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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 ($n=60$).