (585.3 ± 284.7, $n=18$); aripiprazole (843.3 ± 454.8, $n=9$); and quetiapine (662.6 ± 332.4, $n=8$); ($P=0.172$).

3.3. Association between mature BDNF levels and clinical variables

Regression analysis revealed no association between serum levels of mature BDNF and MMP-9 in the combined samples, healthy controls, or patients with schizophrenia ($P>0.05$). In schizophrenics, serum levels of mature BDNF were positively associated with SANS total scores ($\beta=0.40$, $P=0.001$) and the Information subtest of WAIS-R ($\beta=0.29$, $P=0.014$), suggesting that higher serum levels of mature BDNF were independently associated with more severe negative symptoms and better performance in the Information subtest. The Trail Making Test Part A was excluded from the stepwise regression model. These associations continued to be significant after adjustment for age, gender and education (SANS total score, $\beta=0.41$, $P=0.001$; Information subtest, $\beta=0.31$, $P=0.014$). In healthy controls, there was no significant association between serum levels of mature BDNF and any other variables. In combined samples, serum levels of mature BDNF were positively associated with Stroop D test scores ($\beta=0.23$, $P=0.015$), implying that higher serum levels of mature BDNF were associated with lower performances in the Stroop D test (naming colors of dots). However, this association was not significant after adjustment for diagnosis, age, and gender.

3.4. Association between MMP-9 levels and clinical variables

In combined samples, serum levels of MMP-9 were positively associated with age ($\beta=0.20$, $P=0.029$) and smoking status ($\beta=0.18$, $P=0.049$), indicating that higher serum levels of MMP-9 were independently associated with older age and smoking. These associations continued to be significant after adjustment for diagnosis and gender (age, $\beta=0.20$, $P=0.031$; smoking status, $\beta=0.20$, $P=0.035$). In healthy controls, serum levels of MMP-9 were positively associated with age ($\beta=0.49$, $P<0.001$). In schizophrenics, serum levels of MMP-9 showed positive association with smoking status ($\beta=0.28$, $P=0.031$) and negative association with the Category fluency test ($\beta=-0.25$, $P=0.047$), suggesting that higher serum levels of MMP-9 were independently associated with smoking and lower performance in the Category fluency test. These associations continued to be significant after adjustment for age, gender and education (smoking status, $\beta=0.35$, $P=0.011$; the Category fluency test, $\beta=-0.29$, $P=0.025$).

4. Discussion

We found no differences in serum levels of mature BDNF or MMP-9 between chronically medicated patients with schizophrenia and healthy controls. In the male but not female subgroup, we detected an interaction effect between diagnosis and smoking status for serum levels of MMP-9. Post hoc analysis revealed that serum levels of MMP-9 in male smoking schizophrenics were significantly higher than those in male non-smoking patients.

The mature BDNF ELISA kits used in this study are able to distinguish between the two forms of BDNF and identify mature BDNF from human serum (Yoshida et al., 2012a; Yoshida et al., 2012b). Although a previous study suggested increased mature BDNF levels in patients with schizophrenia, as measured by western-blotting (Carlino et al., 2011), we found no difference between serum mature BDNF levels in patients and healthy controls. This finding is consistent with several previous studies (Shimizu et al., 2003; Huang and Lee, 2006; Mackin et al., 2007; Goto et al., 2011; Niitsu et al., 2011), although in these studies there was no distinction between BDNF isoforms. In addition, serum levels of mature BDNF and MMP-9 remained the same, independent of the atypical antipsychotic treatment. To determine the effects of antipsychotics on serum levels of mature BDNF in schizophrenic patients, further studies using drug-naïve samples are needed.

The influence of smoking on serum BDNF levels remains controversial. A previous study which did not distinguish between BDNF isoforms, showed that serum BDNF levels were significantly higher in male smoking schizophrenic patients compared with male non-smoking patients (Zhang et al., 2010). However, we found no such effects of smoking on mature BDNF serum levels, in total samples or gender specific subsamples. Therefore, additional studies that distinguish between BDNF isoforms will be needed to determine the effects of smoking on serum BDNF levels.

No association between levels of mature BDNF and MMP-9 was found, although MMP-9 plays an important role in the conversion of pro-BDNF to mature BDNF (Hwang et al., 2005; Ethell and

Ethell, 2007). We found that higher serum levels of mature BDNF in schizophrenics were associated with more severe negative symptomology. This finding is consistent with previous studies examining the same sample set (Niitsu et al., 2011) and a completely different sample set (Reis et al., 2008), although these studies did not distinguish between BDNF isoforms. Additionally, we found that higher levels of mature BDNF in patients were associated with a better performance on the Information subtest of WAIS-R, but not on other cognitive tests. This finding may support a recent study which suggested that serum BDNF levels were positively associated with immediate memory performance in chronic patients with schizophrenia (Zhang et al., 2012b), although again, this study did not distinguish between BDNF isoforms. With regards to other proteolytic BDNF isoforms, a previous study suggested that reduced serum levels of truncated-BDNF (a proteolytic BDNF isoform of 28 kDa), but not mature BDNF, correlated with higher positive and lower negative PANSS scores and a poorer performance in all cognitive assays (Carlino et al., 2011). Recently, Carlino et al. proposed a hypothesis stating that deficits in pro and mature BDNF expression may lead to different types of cognitive deficits (Carlino et al., 2013), and our findings go some way to support this hypothesis. Thus, further studies examining the association between the BDNF isoforms and clinical symptoms and cognitive impairment are still needed.

We found no difference in serum MMP-9 levels between patients with schizophrenia and healthy controls. This finding is inconsistent with the results of a proteomic study, using plasma samples from a large case-control collection, which found increased levels of MMP-9 in both male and female patients with schizophrenia (Domenici et al., 2010). It is worth noting however, that this study included a significantly higher percentage of active smokers in the schizophrenia group, compared with controls. In this study, the proportion of male smoking schizophrenics was higher than that of male smoking controls, although this difference was not present in the female subgroup. Interestingly, we found that, in male patients, serum MMP-9 levels in smokers were higher than those of non-smokers, while this was not the case in male controls. It is likely therefore that MMP-9 serum levels in male patients with schizophrenia are affected by smoking. In females, the discrepancy in MMP-9 levels between our results and a previous study (Domenici et al., 2010) may be attributed to the difference in the proportion of smokers between the two studies. In summary, we found that serum MMP-9 levels were associated with age in healthy controls, and smoking status and performance of the Category fluency test in patients with schizophrenia. While the role of serum MMP-9 in the pathophysiology of schizophrenia is currently unclear, further studies are needed to confirm its association with these factors.

As with similar studies, this study has a number of limitations, the most prominent being the small sample size. It is clear that future studies will need larger cohort numbers. Next, the proportion of smokers in the male subgroup differed between patients and controls. This limitation may produce biases in our observations. Finally, we did not examine the levels of pro-BDNF and truncated-BDNF. It is possible that serum levels of BDNF isoforms play a role in the pathophysiology of schizophrenia (Carlino et al., 2011), and therefore it would be of great interest to study the relationship between serum levels of BDNF isoforms, as well as levels of extracellular peptidases (e.g., MMP-9, plasmin) that convert pro-BDNF to other BDNF isoforms.

In conclusion, we found that serum levels of mature BDNF and MMP-9 showed no difference between patients with schizophrenia and controls. However, in male patients only, serum MMP-9 levels of smokers were higher than those of non-smokers. We also found that in patients with schizophrenia, serum mature BDNF levels were associated with negative symptoms and Information subscale scores of WAIS-R, while serum MMP-9 levels were associated with smoking status and Category fluency performance. Further studies measuring the serum levels of mature BDNF and MMP-9 using larger cohorts are needed to examine the results of this study.

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Competing interests

The authors declare that they have no competing interests.

Author contributions

Conception and design: TN, DM, YS, KH, MI. Serum sampling and assay: TI, TY, TH. Analysis and interpretation of data: TN, KH. Drafting the article: TN. Critical review: TY, DM, YS, MN, ES, KH, MI. Final approval of the article: TN, TI, TY, TH, DM, YS, MN, ES, KH, MI.

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References

Autry, A.E., Monteggia, L.M., 2012. Brain-derived neurotrophic factor and neuropsychiatric disorders. Pharmacological Reviews 64, 238–258.

Carlino, D., De Vanna, M., Tongiorgi, E., 2013. Is altered BDNF biosynthesis a general feature in patients with cognitive dysfunctions? The Neuroscientist 19, 345–353.

Carlino, D., Leone, E., Di Cola, F., Baj, G., Marin, R., Dinelli, G., Tongiorgi, E., De Vanna, M., 2011. Low serum truncated-BDNF isoform correlates with higher cognitive impairment in schizophrenia. Journal of Psychiatric Research 45, 273–279.

Carter, C.S., Mintun, M., Cohen, J.D., 1995. Interference and facilitation effects during selective attention: an H215O PET study of Stroop task performance. NeuroImage 2, 264–272.

Chan, M.W., Yip, J.T., Lee, T.M., 2004. Differential impairment on measures of attention in patients with paranoid and nonparanoid schizophrenia. Journal of Psychiatric Research 38, 145–152.

Chen da, C., Wang, J., Wang, B., Yang, S.C., Zhang, C.X., Zheng, Y.L., Li, Y.L., Wang, N., Yang, K.B., Xiu, M.H., Kosten, T.R., Zhang, X.Y, 2009. Decreased levels of serum brain-derived neurotrophic factor in drug-naive first-episode schizophrenia: relationship to clinical phenotypes. Psychopharmacology (Berl) 207, 375–380.

Domenici, E., Wille, D.R., Tozzi, F., Prokopenko, I., Miller, S., McKeown, A., Brittain, C., Rujescu, D., Giegling, I., Turck, C.W., Holsboer, F., Bullmore, E.T., Middleton, L., Merlo-Pich, E., Alexander, R.C., Muglia, P., 2010. Plasma protein biomarkers for depression and schizophrenia by multi analyte profiling of case-control collections. PLoS ONE 5, e9166.

Ethell, I.M., Ethell, D.W., 2007. Matrix metalloproteinases in brain development and remodeling: synaptic functions and targets. Journal of Neuroscience Research 85, 2813–2823.

Favalli, G., Li, J., Belmonte-de-Abreu, P., Wong, A.H., Daskalakis, Z.J., 2012. The role of BDNF in the pathophysiology and treatment of schizophrenia. Journal of Psychiatric Research 46, 1–11.

Fernandes, B.S., Massuda, R., Torres, M., Camargo, D., Fries, G.R., Gama, C.S., Belmonte-de-Abreu, P.S., Kapczinski, F., Lobato, M.I., 2010. Improvement of schizophrenia with electroconvulsive therapy and serum brain-derived

neurotrophic factor levels: lack of association in a pilot study. Psychiatry and Clinical Neurosciences 64, 663–665.
Goto, N., Yoshimura, R., Kakeda, S., Moriya, J., Hayashi, K., Ikenouchi-Sugita, A., Umene-Nakano, W., Hori, H., Ueda, N., Korogi, Y., Nakamura, J., 2011. Comparison of brain N-acetylaspartate levels and serum brain-derived neurotrophic factor (BDNF) levels between patients with first-episode schizophrenia psychosis and healthy controls. European Psychiatry 26, 57–63.
Green, M.J., Matheson, S.L., Shepherd, A., Weickert, C.S., Carr, V.J., 2011. Brain-derived neurotrophic factor levels in schizophrenia: a systematic review with meta-analysis. Molecular Psychiatry 16, 960–972.
Hashimoto, K., 2007. BDNF variant linked to anxiety-related behaviors. BioEssays 29, 116–119.
Hashimoto, K., 2010. Brain-derived neurotrophic factor as a biomarker for mood disorders: an historical overview and future directions. Psychiatry and Clinical Neurosciences 64, 341–357.
Hashimoto, K., 2012. A BDNF Val66Met polymorphism and ketamine-induced rapid antidepressant action. Clinical Psychopharmacology and Neuroscience 10, 59–60.
Hashimoto, K., 2013. Sigma-1 receptor chaperone and brain-derived neurotrophic factor: emerging links between cardiovascular disease and depression. Progress in Neurobiology 100, 15–29.
Hori, H., Noguchi, H., Hashimoto, R., Nakabayashi, T., Omori, M., Takahashi, S., Tsukue, R., Anami, K., Hirabayashi, N., Harada, S., Saitoh, O., Iwase, M., Kajimoto, O., Takeda, M., Okabe, S., Kunugi, H., 2006. Antipsychotic medication and cognitive function in schizophrenia. Schizophrenia Research 86, 138–146.
Huang, T.L., Lee, C.T., 2006. Associations between serum brain-derived neurotrophic factor levels and clinical phenotypes in schizophrenia patients. Journal of Psychiatric Research 40, 664–668.
Hwang, J.J., Park, M.H., Choi, S.Y., Koh, J.Y., 2005. Activation of the Trk signaling pathway by extracellular zinc. Role of metalloproteinases. The Journal of Biological Chemistry 280, 11995–12001.
Igarashi, K., Oguni, H., Osawa, M., Awaya, Y., Kato, M., Mimura, M., Kashima, H., 2002. Wisconsin card sorting test in children with temporal lobe epilepsy. Brain and Development 24, 174–178.
Lee, A.H., Lange, C., Ricken, R., Hellweg, R., Lang, U.E., 2011. Reduced brain-derived neurotrophic factor serum concentrations in acute schizophrenic patients increase during antipsychotic treatment. Journal of Clinical Psychopharmacology 31, 334–336.
Lu, B., 2003. Pro-region of neurotrophins: role in synaptic modulation. Neuron 39, 735–738.
Lu, B., Pang, P.T., Woo, N.H., 2005. The yin and yang of neurotrophin action. Nature Reviews Neuroscience 6, 603–614.
Mackin, P., Gallagher, P., Watson, S., Young, A.H., Ferrier, I.N., 2007. Changes in brain-derived neurotrophic factor following treatment with mifepristone in bipolar disorder and schizophrenia. The Australian and New Zealand Journal of Psychiatry 41, 321–326.
Martinotti, G., Di Iorio, G., Marini, S., Ricci, V., De Berardis, D., Di Giannantonio, M., 2012. Nerve growth factor and brain-derived neurotrophic factor concentrations in schizophrenia: a review. Journal of Biological Regulators and Homeostatic Agents 26, 347–356.
Mizoguchi, Y., Monji, A., Kato, T., Seki, Y., Gotoh, L., Horikawa, H., Suzuki, S.O., Iwaki, T., Yonaha, M., Hashioka, S., Kanba, S., 2009. Brain-derived neurotrophic factor induces sustained elevation of intracellular Ca^{2+} in rodent microglia. Journal of Immunology 183, 7778–7786.
Niitsu, T., Shirayama, Y., Matsuzawa, D., Hasegawa, T., Kanahara, N., Hashimoto, T., Shiraishi, T., Shiina, A., Fukami, G., Fujisaki, M., Watanabe, H., Nakazato, M., Asano, M., Kimura, S., Hashimoto, K., Iyo, M., 2011. Associations of serum brain-derived neurotrophic factor with cognitive impairments and negative symptoms in schizophrenia. Progress in Neuro-Psychopharmacology and Biological Psychiatry 35, 1836–1840.
Nurjono, M., Lee, J., Chong, S.A., 2012. A review of brain-derived neurotrophic factor as a candidate biomarker in schizophrenia. Clinical Psychopharmacology and Neuroscience 10, 61–70.
Pillai, A., Kale, A., Joshi, S., Naphade, N., Raju, M.S., Nasrallah, H., Mahadik, S.P., 2010. Decreased BDNF levels in CSF of drug-naive first-episode psychotic subjects: correlation with plasma BDNF and psychopathology. The International Journal of Neuropsychopharmacology 13, 535–539.
Reis, H.J., Nicolato, R., Barbosa, I.G., Teixeira do Prado, P.H., Romano-Silva, M.A., Teixeira, A.L., 2008. Increased serum levels of brain-derived neurotrophic factor in chronic institutionalized patients with schizophrenia. Neuroscience Letters 439, 157–159.
Rizos, E.N., Michalopoulou, P.G., Siafakas, N., Stefanis, N., Douzenis, A., Rontos, I., Laskos, E., Kastania, A., Zoumpourlis, V., Lykouras, L., 2010a. Association of serum brain-derived neurotrophic factor and duration of untreated psychosis in first-episode patients with schizophrenia. Neuropsychobiology 62, 87–90.
Rizos, E.N., Papadopoulou, A., Laskos, E., Michalopoulou, P.G., Kastania, A., Vasilopoulos, D., Katsafouros, K., Lykouras, L., 2010b. Reduced serum BDNF levels in patients with chronic schizophrenic disorder in relapse, who were treated with typical or atypical antipsychotics. The World Journal of Biological Psychiatry 11, 251–255.
Rizos, E.N., Papathanasiou, M., Michalopoulou, P.G., Mazioti, A., Douzenis, A., Kastania, A., Nikolaidou, P., Laskos, E., Vasilopoulou, K., Lykouras, L., 2011. Association of serum BDNF levels with hippocampal volumes in first psychotic episode drug-naive schizophrenic patients. Schizophrenia Research 129, 201–204.
Shimizu, E., Hashimoto, K., Watanabe, H., Komatsu, N., Okamura, N., Koike, K., Shinoda, N., Nakazato, M., Kumakiri, C., Okada, S., Iyo, M., 2003. Serum brain-derived neurotrophic factor (BDNF) levels in schizophrenia are indistinguishable from controls. Neuroscience Letters 351, 111–114.
Sumiyoshi, C., Sumiyoshi, T., Nohara, S., Yamashita, I., Matsui, M., Kurachi, M., Niwa, S., 2005. Disorganization of semantic memory underlies alogia in schizophrenia: an analysis of verbal fluency performance in Japanese subjects. Schizophrenia Research 74, 91–100.
Yang, Y.Q., Sun, S., Yu, Y.Q., Li, W.J., Zhang, X., Xiu, M.H., Chen da, C., De Yang, F., Liu, H., Li, C., Kosten, T.R., Zhang, X.Y., 2011. Decreased serum brain-derived neurotrophic factor levels in schizophrenic patients with tardive dyskinesia. Neuroscience Letters 502, 37–40.
Yoshida, T., Ishikawa, M., Iyo, M., Hashimoto, K., 2012a. Serum levels of mature Brain-Derived Neurotrophic Factor (BDNF) and its precursor proBDNF in healthy subjets. The Open Clinical Chemistry Journal 5, 7–12.
Yoshida, T., Ishikawa, M., Niitsu, T., Nakazato, M., Watanabe, H., Shiraishi, T., Shiina, A., Hashimoto, T., Kanahara, N., Hasegawa, T., Enohara, M., Kimura, A., Iyo, M., Hashimoto, K., 2012b. Decreased serum levels of mature brain-derived neurotrophic factor (BDNF), but not its precursor proBDNF, in patients with major depressive disorder. PLoS ONE 7, e42676.
Zhang, X.Y., Chen da, C., Xiu, M.H., Haile, C.N., Luo, X., Xu, K., Zhang, H.P., Zuo, L., Zhang, Z., Zhang, X., Kosten, T.A., Kosten, T.R., 2012a. Cognitive and serum BDNF correlates of BDNF Val66Met gene polymorphism in patients with schizophrenia and normal controls. Human Genetics 131, 1187–1195.
Zhang, X.Y., Liang, J., Chen da, C., Xiu, M.H., Yang, F.D., Kosten, T.A., Kosten, T.R., 2012b. Low BDNF is associated with cognitive impairment in chronic patients with schizophrenia. Psychopharmacology (Berl) 222, 277–284.
Zhang, X.Y., Xiu, M.H., Chen da, C., Yang, F.D., Wu, G.Y., Lu, L., Kosten, T.A., Kosten, T. R., 2010. Nicotine dependence and serum BDNF levels in male patients with schizophrenia. Psychopharmacology (Berl) 212, 301–307.
Zhang, X.Y., Zhang, W.F., Zhou, D.F., Chen da, C., Xiu, M.H., Wu, H.R., Haile, C.N., Kosten, T.A., Kosten, T.R., 2012c. Brain-derived neurotrophic factor levels and its Val66Met gene polymorphism predict tardive dyskinesia treatment response to Ginkgo biloba. Biological Psychiatry 72, 700–706.

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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

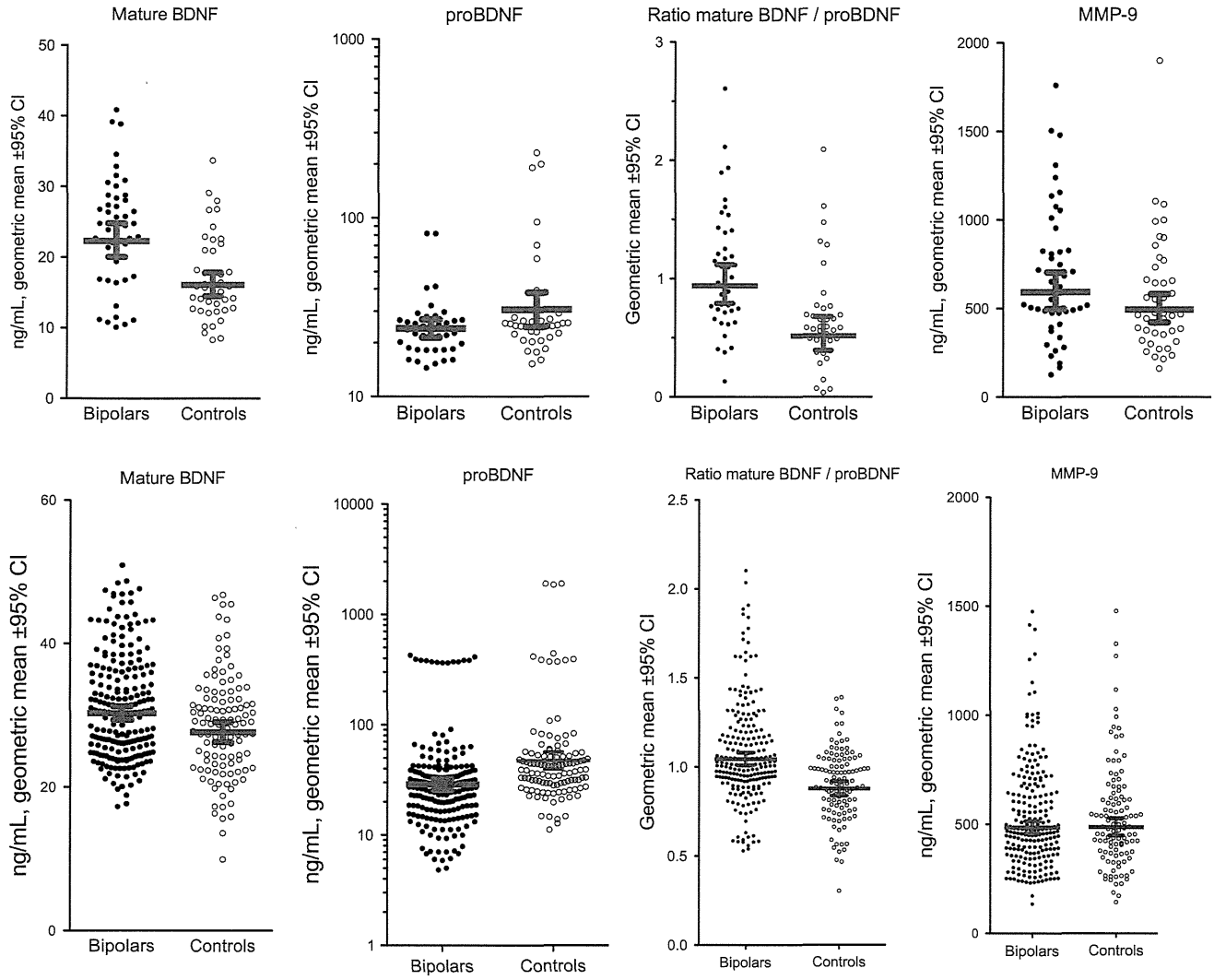

Fig. 1. Distributions and compared geometric means of serum measures.

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.

References

Altar, C.A., 1999. Neurotrophins and depression. Trends Pharmacol. Sci. 20, 59–62.
Angst, F., Angst, J., Stassen, H.H., Clayton, P.J., 2002. Mortality of patients with mood disorders: follow-up over 34–38 years. J. Affective Dis. 68, 167–181.
Bath, K.G., Akins, M.R., Lee, F.S., 2012. BDNF control of adult SVZ neurogenesis. Dev. Psychobiol. 54, 578–589.
Bekinschtein, P., Oomen, C.A., Saksida, L.M., Bussey, T.J., 2011. Effects of environmental enrichment and voluntary exercise on neurogenesis, learning and memory, and pattern separation: BDNF as a critical variable? Semin. Cell Dev. Biol. 22, 536–542.
Belmaker, R.H., 2004. Medical progress: bipolar disorder. N. Engl. J. Med. 351, 476–486.
Cassidy, C.M., Schmitz, N., Malla, A., 2008. Validation of the alcohol use disorders identification test and the drug abuse screening test in first episode psychosis. Can. J. Psychiatry 53, 26–33.
Conigrave, K.M., Hall, W.D., Saunders, J.B., 1995. The AUDIT questionnaire: choosing a cut-off score. Alcohol use disorder identification test. Addiction 90, 1349–1356.
Cunha, A.B., Frey, B.N., Andreazza, A.C., Goi, J.D., Rosa, A.R., Goncalves, C.A., Santin, A., Kapczinski, F., 2006. Serum brain-derived neurotrophic factor is decreased in bipolar disorder during depressive and manic episodes. Neurosci. Lett. 398, 215–219.
de Oliveira, G.S., Cereser, K.M., Fernandes, B.S., Kauer-Sant'Anna, M., Fries, G.R., Stertz, L., Aguiar, B., Pfaffenseller, B., Kapczinski, F., 2009. Decreased brain-derived neurotrophic factor in medicated and drug-free bipolar patients. J. Psychiatric Res. 43, 1171–1174.
Dias, V.V., Brissos, S., Frey, B.N., Andreazza, A.C., Cardoso, C., Kapczinski, F., 2009. Cognitive function and serum levels of brain-derived neurotrophic factor in patients with bipolar disorder. Bipolar Disorders 11, 663–671.
Dieni, S., Frotscher, M., Barde, Y.-A., Matsumoto, T., Dekkers, M., Rauskolb, S., Ionescu, M.S., Deogracias, R., Gundelfinger, E.D., Kojima, M., Nestel, S., 2012. BDNF and its pro-peptide are stored in presynaptic dense core vesicles in brain neurons. J. Cell Biol. 196, 775–788.
Duman, R.S., Heninger, G.R., Nestler, E.J., 1997. A molecular and cellular theory of depression. Arch. Gen. Psychiatry 54, 597–606.
Ekman, C.J., Lind, J., Rydén, E., Ingvar, M., Landén, M., 2010. Manic episodes are associated with grey matter volume reduction—a voxel-based morphometry brain analysis. Acta Psychiatrica Scand. 122, 507–515.
Ernfors, P., Wetmore, C., Olson, L., Persson, H., 1990. Identification of cells in rat brain and peripheral tissues expressing mRNA for members of the nerve growth factor family. Neuron 5, 511–526.
Ethell, I.M., Ethell, D.W., 2007. Matrix metalloproteinases in brain development and remodeling: synaptic functions and targets. J. Neurosci. Res. 85, 2813–2823.
Fernandes, B.S., Kapczinski, F., Gama, C.S., Maria Ceresér, K., Yatham, L.N., Fries, G.R., Colpo, G., de Lucena, D., Kunz, M., Gomes, F.A., 2011. Brain-derived neurotrophic factor as a state-marker of mood episodes in bipolar disorders: a systematic review and meta-regression analysis. J. Psychiatric Res. 45, 995–1004.
Folstein, M.F., Folstein, S.E., McHugh, P.R., 1975. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J. Psychiatric Res. 12, 189–198.
Goldstein, T.R., Keller, M., Birmaher, B., Axelson, D., Goldstein, B.I., Gill, M.K., Esposito-Smythers, C., Ryan, N.D., Strober, M.A., Hunt, J., 2009. Psychosocial functioning among bipolar youth. J. Affective Dis. 114, 174–183.

Guy, W., 1976. Early Clinical Drug Evaluation (ECDEU) Assessment Manual for PsychopharmacologyDepartment of Health, Education and Welfare
Hall, R.C., 1995. Global assessment of functioning. A modified scale. Psychosomatics 36, 267–275.
Hashimoto, K., 2007. BDNF variant linked to anxiety-related behaviors. Bioessays 29, 116–119.
Hashimoto, K., 2010. Brain-derived neurotrophic factor as a biomarker for mood disorders: an historical overview and future directions: BDNF as a biomarker of mood disorders. Psychiatry Clin. Neurosci. 64, 341–357.
Hashimoto, K., 2013. Sigma-1 receptor chaperone and brain-derived neurotrophic factor: emerging links between cardiovascular disease and depression. Prog. Neurobiol. 100, 15–29.
Hashimoto, K., Shimizu, E., Iyo, M., 2004. Critical role of brain-derived neurotrophic factor in mood disorders. Brain Res. Rev. 45, 104–114.
Huang, T.L., Hung, Y.Y., Lee, C.T., Chen, R.F., 2012. Serum protein levels of brain-derived neurotrophic factor and tropomyosin-related kinase B in bipolar disorder: effects of mood stabilizers. Neuropsychobiology 65, 65–69.
Kauer-Sant'Anna, M., Kapczinski, F., Andreazza, A.C., Bond, D.J., Lam, R.W., Young, L.T., Yatham, L.N., 2009. Brain-derived neurotrophic factor and inflammatory markers in patients with early- *vs.* late-stage bipolar disorder. Int. J. Neuropsychopharmacol. 12, 447–458.
Lin, P.Y., 2009. State-dependent decrease in levels of brain-derived neurotrophic factor in bipolar disorder: a meta-analytic study. Neurosci. Lett. 466, 139–143.
Luborsky, L., 1962. Clinician's judgments of mental health. Arch. Gen. Psychiatry 7, 407–417.
Machado-Vieira, R., Dietrich, M.O., Leke, R., Cereser, V.H., Zanatto, V., Kapczinski, F., Souza, D.O., Portela, L.V., Gentil, V., 2007. Decreased plasma brain derived neurotrophic factor levels in unmedicated bipolar patients during manic episode. Biol. Psychiatry 61, 142–144.
Mackin, P., Gallagher, P., Watson, S., Young, A.H., Ferrier, I.N., 2007. Changes in brain-derived neurotrophic factor following treatment with mifepristone in bipolar disorder and schizophrenia. Aust. N. Z. J. Psychiatry 41, 321–326.
Marlatt, M.W., Potter, M.C., Lucassen, P.J., van Praag, H., 2012. Running throughout middle-age improves memory function, hippocampal neurogenesis, and BDNF levels in female C57BL/6J mice. Dev. Neurobiol. 72, 943–952.
Martinowich, K., Manji, H., Lu, B., 2007. New insights into BDNF function in depression and anxiety. Nat. Neurosci. 10, 1089–1093.
Monteleone, P., Serritella, C., Martiadis, V., Maj, M., 2008. Decreased levels of serum brain-derived neurotrophic factor in both depressed and euthymic patients with unipolar depression and in euthymic patients with bipolar I and II disorders. Bipolar Disorders 10, 95–100.
Montgomery, S.A., Åsberg, M., 1979. A new depression scale designed to be sensitive to change. Br. J. Psychiatry 134, 322–389.
Nestler, E.J., Barrot, M., DiLeone, R.J., Eisch, A.J., Gold, S.J., Monteggia, L.M., 2002. Neurobiology of depression. Neuron 34, 13–25.
Palomino, A., Vallejo-Illarramendi, A., Gonzalez-Pinto, A., Aldama, A., Gonzalez-Gomez, C., Mosquera, F., Gonzalez-Garcia, G., Matute, C., 2006. Decreased levels of plasma BDNF in first-episode schizophrenia and bipolar disorder patients. Schizophrenia Res. 86, 321–322.
Pan, W., Banks, W.A., Fasold, M.B., Bluth, J., Kastin, A.J., 1998. Transport of brain-derived neurotrophic factor across the blood–brain barrier. Neuropharmacology 37, 1553–1561.
Pang, P.T., Woo, N.H., 2005. The yin and yang of neurotrophin action. Nat. Rev. Neurosci. 6, 603–614.
Park, H., Poo, M.M., 2013. Neurotrophin regulation of neural circuit development and function. Nat. Rev. Neurosci. 14, 7–23.
Peng, Q., Masuda, N., Jiang, M., Li, Q., Zhao, M., Ross, C.A., Duan, W., 2008. The antidepressant sertraline improves the phenotype, promotes neurogenesis and increases BDNF levels in the R6/2 Huntington's disease mouse model. Exp. Neurol. 210, 154–163.
Poo, M.-m., 2001. Neurotrophins as synaptic modulators. Nat. Rev. Neurosci. 2, 24–32.
Rossi, C., Angelucci, A., Costantin, L., Braschi, C., Mazzantini, M., Babbini, F., Fabbri, M.E., Tessarollo, L., Maffei, L., Berardi, N., Caleo, M., 2006. Brain-derived neurotrophic factor (BDNF) is required for the enhancement of hippocampal neurogenesis following environmental enrichment. Eur. J. Neurosci. 24, 1850–1856.
Rydén, E., Johansson, C., Blennow, K., Landén, M., 2009. Lower CSF HVA and 5-HIAA in bipolar disorder type 1 with a history of childhood ADHD. J. Neural Transm. 116, 1667–1674.
Sachs, G.S., Thase, M.E., Otto, M.W., Bauer, M., Miklowitz, D., Wisniewski, S.R., Lavori, P., Lebowitz, B., Rudorfer, M., Frank, E., Nierenberg, A.A., Fava, M., Bowden, C., Ketter, T., Marangell, L., Calabrese, J., Kupfer, D., Rosenbaum, J.F., 2003. Rationale, design, and methods of the systematic treatment enhancement program for bipolar disorder (STEP-BD). Biol. Psychiatry 53, 1028–1042.
Schmidt, H.D., Duman, R.S., 2010. Peripheral BDNF produces antidepressant-like effects in cellular and behavioral models. Neuropsychopharmacology 35, 2378–2391.
Sheehan, D.V., Lecrubier, Y., Sheehan, K.H., Amorim, P., Janavs, J., Weiller, E., Hergueta, T., Baker, R., Dunbar, G.C., 1998. The mini-international neuropsychiatric interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. J. Clin. Psychiatry 59 (Suppl 20), 22–33 (quiz 34–57).
Spitzer, R.L., 1983. Psychiatric diagnosis: are clinicians still necessary? Compr. Psychiatry 24, 399–411.
Tohen, M., Cohen, B.M., Hennen, J., Zarate, J.C.M., Baldessarini, R.J., Strakowski, S.M., Stoll, A.L., Faedda, G.L., Suppes, T., Gebre-Medhin, P., 2000. Two-year syndromal and functional recovery in 219 cases of first-episode major affective disorder with psychotic features. Am. J. Psychiatry 157, 220–228.
Yoshida, T., Ishikawa, M., Iyo, M., Hashimoto, K., 2012a. Serum levels of mature brain-derived neurotrophic factor (BDNF) and its precursor proBDNF in healthy subjects. Open Clin. Chem. J. 5, 7–12.
Yoshida, T., Ishikawa, M., Niitsu, T., Nakazato, M., Watanabe, H., Shiraishi, T., Shiina, A., Hashimoto, T., Kanahara, N., Hasegawa, T., Enohara, M., Kimura, A., Iyo, M., Hashimoto, K., 2012b. Decreased serum levels of mature brain-derived neurotrophic factor (BDNF), but not its precursor proBDNF, in patients with major depressive disorder. PLoS One 7, e42676.
Yoshimura, R., Nakano, Y., Hori, H., Ikenouchi, A., Ueda, N., Nakamura, J., 2006. Effect of risperidone on plasma catecholamine metabolites and brain-derived neurotrophic factor in patients with bipolar disorders. Hum. Psychopharmacol. 21, 433–438.
Young, R.C., Biggs, J.T., Ziegler, V.E., Meyer, D.A., 1978. A rating scale for mania: reliability, validity and sensitivity. Br. J. Psychiatry 133, 429–435.

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Plasma levels of mature brain-derived neurotrophic factor (BDNF) and matrix metalloproteinase-9 (MMP-9) in treatment-resistant schizophrenia treated with clozapine☆

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HIGHLIGHTS

- Plasma levels of mature BDNF in schizophrenia were measured for the first time.
- No significant difference was observed in mature BDNF levels in schizophrenia.
- MMP-9 plasma levels were significantly increased in patients with schizophrenia.
- Plasma mature BDNF levels were significantly correlated with plasma MMP-9 levels.

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ABSTRACT

Brain-derived neurotrophic factor (BDNF) regulates the survival and growth of neurons, and influences synaptic efficiency and plasticity. Peripheral BDNF levels in patients with schizophrenia have been widely reported in the literature. However, it is still controversial whether peripheral levels of BDNF are altered in patients with schizophrenia. The peripheral BDNF levels previously reported in patients with schizophrenia were total BDNF (proBDNF and mature BDNF) as it was unable to specifically measure mature BDNF due to limited BDNF antibody specificity. In this study, we examined whether peripheral levels of mature BDNF were altered in patients with treatment-resistant schizophrenia. Matrix metalloproteinase-9 (MMP-9) levels were also measured, as MMP-9 plays a role in the conversion of proBDNF to mature BDNF. Twenty-two patients with treatment-resistant schizophrenia treated with clozapine and 22 age- and sex-matched healthy controls were enrolled. The plasma levels of mature BDNF and MMP-9 were measured using ELISA kits. No significant difference was observed for mature BDNF however, MMP-9 was significantly increased in patients with schizophrenia. The significant correlation was observed between mature BDNF and MMP-9 plasma levels. Neither mature BDNF nor MMP-9 plasma levels were associated clinical variables. Our results do not support the view that peripheral BDNF levels are associated with schizophrenia. MMP-9 may play a role in the pathophysiology of schizophrenia and serve as a biomarker for schizophrenia.

Abbreviations: BDNF, brain-derived neurotrophic factor; MMP-9, matrix metalloproteinase-9; MDD, major depressive disorder; DSM-IV, diagnostic and statistical manual of mental disorders, fourth edition; PANSS, positive and negative syndrome scale.

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1. Introduction

Schizophrenia is a severe psychiatric disease characterized by delusions, hallucinations, impairment of cognitive function and incoherent behavior. It affects approximately 1% of the general population worldwide. Mounting evidence suggests that a deficit in neurotrophin supply to cortical neurons may be an underlying factor in the pathophysiology of schizophrenia as adequate neurotrophic support is required for normal brain development, maturation and function [3,4].

Brain-derived neurotrophic factor (BDNF) is a neurotrophin that regulates neuronal survival, differentiation and growth during brain development, with important effects on neurogenesis and neuroplasticity. It is also important for hippocampal-related learning and memory [17]. A common single nucleotide polymorphism (SNP) of the BDNF gene has impact on episodic memory, hippocampal morphology and memory-related hippocampal activity in human [9,16]. Mature BDNF is initially synthesized as a precursor protein, preproBDNF. Following cleavage of the signal peptide, proBDNF is converted to mature BDNF by extracellular proteases, such as matrix metalloproteinase-9 (MMP-9). Mature BDNF and poBDNF each plays important roles in several physiological functions. Recent studies show that mature BDNF and pro BDNF elicit opposing effects via the TrkB and $p75^{NTR}$ receptors respectively. Mature BDNF preferentially binds to the TrkB receptor and plays an important role through BDNF-TrkB signaling which fulfills wide variety of functions such as cell survival, migration, outgrowth of neuritis and synaptic plasticity. In contrast, pro BDNF preferentially binds to the $p75^{NTR}$ receptors and elicit apoptosis rather than cell survival [8,11]. Considering the important roles of mature BDNF, it would be informative to specifically measure mature BDNF. Although BDNF levels in human blood can be measured using commercially available human BDNF ELISA kits, due to the limited specificity of the BDNF antibody, it has not been possible to distinguish between proBDNF and mature BDNF. Recently, peripheral levels of mature BDNF have been reported to be measurable using newly available human BDNF ELISA kits [23].

It is of great interest to assess the potential contribution of BDNF to the pathophysiology of schizophrenia. Several studies report altered BDNF mRNA and protein in prefrontal cortical regions and hippocampus of post-mortem brain tissues [13,21,22]. Peripheral BDNF levels in patients with schizophrenia have also been widely reported in the literature. However, there is no widespread agreement on the degree of peripheral BDNF levels in patients with schizophrenia, as measured in blood serum or plasma. A recent meta-analysis reported that peripheral BDNF levels were reduced in schizophrenia. However, there was considerable heterogeneity in the results [5]. Considering the important roles of mature BDNF such as cell survival, migration, outgrowth of neuritis and synaptic plasticity, it would be informative to specifically measure mature BDNF in patients with schizophrenia because dysfunction of these mature BDNF roles might be an underlying factor in the pathophysiology of schizophrenia. The peripheral BDNF levels previously reported in patients with schizophrenia were total BDNF (proBDNF and mature BDNF); peripheral levels of mature BDNF specifically have not been investigated in patients with schizophrenia. This study aimed to determine whether peripheral levels of mature BDNF were altered in patients with treatment-resistant schizophrenia. We also investigated Matrix metalloproteinase-9 (MMP-9) levels, as MMP-9 plays a role in the conversion of proBDNF to mature BDNF [8].

Table 1
Demographic variables for subjects.

Variables	Control $n=22$	Patients with schizophrenia $n=22$
Age (years)	38.1 ± 12.9	38.1 ± 13.2
Gender (male/female)	(12/10)	(12/10)
Age at onset	–	21.9 ± 8.4
Duration of illness	–	17.2 ± 11.1
PANSS positive	–	23.0 ± 4.6
PANSS negative	–	25.5 ± 5.5
PANSS general	–	52.9 ± 9.6
Clozapine dose (mg)	–	448.6 ± 130.0

Means ± SD are shown.

2. Materials and methods

2.1. Subjects

Twenty-two patients with treatment-resistant schizophrenia who were treated with clozapine were included in this study. Twenty-two age- and sex-matched healthy controls also participated in this study (Table 1). Cases were recruited at Osaka University hospitals. Each subject had been diagnosed and assessed by at least two trained psychiatrists according to the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV) criteria based on structured clinical interview. Treatment-resistant schizophrenia was defined according to the following criteria mentioned in clozapine drug information in Japan: (1) Non- or little response to treatment from at least two adequately dosed antipsychotic trials for at least 4 weeks (including at least one second-generation antipsychotic, >600 mg/day of chlorpromazine equivalent) and patients never had the Global Assessment of Functioning (GAF) scores that were higher than 40. (2) Intolerance to at least two second-generation antipsychotics because of uncontrolled extrapyramidal symptoms. All subjects included in this study met the criteria of non- or little response. Symptoms of schizophrenia were assessed using the Positive and Negative Syndrome Scale (PANSS). Cases of schizophrenia with the comorbidities of substance-related disorders or mental retardation were excluded. Controls were recruited through local advertisements. Psychiatrically, medically and neurologically healthy controls were evaluated using the DSM-IV structured clinical interview, non-patient version. Subjects were excluded if they had neurological or medical conditions that could potentially affect the central nervous system, such as atypical headache, head trauma with loss of consciousness, chronic lung disease, kidney disease, chronic hepatic disease, thyroid disease, active stage cancer, cerebrovascular disease, epilepsy or seizures. Written informed consent was obtained for all subjects after the procedures had been fully explained. This study was carried out in accordance with the World Medical Association's Declaration of Helsinki and approved by the Research Ethical Committee of Osaka University and Chiba University.

2.2. Measurement of mature BDNF and MMP-9

Plasma levels of mature BDNF and MMP-9 were measured using the human BDNF ELISA Kit (Adipo Bioscience, Santa Clara, CA, USA), and the human MMP-9 ELISA Kit (R&D Systems, Minneapolis, MN, USA), respectively. To minimize assay variance, plasma levels of mature BDNF and MMP-9 were measured in each subject on the same day. All experiments were performed in duplicate. Protocols were performed according to the manufacturer's instructions. The optical density of each well was measured using an automated microplate reader (Emax; Molecular Devices, Sunnyvale, CA, USA). As plasma levels of proBDNF are not measurable by the newly available proBDNF ELISA kit due to low sensitivity, we measured only mature BDNF.

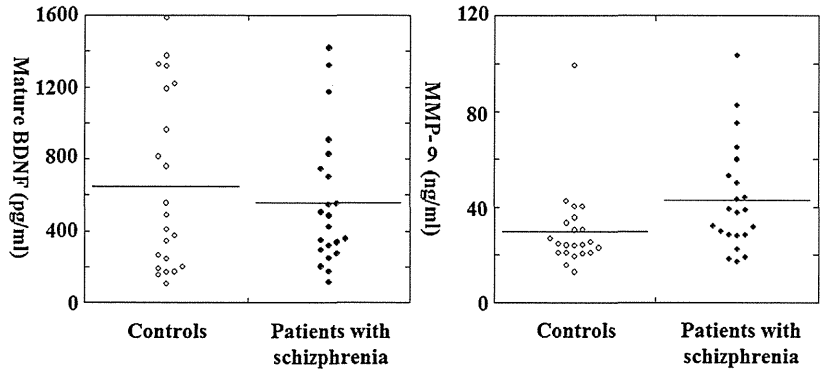

Fig. 1. Plasma levels of mature BDNF and MMP-9 in treatment-resistant schizophrenia treated with clozapine. The plasma levels of mature BDNF and MMP-9 in the controls and treatment-resistant patients with schizophrenia who were treated with clozapine (control, $n = 22$, schizophrenia, $n = 22$).

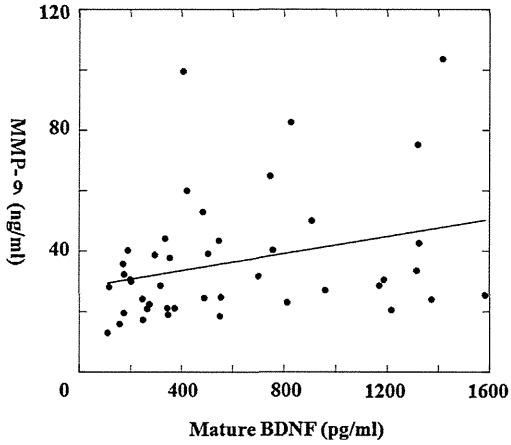

Fig. 2. Correlation between plasma levels of mature BDNF and MMP-9. Positive correlation was observed between plasma levels of mature BDNF and MMP-9 (patients with schizophrenia and controls, $n = 44$, $r = 0.333$, $p = 0.027$).

2.3. Statistical analysis

Statistical analyses were performed using SPSS 20.0J software (SPSS Japan Inc., Tokyo, Japan). Differences in clinical characteristics between patients and controls were analyzed using χ^2 tests for categorical variables. The groups did not differ with respect to age or gender (Table 1). Kolmogrov–Smirnov test was used to test the normality of data distribution. Mature BDNF did not normally distribute in both patients with schizophrenia and controls (patients with schizophrenia; $p = 0.041$, controls; $p = 0.042$). MMP-9 distributed normally in patients with schizophrenia, however did not distribute normally in controls (patients with schizophrenia; $p = 0.130$, controls; $p = 0.012$). And differences between patients and controls were analyzed using the Mann–Whitney U-test for continuous variables. Homogeneity of variance was assessed by Levene's test. The assumption of homogeneity of group variance was not violated in both mature BDNF and MMP-9 levels (mature BDNF; $p = 0.052$, MMP-9; $p = 0.112$). Test of rejection of Smirnoff–Grubbs was performed. Spearman rank order correlation test was performed to assess the possible correlation between plasma levels of mature BDNF and MMP-9 and clinical characteristics. The significant level for statistical tests was set at $p < 0.05$.

3. Results

The plasma levels of mature BDNF and MMP-9 were compared between patients with treatment-resistant schizophrenia who were treated with clozapine and controls, and no significant difference was observed for mature BDNF (Fig. 1, Mann–Whitney test; $U = 238$, $p = 0.925$). However, MMP-9 was significantly increased in patients with schizophrenia (Fig. 1, Mann–Whitney test; $U = 139$, $p = 0.016$). When we exclude each one sample in both groups by test of rejection of Smirnoff–Grubbs, MMP-9 was still significantly increased in patients with schizophrenia (Mann–Whitney test; $U = 118$, $p = 0.010$). As MMP-9 plays a role in the conversion of proBDNF to mature BDNF, the correlation between the levels of mature BDNF and MMP-9 was examined. There were significant correlation between the levels of mature BDNF and MMP-9 in (Fig. 2, patients with schizophrenia and controls, $n = 44$, $r = 0.333$, $p = 0.027$). When we investigate this correlation in patients and controls groups separately, significant correlation was observed in patients with schizophrenia ($n = 22$, $r = 0.585$, $p = 0.004$) but not in controls ($n = 22$, $r = 0.322$, $p = 0.143$). To determine the effect of clozapine on mature BDNF and MMP-9 levels, we also examined the correlation between the plasma levels of mature BDNF or MMP-9 and clozapine dosage. No significant correlation was observed between the plasma levels of mature BDNF or MMP-9 and clozapine dosage (Table 2, BDNF and clozapine dosage; $n = 22$, $r = 0.028$, $p = 0.901$, MMP-9 and clozapine dosage; $n = 22$, $r = 0.131$, $p = 0.562$). The correlations between the plasma levels of mature BDNF or MMP-9 and positive and negative symptom scores on the PANSS were also investigated; no significant correlations were observed (Table 2, BDNF and PANSS positive; $n = 22$, $r = -0.014$, $p = 0.952$, BDNF and PANSS negative; $n = 22$, $r = -0.079$, $p = 0.726$, MMP-9 and PANSS positive; $n = 22$, $r = 0.306$, $p = 0.167$, BDNF and PANSS negative; $n = 22$, $r = 0.127$, $p = 0.574$). The correlations between the plasma levels of mature BDNF or MMP-9 and duration of illness were also investigated; no significant correlations were observed (Table 2, BDNF and duration of illness; $n = 22$, $r = 0.121$, $p = 0.592$, MMP-9 and duration of illness; $n = 22$, $r = 0.087$, $p = 0.699$).

Table 2
Correlation analysis.

	Clozapine dosage	PANSS positive	PANSS negative	PANSS general	Age at onset	Duration of illness
Mature BDNF	0.901	0.952	0.726	0.865	0.332	0.592
MMP-9	0.562	0.167	0.574	0.454	0.685	0.699

p values are shown.

4. Discussion

In this study, for the first time, we measured the plasma levels of mature BDNF in patients with schizophrenia. The plasma levels of mature BDNF were decreased in treatment-resistant schizophrenia, however the difference did not reach statistical significance. Our result was consistent with some previous studies that investigated the serum levels of total BDNF in patients with schizophrenia [10,20]. Treatment-resistant schizophrenia patients treated with clozapine were enrolled because some studies suggest that peripheral BDNF levels increase in association with antipsychotics treatment including clozapine which is used for the treatment of poorly responsive patients with schizophrenia [6,10] and serum BDNF levels were reported to be significantly correlated with clozapine daily dose but not with typical antipsychotics [15]. However, we found no effect of clozapine treatment on the plasma levels of mature BDNF. A possible explanation would be the difference in race. This is the first study investigating the effect of clozapine treatment on the plasma levels of mature BDNF in Japanese population. Accumulating evidence suggests that BDNF plays a key role in the pathophysiology of major depressive disorder (MDD). It was reported that BDNF serum levels in patients with MDD were significantly lower than those of healthy controls, and that there was a negative correlation between BDNF serum levels and the severity of depression in patients [19]. Furthermore, decreased serum levels of BDNF in antidepressant naive patients with MDD, recovered to levels associated with amelioration of depressive symptoms, after antidepressant treatment. Three meta-analyses and a study using a large sample size confirmed these findings [7]. Recently, peripheral levels of mature BDNF have been reported to be decreased in MDD [23]. Further study using larger samples is needed to see whether peripheral levels of mature BNDF are not altered in schizophrenia and mature BDNF levels are not associated with clozapine.

We also investigated MMP-9 plasma levels, as MMP-9 plays a role in the conversion of proBDNF to mature BDNF [8]. The significant correlation was observed between mature BDNF and MMP-9 plasma levels, suggesting that MMP-9 plays a role in the conversion of proBDNF to mature BDNF in the samples of this study. The serum levels of MMP-9 have been reported to be increased in patients with schizophrenia [2]. A higher frequency of positive MMP-9 activity in serum from patients with schizophrenia has also been reported [1]. We confirmed the presence of elevated plasma MMP-9 levels in patients with treatment-resistant schizophrenia. In patients with schizophrenia, MMP9 might be induced to recover the decreased mature BDNF. The finding that significant correlation between mature BDNF and MMP-9 was observed only in patients with schizophrenia but not in controls supports this idea. Plasma levels of MMP-9 have been proposed to be a useful biomarker for assessing pathological event in brain. It was reported that levels of MMP-9 in plasma and brain were significantly correlated after cerebral ischemia in rats [14]. MMP-9 is an enzyme implicated in a number of pathological conditions including neuropsychiatric disorders [18]. A role of MMP-9 in the plasticity of the central nervous system has been investigated in experimental studies and MMP-9 is reported to be required for hippocampal long-term potentiation and memory [12]. MMP-9 may have some roles in pathophysiology of schizophrenia.

Our study must be interpreted in lights of its limitations. Firstly, the sample size of this study is small. Secondly, only treatment-resistant schizophrenia patients treated with clozapine were included and patients treated with other antipsychotics or patients without antipsychotics treatment were not included in this study. Further studies are needed to evaluate the relationship between plasma levels of mature BDNF and schizophrenia and clozapine treatment.

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References

[1] S.H. Chang, S.Y. Chiang, C.C. Chiu, C.C. Tsai, H.H. Tsai, C.Y. Huang, T.C. Hsu, B.S. Tzang, Expression of anti-cardiolipin antibodies and inflammatory associated factors in patients with schizophrenia, Psychiatry Res. 187 (2011) 341–346.
[2] E. Domenici, D.R. Wille, F. Tozzi, I. Prokopenko, S. Miller, A. McKeown, C. Brittain, D. Rujescu, I. Giegling, C.W. Turck, F. Holsboer, E.T. Bullmore, L. Middleton, E. Merlo-Pich, R.C. Alexander, P. Muglia, Plasma protein biomarkers for depression and schizophrenia by multi analyte profiling of case-control collections, PLoS ONE 5 (2010) e9166.
[3] A. Ghosh, J. Carnahan, M.E. Greenberg, Requirement for BDNF in activity-dependent survival of cortical neurons, Science 263 (1994) 1618–1623.
[4] J.A. Gorski, S.R. Zeiler, S. Tamowski, K.R. Jones, Brain-derived neurotrophic factor is required for the maintenance of cortical dendrites, J. Neurosci. 23 (2003) 6856–6865.
[5] M.J. Green, S.L. Matheson, A. Shepherd, C.S. Weickert, V.J. Carr, Brain-derived neurotrophic factor levels in schizophrenia: a systematic review with meta-analysis, Mol. Psychiatry 16 (2011) 960–972.
[6] R.W. Grillo, G.L. Ottoni, R. Leke, D.O. Souza, L.V. Portela, D.R. Lara, Reduced serum BDNF levels in schizophrenic patients on clozapine or typical antipsychotics, J. Psychiatr. Res. 41 (2007) 31–35.
[7] K. Hashimoto, Brain-derived neurotrophic factor as a biomarker for mood disorders: an historical overview and future directions, Psychiatry Clin. Neurosci. 64 (2010) 341–357.
[8] K. Hashimoto, Sigma-1 receptor chaperone and brain-derived neurotrophic factor: emerging links between cardiovascular disease and depression, Prog. Neurobiol. 100 (2013) 15–29.
[9] R. Hashimoto, Y. Moriguchi, F. Yamashita, T. Mori, K. Nemoto, T. Okada, H. Hori, H. Noguchi, H. Kunugi, T. Ohnishi, Dose-dependent effect of the Val66Met polymorphism of the brain-derived neurotrophic factor gene on memory-related hippocampal activity, Neurosci. Res. 61 (2008) 360–367.
[10] B.H. Lee, Y.K. Kim, Increased plasma brain-derived neurotropic factor, not nerve growth factor-Beta, in schizophrenia patients with better response to risperidone treatment, Neuropsychobiology 59 (2009) 51–58.
[11] K. Martinowich, H. Manji, B. Lu, New insights into BDNF function in depression and anxiety, Nat. Neurosci. 10 (2007) 1089–1093.
[12] V. Nagy, O. Bozdagi, A. Matynia, M. Balcerzyk, P. Okulski, J. Dzwonek, R.M. Costa, A.J. Silva, L. Kaczmarek, G.W. Huntley, Matrix metalloproteinase-9 is required for hippocampal late-phase long-term potentiation and memory, J. Neurosci. 26 (2006) 1923–1934.
[13] H. Nawa, M. Takahashi, P.H. Patterson, Cytokine and growth factor involvement in schizophrenia – support for the developmental model, Mol. Psychiatry 5 (2000) 594–603.
[14] K.P. Park, A. Rosell, C. Foerch, C. Xing, W.J. Kim, S. Lee, G. Opdenakker, K.L. Furie, E.H. Lo, Plasma and brain matrix metalloproteinase-9 after acute focal cerebral ischemia in rats, Stroke 40 (2009) 2836–2842.
[15] M. Pedrini, I. Chendo, I. Grande, M.I. Lobato, P.S. Belmonte-de-Abreu, C. Lersch, J. Walz, M. Kauer-Sant'anna, F. Kapczinski, C.S. Gama, Serum brain-derived neurotrophic factor and clozapine daily dose in patients with schizophrenia: a positive correlation, Neurosci. Lett. 491 (2011) 207–210.
[16] L. Pezawas, B.A. Verchinski, V.S. Mattay, J.H. Callicott, B.S. Kolachana, R.E. Straub, M.F. Egan, A. Meyer-Lindenberg, D.R. Weinberger, The brain-derived neurotrophic factor val66met polymorphism and variation in human cortical morphology, J. Neurosci. 24 (2004) 10099–10102.
[17] M.M. Poo, Neurotrophins as synaptic modulators, Nat. Rev. Neurosci. 2 (2001) 24–32.
[18] J.K. Rybakowski, Matrix metalloproteinase-9 (MMP9) – a mediating enzyme in cardiovascular disease, cancer, and neuropsychiatric disorders, Cardiovasc. Psychiatry Neurol. 2009 (2009) 904836.
[19] E. Shimizu, K. Hashimoto, N. Okamura, K. Koike, N. Komatsu, C. Kumakiri, M. Nakazato, H. Watanabe, N. Shinoda, S. Okada, M. Iyo, Alterations of serum

levels of brain-derived neurotrophic factor (BDNF) in depressed patients with or without antidepressants, Biol. Psychiatry 54 (2003) 70–75.
[20] E. Shimizu, K. Hashimoto, H. Watanabe, N. Komatsu, N. Okamura, K. Koike, N. Shinoda, M. Nakazato, C. Kumakiri, S. Okada, M. Iyo, Serum brain-derived neurotrophic factor (BDNF) levels in schizophrenia are indistinguishable from controls, Neurosci. Lett. 351 (2003) 111–114.
[21] C.S. Weickert, T.M. Hyde, B.K. Lipska, M.M. Herman, D.R. Weinberger, J.E. Kleinman, Reduced brain-derived neurotrophic factor in prefrontal cortex of patients with schizophrenia, Mol. Psychiatry 8 (2003) 592–610.
[22] J. Wong, T.M. Hyde, H.L. Cassano, A. Deep-Soboslay, J.E. Kleinman, C.S. Weickert, Promoter specific alterations of brain-derived neurotrophic factor mRNA in schizophrenia, Neuroscience 169 (2010) 1071–1084.
[23] T. Yoshida, M. Ishikawa, T. Niitsu, M. Nakazato, H. Watanabe, T. Shiraishi, A. Shiina, T. Hashimoto, N. Kanahara, T. Hasegawa, M. Enohara, A. Kimura, M. Iyo, K. Hashimoto, Decreased serum levels of mature brain-derived neurotrophic factor (BDNF), but not its precursor proBDNF, in patients with major depressive disorder, PLoS ONE 7 (2012) e42676.