Heaton, R.K., 1981. The Wisconsin Card Sorting Test (Manual). Psychological Assessment Resources, Florida.

Hori, H., Ozeki, Y., Terada, S., Kunugi, H., 2008a. Functional near-infrared spectroscopy reveals altered hemispheric laterality in relation to schizotypy during verbal fluency task. Progress in Neuro-Psychopharmacology and Biological Psychiatry 32, 1944–1951.

Hori, H., Noguchi, H., Hashimoto, R., Nakabayashi, T., Saitoh, O., Murray, R.M., Okabe, S., Kunugi, H., 2008b. Personality in schizophrenia assessed with the Temperament and Character Inventory (TCI). Psychiatry Research 160, 175–183.

Hori, H., Teraishi, T., Ozeki, Y., Hattori, K., Sasayama, D., Matsuo, J., Kawamoto, Y., Kinoshita, Y., Higuchi, T., Kunugi, H., 2011. Schizotypal personality in healthy adults is related to blunted cortisol responses to the combined dexamethasone/corticotropin-releasing hormone test. Neuropsychobiology 63, 232–241.

Kamata, M., Suzuki, A., Matsumoto, Y., Togashi, H., Otani, K., 2009. Effect of month of birth on personality traits of healthy Japanese. European Psychiatry 24, 86–90.

Kashima, H., Handa, T., Kato, M., Sakura, K., Yokoyama, N., Murakami, M., Shigemori, K., Muramatsu, T., Saito, H., Ooe, Y., Mimura, M., Asai, M., Hosaki, H., 1987. Neuropsychological investigation on chronic schizophrenia—aspects of its frontal functions. In: Takahashi, R., Flor-Henry, P., Gruzelier, J., Niwa, S. (Eds.), Cerebral Dynamics, Laterality and Psychopathology. Elsevier, Amsterdam, pp. 337–345.

Kendler, K.S., Ochs, A.L., Gorman, A.M., Hewitt, J.K., Ross, D.E., Mirsky, A.F., 1991. The structure of schizotypy: a pilot multitrait twin study. Psychiatry Research 36, 19–36.

Kiang, M., Kutas, M., 2005. Association of schizotypy with semantic processing differences: an event-related brain potential study. Schizophrenia Research 77, 329–342.

Kijima, N., Saito, R., Takeuchi, M., Yoshino, A., Ono, Y., Kato, M., Kitamura, T., 1996. Cloninger's seven-factor model of temperament and character and Japanese version of Temperament and Character Inventory (TCI). Archives of Psychiatric Diagnostic and Clinical Evaluations 7, 379–399 (in Japanese).

Kijima, N., Tanaka, E., Suzuki, N., Higuchi, H., Kitamura, T., 2000. Reliability and validity of the Japanese version of the Temperament and Character Inventory. Psychological Reports 86, 1050–1058.

Kirkpatrick, B., Tek, C., Allardyce, J., Morrison, G., McCreadie, R.G., 2002. Summer birth and deficit schizophrenia in Dumfries and Galloway, southwestern Scotland. American Journal of Psychiatry 159, 1382–1387.

Kirkpatrick, B., Messias, E., LaPorte, D., 2008. Schizoid-like features and season of birth in a nonpatient sample. Schizophrenia Research 103, 151–155.

Kunugi, H., Nanko, S., Hayashi, N., Saito, K., Hirose, T., Kazamatsuri, H., 1997. Season of birth of schizophrenics in a recent Japanese sample. Psychiatry and Clinical Neurosciences 51, 213–216.

Lahti, J., Raïkkönen, K., Sovio, U., Miettunen, J., Hartikainen, A.L., Pouta, A., Taanila, A., Joukamaa, M., Järvelin, M.R., Veijola, J., 2009. Early-life origins of schizotypal traits in adulthood. British Journal of Psychiatry 195, 132–137.

Lenzenweger, M.F., 2001. Reaction time slowing during high-load, sustained-attention task performance in relation to psychometrically identified schizotypy. Journal of Abnormal Psychology 110, 290–296.

Lenzenweger, M.F., Korfine, L., 1994. Perceptual aberrations, schizotypy, and the Wisconsin Card Sorting Test. Schizophrenia Bulletin 20, 345–357.

Martin, R.P., Foels, P., Clanton, G., Moon, K., 2004. Season of birth is related to child retention rates, achievement, and rate of diagnosis of specific LD. Journal of Learning Disabilities 37, 307–317.

Matheson, S., Langdon, R., 2008. Schizotypal traits impact upon executive working memory and aspects of IQ. Psychiatry Research 159, 207–214.

McGrath, J.J., Welham, J.L., 1999. Season of birth and schizophrenia: a systematic review and meta-analysis of data from the Southern Hemisphere. Schizophrenia Research 35, 237–242.

McGrath, J.J., Saha, S., Lieberman, D.E., Buka, S., 2006. Season of birth is associated with anthropometric and neurocognitive outcomes during infancy and childhood in a general population birth cohort. Schizophrenia Research 81, 91–100.

Messias, E., Kirkpatrick, B., Bromet, E., Ross, D., Buchanan, R.W., Carpenter Jr., W.T., Tek, C., Kendler, K.S., Walsh, D., Dollfus, S., 2004. Summer birth and deficit schizophrenia: a pooled analysis from 6 countries. Archives of General Psychiatry 61, 985–989.

Mitropoulou, V., Goodman, M., Sevy, S., Elman, I., New, A.S., Iskander, E.G., Silverman, J.M., Breier, A., Siever, L.J., 2004. Effects of acute metabolic stress on the dopaminergic and pituitary-adrenal axis activity in patients with schizotypal personality disorder. Schizophrenia Research 70, 27–31.

Noguchi, H., Hori, H., Kunugi, H., 2008. Schizotypal traits and cognitive function in healthy adults. Psychiatry Research 161, 162–169.

O'Driscoll, G.A., Lenzenweger, M.F., Holzman, P.S., 1998. Antisaccades and smooth pursuit eye tracking and schizotypy. Archives of General Psychiatry 55, 837–843.

Otsubo, T., Tanaka, K., Koda, R., Shinoda, J., Sano, N., Tanaka, S., Aoyama, H., Mimura, M., Kamijima, K., 2005. Reliability and validity of Japanese version of the Mini-International Neuropsychiatric Interview. Psychiatry and Clinical Neurosciences 59, 517–526.

Park, S., McTigue, K., 1997. Working memory and the syndromes of schizotypal personality. Schizophrenia Research 26, 213–220.

Raine, A., 1991. The SPQ: a scale for the assessment of schizotypal personality based on DSM-III-R criteria. Schizophrenia Bulletin 17, 555–564.

Raine, A., Reynolds, C., Lencz, T., Scerbo, A., Triphon, N., Kim, D., 1994. Cognitive–perceptual, interpersonal, and disorganized features of schizotypal personality. Schizophrenia Bulletin 20, 191–201.

Raine, A., Lencz, T., Mednick, S.A., 1995. Schizotypal Personality. Cambridge University Press, Cambridge.

Reid, H.M., Zborowski, M.J., 2006. Schizophrenia-proneness, season of birth and sleep: elevated schizotypy scores are associated with spring births and extremes of sleep. Personality and Individual Differences 41, 1185–1193.

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. Journal of Clinical Psychiatry 59 (suppl. 20), 22–57.

Shinagawa, F., Kobayashi, S., Fujita, K., Maekawa, H., 1990. Japanese Wechsler Adult Intelligence Scale-Revised. Nihonbunkakagakusha, Tokyo. in Japanese.

Siever, L.J., Davis, K.L., 2004. The pathophysiology of schizophrenia disorders: perspectives from the spectrum. American Journal of Psychiatry 161, 398–413.

Smith, M.J., Cloninger, C.R., Harms, M.P., Csernansky, J.G., 2008. Temperament and character as schizophrenia-related endophenotypes in non-psychotic siblings. Schizophrenia Research 104, 198–205.

Someya, T., Sasaki, T., Hosoda, S., Takahashi, S., 1994. Reliability and validity of schizotypal personality questionnaire. Archives of Psychiatric Diagnostic and Clinical Evaluations 5, 98 (in Japanese).

Sugishita, M., 2001. Japanese Wechsler Memory Scale-Revised. Nihonbunkakagakusha, Tokyo. in Japanese.

Svrakic, D.M., Whitehead, C., Przybeck, T.R., Cloninger, C.R., 1993. Differential diagnosis of personality disorders by the seven-factor model of temperament and character. Archives of General Psychiatry 50, 991–999.

Takahashi, H., Iwase, M., Canuet, L., Yasuda, Y., Ohi, K., Fukumoto, M., Iike, N., Nakahachi, T., Ikezawa, K., Azechi, M., Kurimoto, R., Ishii, R., Yoshida, T., Kazui, H., Hashimoto, R., Takeda, M., 2010. Relationship between prepulse inhibition of acoustic startle response and schizotypy in healthy Japanese subjects. Psychophysiology 47, 831–837.

Takei, N., Sham, P., Callaghan, E., Glover, G., Murray, R., 1995. Early risk factors in schizophrenia: place and season of birth. European Psychiatry 10, 165–170.

Tatsumi, M., Sasaki, T., Iwanami, A., Kosuga, A., Tanabe, Y., Kamijima, K., 2002. Season of birth in Japanese patients with schizophrenia. Schizophrenia Research 54, 213–218.

Tochigi, M., Okazaki, Y., Kato, N., Sasaki, T., 2004. What causes seasonality of birth in schizophrenia? Neuroscience Research 48, 1–11.

Torrey, E.F., Miller, J., Rawlings, R., Yolken, R.H., 1997. Seasonality of births in schizophrenia and bipolar disorder: a review of the literature. Schizophrenia Research 28, 1–38.

Wang, J., Miyazato, H., Hokama, H., Hiramatsu, K., Kondo, T., 2004. Correlation between P50 suppression and psychometric schizotypy among non-clinical Japanese subjects. International Journal of Psychophysiology 52, 147–157.

Wechsler, D., 1981. Wechsler Adult Intelligence Scale, Revised. Psychological Corporation, New York.

Wechsler, D., 1987. Wechsler Memory Scale Manual, Revised. Psychological Corporation, San Antonio.

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Contents lists available at SciVerse ScienceDirect

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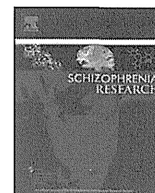

Negative correlation between cerebrospinal fluid oxytocin levels and negative symptoms of male patients with schizophrenia

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ARTICLE INFO

Article history:
Received 6 March 2012
Received in revised form 13 May 2012
Accepted 7 June 2012
Available online 27 June 2012

Keywords:
Schizophrenia
Depression
Oxytocin
Cerebrospinal fluid
Antipsychotics

ABSTRACT

Background: Accumulating evidence indicates that oxytocin plays an important role in social interactions. Previous studies also suggest altered oxytocin function in patients with schizophrenia and depression. However, few studies have examined the central oxytocin levels in these disorders.
Methods: Cerebrospinal fluid (CSF) oxytocin levels were measured by ELISA in male participants consisting of 27 patients with schizophrenia, 17 with major depressive disorder (MDD), and 21 healthy controls.
Results: CSF oxytocin levels of patients with schizophrenia or MDD did not differ significantly with healthy controls. The antidepressant dose or the Hamilton depression rating scale score did not significantly correlate with the oxytocin levels in MDD patients. CSF oxytocin levels in schizophrenic patients significantly negatively correlated with second generation antipsychotic dose ($r = -0.49$, $P = 0.010$) but not with first generation antipsychotic dose ($r = -0.13$, $P = 0.50$). A significant correlation was observed between oxytocin levels and negative subscale of PANSS ($r = -0.38$, $P = 0.050$). This correlation remained significant even after controlling for second generation antipsychotic dose ($r = -0.47$, $P = 0.016$).
Conclusions: We obtained no evidence of altered CSF oxytocin levels in patients with schizophrenia or those with MDD. However, lower oxytocin levels may be related to higher second generation antipsychotic dose and more severe negative symptoms in schizophrenia.

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1. Introduction

Oxytocin is produced in the supraoptic and paraventricular nuclei of hypothalamus and is secreted into the blood stream from the posterior pituitary. Its release is induced by a variety of stressful stimuli, including noxious stimuli, conditioned fear, and exposure to novel environments (Onaka, 2004). Accumulating evidence indicates that oxytocin plays an important role in social interactions (Lim and Young, 2006; Bartz et al., 2010). Deficits in social functioning observed in psychiatric disorders including schizophrenia (Couture et al., 2006; Sparks et al., 2010) and mood disorders (Inoue et al., 2004; Montag et al., 2010; Wolkenstein et al., 2011) imply the possible involvement of oxytocin in the pathophysiology of these disorders.

Many studies have investigated the possible link between oxytocin and psychiatric disorders. Some previous studies reported altered oxytocin function in patients with schizophrenia (Linkowski et al., 1984; Beckmann et al., 1985; Mai et al., 1993). Higher plasma oxytocin levels in schizophrenic patients were associated with lower symptom severity (Rubin et al., 2010). A clinical study showed that administration of this hormone ameliorated symptoms of schizophrenia (Feifel et al., 2010). In a preclinical study, systemically administered oxytocin reversed prepulse inhibition deficits induced by amphetamine and the phencyclidine analog in rats (Feifel and Reza, 1999). Oxytocin dysfunction has been implicated in the pathophysiology of depression as well. Two studies have shown that peripheral oxytocin levels and depressive symptoms were significantly correlated in patients with major depressive disorder (MDD) (Scantamburlo et al., 2007; Cyranowski et al., 2008). Moreover, oxytocin knock-out mice have shown dysregulated stress responses to psychological stimuli (Mantella et al., 2005) and enhanced anxiety behaviors (Mantella et al., 2003).

Oxytocin secreted from the pituitary gland generally does not re-enter the brain through the blood-brain barrier (Ermisch et al., 1985). Therefore, the behavioral effects of oxytocin are likely to be due to the release from centrally projecting oxytocin neurons. Since

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doi:10.1016/j.schres.2012.06.016

oxytocin in the nervous system can be transported to blood (Durham et al., 1991), peripheral oxytocin levels may reflect brain levels to some extent. However, central and peripheral oxytocin is regulated independently, and the half-life of oxytocin is less than 5 minutes in the blood (Ryden and Sjoholm, 1969) while that in the brain is 19.1 -minutes (Durham et al., 1991). Therefore, measurement in the CSF is necessary for the direct assessment of central oxytocin levels.

To our knowledge, two studies have previously examined the cerebrospinal fluid (CSF) levels of oxytocin in patients with schizophrenia. One reported elevated oxytocin levels in schizophrenia compared with controls (Beckmann et al., 1985), while the other did not obtain such a finding (Glovinsky et al., 1994). Only one study has examined the CSF levels of oxytocin in patients with depression, in which no difference was found compared with controls (Pitts et al., 1995). No study to date has examined the association of CSF oxytocin levels with symptom severity of these disorders. Since symptom severity forms a continuous spectrum ranging from mild to severe state, an association with the severity of the disease would suggest that oxytocin levels reflect the state of the disease.

In the present study, the oxytocin levels in the CSF of patients with schizophrenia and those with depression were measured and compared to that of healthy controls. Furthermore, we investigated the correlation between CSF oxytocin levels and symptom severity of these disorders. From the findings of previous studies examining peripheral oxytocin levels (Scantamburlo et al., 2007; Rubin et al., 2010), we hypothesized that CSF oxytocin levels would be lower in patient groups compared to healthy controls and that symptom severity would be negatively correlated with the oxytocin levels.

2. Materials and methods

2.1. Subjects

Participants were 27 patients with schizophrenia (mean age (standard deviation): 42.6 (8.5) years), 17 patients with major depressive disorder (MDD) (age: 39.5 (8.0) years), and 21 healthy controls (age: 38.3 (15.3) years). Demographic and clinical characteristics of the subjects are summarized in Table 1. All subjects were males to avoid gender effects and were biologically unrelated Japanese recruited from the outpatient clinic of the National Center of Neurology and Psychiatry Hospital, Tokyo, Japan or through advertisements in free local information magazines and by our website announcement. None of the healthy controls were on psychotropic medication, while 70.6% of the patients with MDD were treated with antidepressant medication at the time of the study. Most of the schizophrenic patients were prescribed antipsychotic medication, and all of those prescribed antipsychotics were on the medication for more than 3 years. Consensus diagnosis by at least 2 psychiatrists was made for each patient according to the Diagnostic and Statistical Manual of Mental Disorders, 4th edition criteria (American Psychiatric Association, 1994), on the basis of unstructured interviews and information from medical records. The controls were healthy volunteers with no current or past history of psychiatric treatment and were screened using the Japanese version of the Mini International Neuropsychiatric Interview (Sheehan et al., 1998; Otsubo et al., 2005) by a research psychiatrist to eliminate the possibility of any axis I psychiatric disorders. Participants were excluded if they had prior medical histories of central nervous system diseases or severe head injury or if they met the criteria for substance abuse or dependence or mental retardation. The study protocol was approved by the ethics committee at the National Center of Neurology and Psychiatry, Japan. After describing the study, written informed consent was obtained from every subject.

2.2. Clinical measures

Schizophrenic symptoms and depressive symptoms were assessed immediately after the lumbar puncture by an experienced research psychiatrist using the Japanese version of the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987; Yamada et al., 1991) and the Japanese version of the GRID Hamilton Depression Rating Scale, 17-item version (HAMD-17) (Hamilton, 1967), which have both been demonstrated to show good inter-rater reliability (Igarashi et al., 1998; Tabuse et al., 2007). Medication status at the time of lumbar puncture was recorded. Daily doses of antipsychotics in patients with schizophrenia and antidepressants in patients with MDD were

Table 1
Demographic and clinical characteristics.

	Controls ($N=21$)	Schizophrenia ($N=27$)	Depression ($N=17$)	Analysis
Age (years)	38.3 (15.3)	42.6 (8.5)	39.5 (8.0)	ANOVA: $F=0.97$, n.s.
BMI	23.9 (4.1)	26.0 (6.2)	23.9 (4.5)	ANOVA: $F=1.06$, n.s.
Duration of illness (years)		16.3 (9.8)	7.7 (7.3)	*t*-test: $t=2.8$, $P<0.01$
Treatment duration (years)		15.5 (9.1)	5.8 (6.9)	*t*-test: $t=3.4$, $P<0.01$
Medication status				
on antipsychotic medication				
first generation (%)	0	59.3	11.8	
second generation (%)	0	66.7	23.5	
first and/or second generation (%)	0	96.3	35.3	
on antidepressant medication (%)	0	25.9	70.6	
on benzodiazepine medication (%)	0	81.5	76.5	
on mood stabilizer medication (%)	0	14.8	5.9	
CP equivalent dose				
first generation (mg/day)		361.8 (445.0)		
second generation (mg/day)		402.4 (498.3)		
total (mg/day)		764.2 (591.6)		
IMI equivalent dose (mg/day)			167.2 (141.5)	
PANSS				
Positive symptoms score		12.5 (3.8)		
Negative symptom score		16.0 (5.8)		
General symptom score		6.8 (1.3)		
Total score		55.6 (12.6)		
HAMD-17 score			13.4 (9.6)	

Values are shown as mean (standard deviation).
BMI: body mass index; CP: chlorpromazine; IMI: imipramine.
PANSS: Positive and Negative Syndrome Scale; HAMD-17: 17 item Hamilton Rating Scale for Depression.
ANOVA: analysis of variance; n.s.: not significant.

converted to chlorpromazine and imipramine equivalent doses, respectively, using published guidelines (Inagaki et al., 1999).

2.3. Lumbar puncture and oxytocin assay

Lumbar puncture was performed with the subject in the left decubitus position. CSF was withdrawn from the L3–L4 or L4–L5 interspace. After the removal of 2 ml of CSF, a further 6 ml of CSF was collected and immediately transferred on ice to be centrifuged at 4 °C and aliquoted for storage at −80 °C until assay. CSF oxytocin levels were analyzed using a commercial ELISA kit (Enzo Life Sciences, INC., NY). Using the results from two separate runs of standard concentrations, the inter-assay coefficient of variation (CV) was less than 10%.

2.4. Statistical analysis

Statistical differences between groups were calculated using Student's *t*-test, Welch's *t*-test, or one-way analysis of variance (ANOVA). Correlations were assessed using Pearson's correlation coefficient. Since the CSF oxytocin levels were not normally distributed, log transformation was applied prior to statistical analyses to achieve normal distribution. Because previous studies suggest that some antipsychotic and antidepressant medications increase oxytocin secretion (Uvnas-Moberg et al., 1992, 1999), chlorpromazine and imipramine equivalent doses were examined as possible confounders. Statistical analyses were performed using the Statistical Package for the Social Sciences version 11.0 (SPSS Japan, Tokyo, Japan). All statistical tests were two-tailed, and $P<0.05$ indicated statistical significance.

3. Results

Fig. 1 shows the CSF oxytocin levels in each diagnostic group. A one-way ANOVA using the transformed oxytocin levels as the dependent variable indicated no significant difference between diagnostic groups ($F=1.08$, $P=0.35$). The transformed oxytocin levels showed no significant correlation with age or body weight. Figs. 2 and 3 show the relation of CSF oxytocin levels with symptom severity and psychotropic dose, respectively. The antidepressant dose or the HAMD-17 score did not significantly correlate with the transformed oxytocin levels in patients with MDD (antidepressant dose: $r=-0.15$, $P=0.57$; HAMD-17: $r=-0.19$, $P=0.46$). The transformed oxytocin levels were significantly negatively correlated with negative subscale of PANSS ($r=-0.38$, $P=0.050$). Correlations between transformed oxytocin levels and other subscales of PANSS were not statistically significant. The transformed oxytocin levels in schizophrenic patients were significantly negatively correlated with chlorpromazine equivalents of total antipsychotic dose ($r=-0.51$, $P=0.0064$) and second generation antipsychotic (SGA) dose ($r=-0.49$, $P=0.010$) but not with chlorpromazine equivalents of first generation antipsychotic (FGA) dose ($r=-0.13$, $P=0.50$). Those prescribed SGA had significantly lower CSF oxytocin levels compared to those not prescribed SGA (Welch's *t* test: $t=2.6$, $df=10.4$, $P=0.024$). Comparison between patients prescribed and not prescribed FGA did not yield significant difference (Student's t test: $t=1.1$, $df=25$, $P=0.27$). Although none of the subscales of PANSS were correlated with FGA, SGA, or total chlorpromazine equivalent dose in the present study (all $P>0.1$), a previous study (Sim et al., 2009) reported an association between antipsychotic dose and the severity of positive as well as negative symptoms of schizophrenia. Therefore, we considered antipsychotic dose as a confounding factor for the association between oxytocin levels and symptom severity. Thus, we also examined the correlation between the oxytocin levels and PANSS scores controlling for prescribed antipsychotic dose. Partial correlation between transformed oxytocin levels and negative subscale of PANSS, removing the linear effects of total antipsychotic dose, was statistically significant ($r=-0.39$, $P=0.047$). Removing the linear effects of SGA dose instead of total antipsychotic dose also resulted in significant correlation of transformed CSF oxytocin levels with negative subscale ($r=-0.47$, $P=0.016$) as well as with total PANSS score ($r=-0.47$, $P=0.016$). SGA dose-controlled partial correlations between transformed oxytocin levels and other subscales of PANSS were not statistically significant (positive subscale: $r=-0.24$, $P=0.23$; general subscale: $r=-0.33$, $P=0.099$).

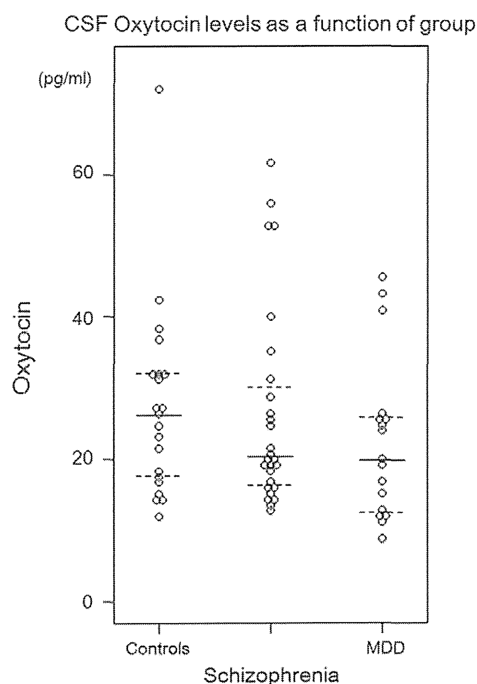

Fig. 1. Cerebrospinal fluid oxytocin levels as a function of group. The cerebrospinal fluid oxytocin levels in healthy controls and patients with schizophrenia and major depressive disorder are shown. Solid bars indicate median values and the dotted lines indicate interquartile range. No significant difference was observed between the diagnostic groups.

4. Discussion

Consistent with some previous studies (Glovinsky et al., 1994; Pitts et al., 1995), CSF oxytocin levels did not significantly differ between healthy controls and patients with schizophrenia and MDD. However, the present results showed that higher levels of CSF oxytocin may be associated with less severe symptoms of schizophrenia.

The observed negative correlation between antipsychotic dose and CSF oxytocin levels points to the possibility that antipsychotic medication lowers oxytocin levels. A recent study suggests that an inhibitory feedback loop may exist between prolactin-secreting lactrophs and oxytocinergic paraventricular neurons (Sirzen-Zelenskaya et al., 2011). Therefore, the disinhibition of prolactin secretion due to the D_2 receptor blockade by antipsychotics may have resulted in the suppression of oxytocin secretion. This, however, does not explain the stronger correlation of SGA dose compared to FGA dose. Kiss et al (2010) showed that SGAs have a more potent influence than haloperidol on the activity of oxytocin magnocellular neurons. This also seems contradictory to the present finding that SGA is negatively correlated with oxytocin levels. An alternative explanation for this negative correlation is that patients with low oxytocin levels may respond poorly to antipsychotic medication, and thus, higher dose was prescribed to such patients. Nevertheless, despite the relatively strong correlation with the antipsychotic dose, the cross-sectional design of the present study hinders any causal inferences. One previous study (Glovinsky et al., 1994) demonstrated that CSF oxytocin levels were unchanged by antipsychotic medication. Thus, further investigation is necessary to elucidate the effects of antipsychotic medication on oxytocin levels.

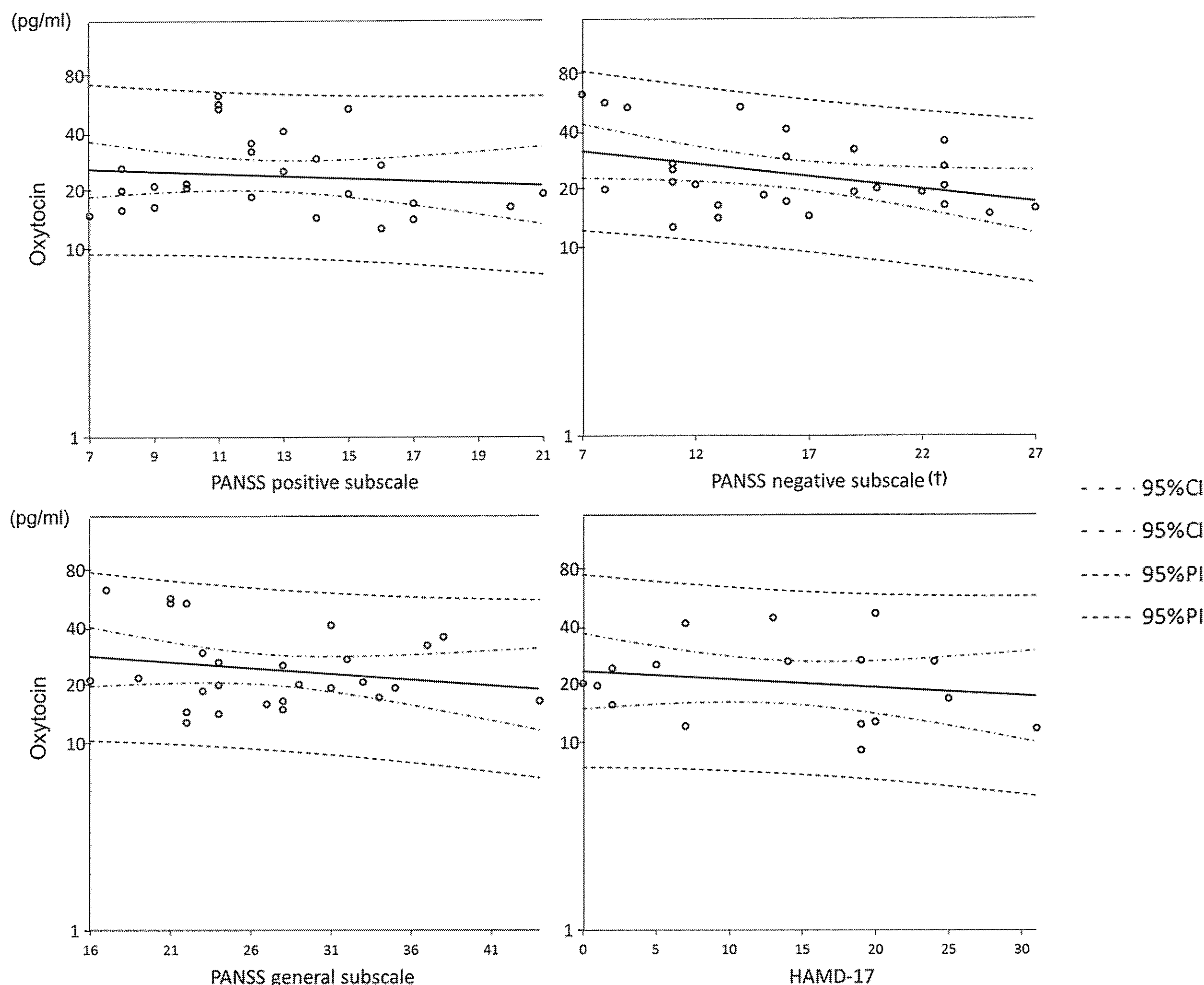

Fig. 2. Relationship between cerebrospinal fluid oxytocin levels and symptom severity. The association between cerebrospinal oxytocin levels and symptom severity is shown. Oxytocin levels are shown in logarithmic scale. Solid lines indicate fitted regression lines, unevenly dashed lines indicate 95% confidence intervals, and evenly dashed lines indicate 95% prediction intervals. (†): Correlation at significance level of $P<0.05$. PANSS: Positive and Negative Syndrome Scale, HAMD-17: Hamilton Depression Rating Scale, 17-item version, 95%CI: 95% confidence interval, 95%PI: 95% prediction interval.

The present results showed that the negative symptoms of schizophrenia were negatively correlated with CSF oxytocin levels. The correlation coefficient between CSF oxytocin levels and total PANSS score was also significant, controlling for SGA dose. Rubin et al. (2010) reported that higher peripheral oxytocin levels were associated with more prosocial behaviors in female patients with schizophrenia. Furthermore, previous studies have demonstrated improvement of social behaviors with administration of intranasal oxytocin (Macdonald and Macdonald, 2010; Pedersen et al., 2011). Since strong relationships between negative symptoms and social difficulties have been demonstrated in schizophrenia (Weinberg et al., 2009), the present finding associating higher CSF oxytocin levels with lower negative subscale is in accord with what has previously been described for peripheral oxytocin. Whether the peripheral oxytocin levels reflect the CSF oxytocin levels, or whether a different mechanisms of action in the brain and the peripheral result in a similar effect, remains to be explored.

Previous studies examining CSF oxytocin levels in patients with schizophrenia (Beckmann et al., 1985; Glovinsky et al., 1994) and depression (Pitts et al., 1995) showed mean oxytocin levels of less than 10 pg/ml, which is lower than that in the present study (>20 pg/ml). Such outcome may have resulted from some of the methodological differences between previous studies and the present one. Previous three studies measured oxytocin levels using radioimmunoassay (RIA), while the present study used a commercially available ELISA kit. A recent study that used the same ELISA kit to measure CSF oxytocin levels (Heim et al., 2009) also demonstrated higher levels of oxytocin (mean oxytocin levels of 17 pg/ml in women without a history of emotional abuse) compared to the previous studies using RIA. Thus, the different measurement techniques may have influenced the values.

A number of other methodological differences exist between the present study and previous ones examining CSF oxytocin levels (Beckmann et al., 1985; Glovinsky et al., 1994; Pitts et al., 1995). One of the major differences was that the present study did not require fasting prior to lumbar puncture, while Beckmann et al (Beckmann et al., 1985) collected CSF in patients with schizophrenia after 12 hours fasting. Although a previous study (Challinor et al., 1994) reported that peripheral oxytocin levels were not affected by 20 hours of fasting, the influence of fasting on CSF levels is unknown. Furthermore, Beckmann et al used Research Diagnostic Criteria to select a patient group consisting entirely of paranoid schizophrenia. Such difference in composition of participants may have affected the outcome of the study by Beckmann et al (1985), which showed significantly higher CSF oxytocin levels in schizophrenic patients compared to healthy controls. The findings by Glovinsky et al (1994) and Pitts et al (1995) were consistent with the present study in that no significant difference in CSF oxytocin levels was found between patients and controls. However,

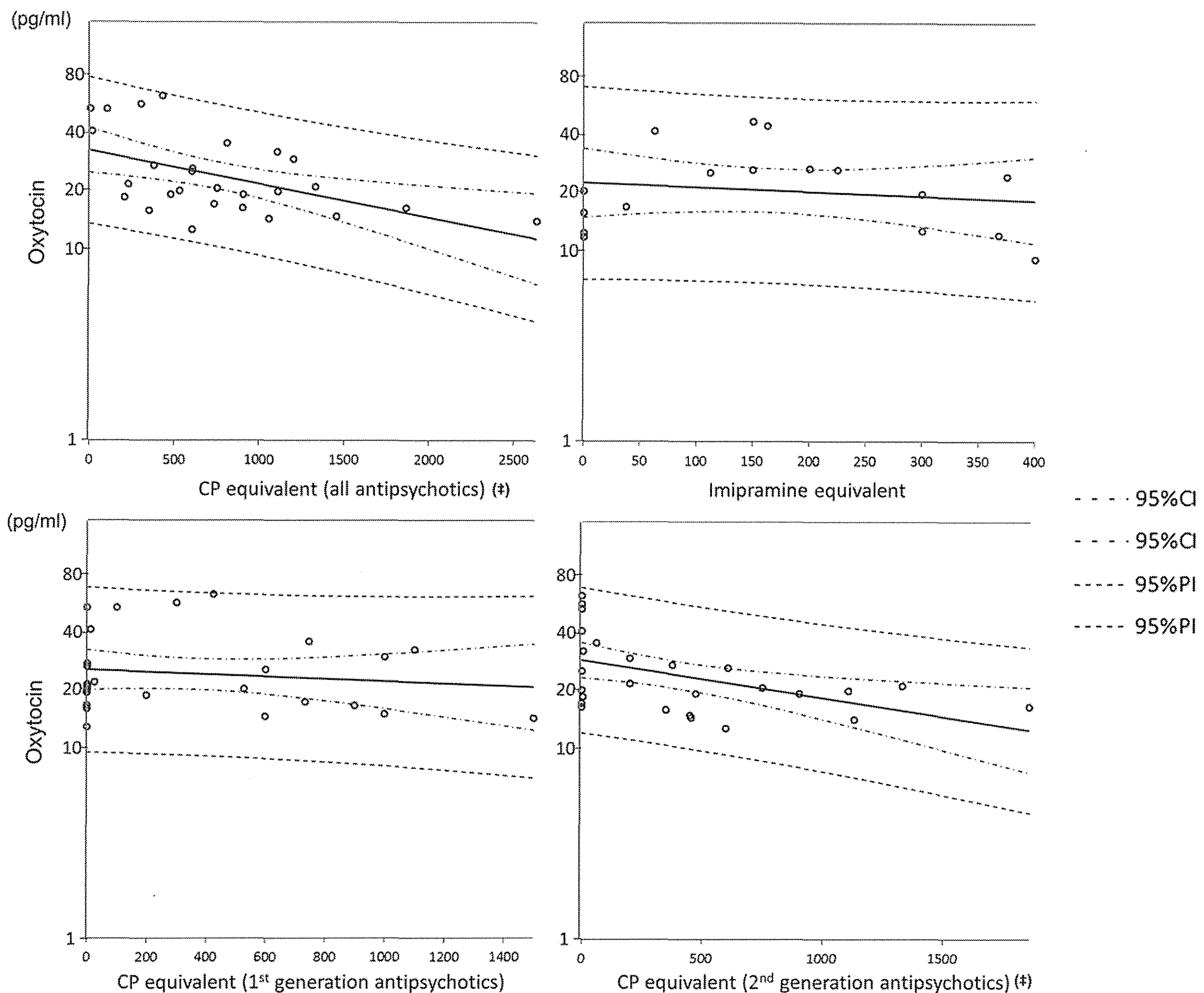

Fig. 3. Relationship between cerebrospinal fluid oxytocin levels and dose of psychotropics. The association between cerebrospinal oxytocin levels and dose of psychotropics is shown. Oxytocin levels are shown in logarithmic scale. Solid lines indicate fitted regression lines, unevenly dashed lines indicate 95% confidence intervals, and evenly dashed lines indicate 95% prediction intervals. (‡): Correlation at significance level of $P<0.01$. CP equivalent: chlorpromazine equivalent, 95%CI: 95% confidence interval, 95%PI: 95% prediction interval.

participants in these studies also differed from that of the present study in that both genders were included. Furthermore, MDD patients in the study by Pitts et al (1995) all scored 18 or above on the HAMD-17, while the MDD patients in the present study included those in a remitted state. These differences in composition of study samples should be carefully considered when comparing findings across studies.

Some limitations must be considered when interpreting the results of this study. First, the effects of medication could not be fully controlled due to the variability in types and doses. Future studies should examine oxytocin levels in untreated patients to elucidate the role of oxytocin in the pathophysiology of schizophrenia and depression. Treatment duration may also affect oxytocin levels. However, since all of the schizophrenic patients that were prescribed antipsychotics were on chronic treatment with the medication, treatment duration is unlikely to have confounded the main findings of the present study. Secondly, as mentioned above, the cross-sectional design did not allow for any definitive conclusions regarding the causal relationship between the CSF oxytocin levels, psychotropic medication, and symptom severity. Thirdly, only male participants were included in the present study. Previous studies suggest that effects of peripheral and intranasal oxytocin may differ between men and women (Domes et al., 2010; Rubin et al., 2010, 2011). Therefore, the present findings cannot be generalized to women. Finally, the risk of type II error was high due to the small sample size. The sample size in the present study was comparable to those of the previous studies that examined CSF oxytocin levels in patients with schizophrenia and depression (Beckmann et al., 1985; Glovinsky et al., 1994; Pitts et al., 1995). However, the power to detect a moderate difference (effect size of 0.50) in CSF oxytocin levels between patients and controls was relatively low (schizophrenia: 39%; MDD: 32%; calculated by G*Power 3.1.3 (Faul et al., 2007)). A larger sample may be necessary to detect small to moderate change in CSF oxytocin levels in psychiatric disorders.

In conclusion, we obtained no evidence of altered CSF oxytocin levels in patients with schizophrenia or those with MDD. However, lower CSF oxytocin levels may be related to higher SGA dose and more severe negative symptoms in schizophrenia, which is in line with the possibility that central oxytocin may ameliorate the severity of some symptoms of schizophrenia by improving social functioning.

Role of the funding source

This study was supported by Health and Labor Sciences Research Grants (Comprehensive Research on Disability, Health, and Welfare), the Strategic Research Program for Brain Sciences by the Ministry of Education, Culture, Sports, Science and Technology of Japan (Understanding of molecular and environmental bases for brain health), Intramural Research Grant for Neurological and Psychiatric Disorders of NCNP (H.K.), Takeda Science Foundation, and Mitsubishi Pharma Research Foundation. They played

no role in the study design; the collection, analysis and interpretation of data, in the writing of the report; and in the decision to submit the paper for publication.

Contributors

Daimei Sasayama and Kotaro Hattori designed the study. Daimei Sasayama, Kotaro Hattori, and Toshiya Teraishi performed the lumbar punctures. Daimei Sasayama, Kotaro Hattori, Toshiya Teraishi, Hiroaki Hori, Miho Ota, Sumiko Yoshida, Kunimasa Arima, and Hiroshi Kunugi screened and diagnosed the study participants. Daimei Sasayama wrote the draft of the manuscript. Hiroshi Kunugi supervised the writing of the paper. Teruhiko Higuchi and Naoji Amano gave critical comments on the manuscript. All authors contributed to and have approved the final manuscript.

Conflict of interest statement

The authors declare no conflicts of interest.

Acknowledgements

The authors thank the members of the Translational Medical Center, National Center of Neurology and Psychiatry for their dedicated efforts in achieving the reported results.

References

American Psychiatric Association, 1994. DSM-IV: Diagnostic and Statistical Manual of Mental Disorders, 4th edition. American Psychiatric Press, Washington D.C.

Bartz, J.A., Zaki, J., Ochsner, K.N., Bolger, N., Kolevzon, A., Ludwig, N., Lydon, J.E., 2010. Effects of oxytocin on recollections of maternal care and closeness. Proc. Natl. Acad. Sci. U. S. A. 107 (50), 21371–21375.

Beckmann, H., Lang, R.E., Gattaz, W.F., 1985. Vasopressin–oxytocin in cerebrospinal fluid of schizophrenic patients and normal controls. Psychoneuroendocrinology 10 (2), 187–191.

Challinor, S.M., Winters, S.J., Amico, J.A., 1994. Pattern of oxytocin concentrations in the peripheral blood of healthy women and men: effect of the menstrual cycle and short-term fasting. Endocr. Res. 20 (2), 117–125.

Couture, S.M., Penn, D.L., Roberts, D.L., 2006. The functional significance of social cognition in schizophrenia: a review. Schizophr. Bull. 32 (Suppl. 1), S44–S63.

Cyranowski, J.M., Hofkens, T.L., Frank, E., Seltman, H., Cai, H.M., Amico, J.A., 2008. Evidence of dysregulated peripheral oxytocin release among depressed women. Psychosom. Med. 70 (9), 967–975.

Domes, G., Lischke, A., Berger, C., Grossmann, A., Hauenstein, K., Heinrichs, M., Herpertz, S.C., 2010. Effects of intranasal oxytocin on emotional face processing in women. Psychoneuroendocrinology 35 (1), 83–93.

Durham, D.A., Banks, W.A., Kastin, A.J., 1991. Carrier-mediated transport of labeled oxytocin from brain to blood. Neuroendocrinology 53 (5), 447–452.

Ermisch, A., Barth, T., Ruhle, H.J., Skopkova, J., Hrbas, P., Landgraf, R., 1985. On the blood–brain barrier to peptides: accumulation of labelled vasopressin, DesGlyNH2-vasopressin and oxytocin by brain regions. Endocrinol. Exp. 19 (1), 29–37.

Faul, F., Erdfelder, E., Lang, A.G., Buchner, A., 2007. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav. Res. Methods 39 (2), 175–191.

Feifel, D., Reza, T., 1999. Oxytocin modulates psychotomimetic-induced deficits in sensorimotor gating. Psychopharmacology (Berl.) 141 (1), 93–98.

Feifel, D., Macdonald, K., Nguyen, A., Cobb, P., Warlan, H., Galangue, B., Minassian, A., Becker, O., Cooper, J., Perry, W., Lefebvre, M., Gonzales, J., Hadley, A., 2010. Adjunctive intranasal oxytocin reduces symptoms in schizophrenia patients. Biol. Psychiatry 68 (7), 678–680.

Glovinsky, D., Kalogeras, K.T., Kirch, D.G., Suddath, R., Wyatt, R.J., 1994. Cerebrospinal fluid oxytocin concentration in schizophrenic patients does not differ from control subjects and is not changed by neuroleptic medication. Schizophr. Res. 11 (3), 273–276.

Hamilton, M., 1967. Development of a rating scale for primary depressive illness. Br. J. Soc. Clin. Psychol. 6 (4), 278–296.

Heim, C., Young, L.J., Newport, D.J., Mletzko, T., Miller, A.H., Nemeroff, C.B., 2009. Lower CSF oxytocin concentrations in women with a history of childhood abuse. Mol. Psychiatry 14 (10), 954–958.

Igarashi, Y., Hayashi, N., Yamashina, M., Otsuka, N., Kuroki, N., Anzai, N., Kazamatsuri, H., 1998. Interrater reliability of the Japanese version of the Positive and Negative Syndrome Scale and the appraisal of its training effect. Psychiatry Clin. Neurosci. 52 (5), 467–470.

Inagaki, A., Inada, T., Fujii, Y., Yagi, G., 1999. Equivalent Dose of Psychotropics. Seiwa Shoten, Tokyo.

Inoue, Y., Tonooka, Y., Yamada, K., Kanba, S., 2004. Deficiency of theory of mind in patients with remitted mood disorder. J. Affect. Disord. 82 (3), 403–409.

Kay, S.R., Fiszbein, A., Opler, L.A., 1987. The Positive and Negative Syndrome Scale (PANSS) for schizophrenia. Schizophr. Bull. 13 (2), 261–276.

Kiss, A., Bundzikova, J., Pirnik, Z., Mikkelsen, J.D., 2010. Different antipsychotics elicit different effects on magnocellular oxytocinergic and vasopressinergic neurons as revealed by Fos immunohistochemistry. J. Neurosci. Res. 88 (3), 677–685.

Lim, M.M., Young, L.J., 2006. Neuropeptidergic regulation of affiliative behavior and social bonding in animals. Horm. Behav. 50 (4), 506–517.

Linkowski, P., Geenen, V., Kerkhofs, M., Mendlewicz, J., Legros, J.J., 1984. Cerebrospinal fluid neurophysins in affective illness and in schizophrenia. Eur. Arch. Psychiatry Neurol. Sci. 234 (3), 162–165.

Macdonald, K., Macdonald, T.M., 2010. The peptide that binds: a systematic review of oxytocin and its prosocial effects in humans. Harv. Rev. Psychiatry 18 (1), 1–21.

Mai, J.K., Berger, K., Sofroniew, M.V., 1993. Morphometric evaluation of neurophysin-immunoreactivity in the human brain: pronounced inter-individual variability and evidence for altered staining patterns in schizophrenia. J. Hirnforsch. 34 (2), 133–154.

Mantella, R.C., Vollmer, R.R., Li, X., Amico, J.A., 2003. Female oxytocin-deficient mice display enhanced anxiety-related behavior. Endocrinology 144 (6), 2291–2296.

Mantella, R.C., Vollmer, R.R., Amico, J.A., 2005. Corticosterone release is heightened in food or water deprived oxytocin deficient male mice. Brain Res. 1058 (1–2), 56–61.

Montag, C., Ehrlich, A., Neuhaus, K., Dziobek, I., Heekeren, H.R., Heinz, A., Gallinat, J., 2010. Theory of mind impairments in euthymic bipolar patients. J. Affect. Disord. 123 (1–3), 264–269.

Onaka, T., 2004. Neural pathways controlling central and peripheral oxytocin release during stress. J. Neuroendocrinol. 16 (4), 308–312.

Otsubo, T., Tanaka, K., Koda, R., Shinoda, J., Sano, N., Tanaka, S., Aoyama, H., Mimura, M., Kamijima, K., 2005. Reliability and validity of Japanese version of the Mini-International Neuropsychiatric Interview. Psychiatry Clin. Neurosci. 59 (5), 517–526.

Pedersen, C.A., Gibson, C.M., Rau, S.W., Salimi, K., Smedley, K.L., Casey, R.L., Leserman, J., Jarskog, L.F., Penn, D.L., 2011. Intranasal oxytocin reduces psychotic symptoms and improves Theory of Mind and social perception in schizophrenia. Schizophr. Res. 132 (1), 50–53.

Pitts, A.F., Samuelson, S.D., Meller, W.H., Bissette, G., Nemeroff, C.B., Kathol, R.G., 1995. Cerebrospinal fluid corticotropin-releasing hormone, vasopressin, and oxytocin concentrations in treated patients with major depression and controls. Biol. Psychiatry 38 (5), 330–335.

Rubin, L.H., Carter, C.S., Drogos, L., Pournajafi-Nazarloo, H., Sweeney, J.A., Maki, P.M., 2010. Peripheral oxytocin is associated with reduced symptom severity in schizophrenia. Schizophr. Res. 124 (1–3), 13–21.

Rubin, L.H., Carter, C.S., Drogos, L., Jamadar, R., Pournajafi-Nazarloo, H., Sweeney, J.A., Maki, P.M., 2011. Sex-specific associations between peripheral oxytocin and emotion perception in schizophrenia. Schizophr. Res. 130 (1–3), 266–270.

Ryden, G., Sjoholm, I., 1969. Half-life of oxytocin in blood of pregnant and non-pregnant women. Acta Endocrinol. (Copenh.) 61 (3), 425–431.

Scantamburlo, G., Hansenne, M., Fuchs, S., Pitchot, W., Marechal, P., Pequeux, C., Ansseau, M., Legros, J.J., 2007. Plasma oxytocin levels and anxiety in patients with major depression. Psychoneuroendocrinology 32 (4), 407–410.

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.

Sim, K., Su, H.C., Fujii, S., Yang, S.Y., Chong, M.Y., Ungvari, G., Si, T., He, Y.L., Chung, E.K., Chan, Y.H., Shinfuku, N., Kua, E.H., Tan, C.H., Sartorius, N., 2009. High-dose antipsychotic use in schizophrenia: a comparison between the 2001 and 2004 Research on East Asia Psychotropic Prescription (REAP) studies. Br. J. Clin. Pharmacol. 67 (1), 110–117.

Sirzen-Zelenskaya, A., Gonzalez-Iglesias, A.E., Boutet de Monvel, J., Bertram, R., Freeman, M.E., Gerber, U., Egli, M., 2011. Prolactin induces a hyperpolarising current in rat paraventricular oxytocinergic neurones. J. Neuroendocrinol. 23 (10), 883–893.

Sparks, A., McDonald, S., Lino, B., O'Donnell, M., Green, M.J., 2010. Social cognition, empathy and functional outcome in schizophrenia. Schizophr. Res. 122 (1–3), 172–178.

Tabuse, H., Kalali, A., Azuma, H., Ozaki, N., Iwata, N., Naitoh, H., Higuchi, T., Kanba, S., Shioe, K., Akechi, T., Furukawa, T.A., 2007. The new GRID Hamilton Rating Scale for Depression demonstrates excellent inter-rater reliability for inexperienced and experienced raters before and after training. Psychiatry Res. 153 (1), 61–67.

Uvnas-Moberg, K., Alster, P., Svensson, T.H., 1992. Amperozide and clozapine but not haloperidol or raclopride increase the secretion of oxytocin in rats. Psychopharmacology (Berl.) 109 (4), 473–476.

Uvnas-Moberg, K., Bjokstrand, E., Hillegaart, V., Ahlenius, S., 1999. Oxytocin as a possible mediator of SSRI-induced antidepressant effects. Psychopharmacology (Berl.) 142 (1), 95–101.

Weinberg, D., Shahar, G., Davidson, L., McGlashan, T.H., Fennig, S., 2009. Longitudinal associations between negative symptoms and social functioning in schizophrenia: the moderating role of employment status and setting. Psychiatry 72 (4), 370–381.

Wolkenstein, L., Schonenberg, M., Schirm, E., Hautzinger, M., 2011. I can see what you feel, but I can't deal with it: impaired theory of mind in depression. J. Affect. Disord. 132 (1–2), 104–111.

Yamada, H., Masui, K., Kikuimoto, K., 1991. The Japanese version of The Positive and Negative Syndrome Scale (PANSS) Rating Manual. Seiwa, Tokyo.

Biological Psychology 89 (2012) 117–122

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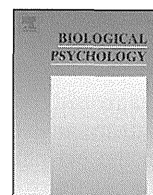

Blood *CADPS2ΔExon3* expression is associated with intelligence and memory in healthy adults

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ARTICLE INFO

Article history:
Received 7 June 2011
Accepted 26 September 2011
Available online 12 October 2011

Keywords:
CAPS2
Splicing variant
Intelligence quotient
Memory
Autistic disorder
BDNF
Dopamine

ABSTRACT

Ca^{2+}-dependent activator protein for secretion 2 (CADPS2), a secretory granule associate protein, mediates monoamine transmission and neurotrophin release. Both monoamines and neurotrophins play a crucial role in cognition, learning and memory. An aberrant splice variant of *CADPS2*, *CADPS2ΔExon3*, was reported to be associated with autism. Therefore, we examined the possible association between the expression of *CADPS2/CADPS2ΔExon3* in peripheral blood and brain functions such as intelligence and memory. Quantitative polymerase chain reaction analysis was performed in 271 healthy adults (age range 20–74 years, mean ± SD 43.3 ± 15.3). Data on intelligence quotient (IQ) and memory were obtained by using full versions of the Wechsler Adult Intelligence Scale-Revised (WAIS-R), and the Wechsler Memory Scale-Revised (WMS-R), respectively. *CADPS2* expression levels were not significantly associated with any scores/sub-scores of these scales. However, *CADPS2ΔExon3* expression was significantly associated with lower IQ ($p = 0.022$; effect size: $\eta_p^2 = 0.031$), particularly verbal IQ of WAIS-R ($p = 0.019$; $\eta_p^2 = 0.032$), lower verbal memory ($p = 0.026$; $\eta_p^2 = 0.026$) and delayed recall ($p = 0.042$; $\eta_p^2 = 0.021$) of WMS-R. Our results suggest that CADPS2ΔExon3 affects intelligence and memory in the non-clinical population.

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1. Introduction

Ca^{2+}-dependent activator protein for secretion 2 (CADPS2) is a secretory granule-associated protein involved in the release of neurotrophins such as brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3). Mouse CADPS2 protein is associated with BDNF-containing secretory vesicles and promotes activity-dependent release of BDNF (Sadakata et al., 2004). Accordingly, BDNF release is significantly reduced in the cultured neurons prepared from the cerebellum, neocortex and hippocampus of CADPS2 deficient mice (Sadakata et al., 2007a, 2007b).

BDNF plays a crucial role in the development and maintenance of brain function, including formation of synapses and neural circuits. Reduced long-term potentiation and impaired spatial memory have been reported in conditional BDNF deficient mice or mice after infusion of antisense BDNF (Mizuno et al., 2000; Monteggia et al., 2004). A polymorphism in *BDNF*, Val66Met was reported to affect human memory and hippocampal activity (Egan et al., 2003). That polymorphism may also affect intelligence (Tsai et al., 2004), and susceptibility to psychiatric disorders including depression, schizophrenia (Rybakowski, 2008) and Alzheimer's disease (Fukumoto et al., 2010), although there are also negative reports; i.e. cognition (Houlihan et al., 2009), memory (Strauss et al., 2004), psychiatric disorders (Naoe et al., 2007; Zhang et al., 2006).

CADPS2 also mediates monoamine transmission. CADPS2, together with its family protein, CADPS1, mediates the refilling of catecholamine to the releasable vesicles, and catecholamine secretion is significantly suppressed in the CADPS1/2 double deficient cells (Liu et al., 2008). Another study supports that CADPS2 is involved in monoamine storage as antibodies against CADPS2 inhibit monoamine sequestration by synaptic vesicles (Brunk et al., 2009). Monoamine-containing neurons project to diverse brain regions including the hippocampus, neocortex, amygdala and neocortex, and regulate the mode of their function (Robbins and Arnsten, 2009). Dopamine neurotransmission is critical for basic reinforcement learning, noradrenalin modulate attention/concentration, while serotonin mediates cognitive flexibility (Kehagia et al., 2010). CADPS2's roles in synaptic functions suggest that CADPS2 may also mediate human brain functions, especially in learning, memory and cognition.

The regulation of learning/memory by CADPS2 could also be developmental. A comprehensive voxelwise genome-wide association study (GWAS) study found that a single nucleotide polymorphism (SNP) in *CADPS2* was associated with brain structure (Stein et al., 2010). In that study, the association between whole voxels from brain images of the 740 elderly subjects and SNPs were

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doi:10.1016/j.biopsycho.2011.09.017

analyzed, and the *CADPS2* SNP was found to be associated with temporal lobe volume, the region especially crucial for memory.

There is also a link between *CADPS2* expression and human brain disorders. Aberrant splicing of *CADPS2* mRNA was reported in autism; an exon-3 skipped isoform, *CADPS2ΔExon3* was detected in the peripheral blood samples of several autistic patients but not in those of healthy controls (Sadakata et al., 2007b). The authors showed that CADPS2ΔExon3 protein was deficient in proper axonal transport, which results in the loss of local synaptic BDNF release. While the relationship of *CADPS2ΔExon3* expression in the brains and autism is unclear, the aberrant splicing of *CADPS2* could contribute to autism susceptibility by affecting neurotrophin and/or monoamine release.

Previously, we found that both *CADPS2* and *CADPS2ΔExon3* expression were increased in the post-mortem brains of schizophrenic patients (Hattori et al., 2011). We also detected *CADPS2ΔExon3* in the blood of both schizophrenic patients and control subjects. There were more *CADPS2ΔExon3* positive subjects in the schizophrenic patients than in the controls, although the difference was not statistically significant.

To get more insight into CADPS2's role in human brain function, the present study examined the possible association between the blood expression levels of *CADPS2/CADPS2ΔExon3* and intelligence/memory in healthy subjects. Considering the continuity between developmental disorders and healthy state (Bishop, 1989; Volkmar et al., 2004), intermediate phenotypes related to the developmental disorders should also be expressed in "healthy" subjects and might be associated with *CADPS2/CADPS2ΔExon3* expression levels. We applied quantitative PCR, a more reliable method, to detect each transcript rather than evaluating electrophoresis bands, applied in the past studies (Eran et al., 2009; Sadakata et al., 2007b). As a result, we found that *CADPS2ΔExon3* expression was associated with lower intelligence and memory. To our knowledge, this is a novel finding, which is likely to have relevance to the susceptibility to autism and learning disorders.

2. Subjects and methods

2.1. Participants

Subjects were 271 healthy volunteers [67 males and 204 females; age range 20–74 years; mean age 43.3 ± 15.3 (standard deviation: SD) years] recruited through advertisements in free local magazines and our website announcement. All subjects were biologically unrelated healthy Japanese from the same geographical area (Western part of Tokyo Metropolitan). They were interviewed by the Japanese version of the Mini-International Neuropsychiatric Interview (M.I.N.I.) (Otsubo et al., 2005; Sheehan et al., 1998) by a research psychiatrist, and those who had a current history of psychiatric disorder were not enrolled in the study. In addition, those individuals who demonstrated one or more of the following conditions in a non-structured interview performed by an experienced psychiatrist were excluded from this study: past or current regular contact to psychiatric services, having a history of regular use of psychotropics or substance abuse/dependence, presenting other obvious self-reported signs of past primary psychotic and mood disorders, and having a prior medical history of central nervous system disease or severe head injury. After the nature of the study procedures had been fully explained, written informed consent was obtained from every subject. The study was conducted in accordance with the Declaration of Helsinki and approved by the ethics committee of the National Center of Neurology and Psychiatry, Japan.

2.2. Sample preparation

Blood collection and RNA isolation was performed using the PAXgene blood RNA system (Qiagen, Valencia, CA) as described previously (Hattori et al., 2011). Blood samples were collected around 11 A.M. Extracted RNA was quantified by optical density reading at 260 nm using NanoDrop ND-1000 (Thermo Scientific, Rockford, IL). Samples that contained more than 40 ng/μl of total RNA were used for analysis; 8 μl from each sample was reverse transcribed using SuperScript VILO cDNA Synthesis Kit (Invitrogen, Carlsbad, CA).

2.3. Quantitative real-time polymerase chain reaction

Polymerase chain reaction (PCR) amplifications were performed in triplicate (5 μl volume) on 384-well plates using ABI prism 7900HT (Applied Biosystems, Foster City, CA) as described previously (Hattori et al., 2011). Each reaction contained 0.28 μl of cDNA sample, qPCR QuickGoldStar Mastermix Plus (Eurogentec, Seraing, Belgium) and a primer of the target, i.e. *CADPS2* (Hs01095968_m1 at Exons 4–5, on NM_017954.9), *CADPS2ΔExon3* (forward primer: GTAGCTGACGAAGCATTTTGCA, reverse Primer: TGATCTGGGCTGCTTGTTCAT, reporter: CTGCGTTATCCAGCTCAT) and a primer of the housekeeping gene, Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) (4326317E), all purchased from Applied Biosystems. Negative control reactions were carried out with "no RNA" samples. The real time PCR reactions ran at 50 °C for 2 min, 95 °C for 10 min and in 40 (for *CADPS2* and *GAPDH*) or 45 (for *CADPS2ΔExon3*) cycles changing between 95 °C for 15 s and 60 °C for 1 min. Data were analyzed using the Sequence Detection System (SDS) 2.0 software (Applied Biosystems) as follows. A standard amplification curve was made by serial dilution of a "standard" pooled cDNA sample in each plate. The mean value of triplicate of each sample was normalized to the standard curve. Then the values of *CADPS2* from each sample were normalized to those of *GAPDH*. With respect to *CADPS2ΔExon3*, we counted the number of tubes in which signals were detected, among triplicates, as reported previously (Hattori et al., 2011). In brief, for each tube, we defined 'detected' if the signal reached a threshold automatically set by the SDS 2.0 software within 45 cycles, and a threshold cycle (Ct) value was obtained. Second, we counted the number of 'detected' tubes of each triplicate (Supplemental Fig. S1). Third, we defined 'positive' when 2 or 3 tubes in triplicate analysis of each sample were detected as we assumed that the 'detection' should be repeated at least once. We defined 'negative' when no tube was detected. The samples with only one-tube detection were excluded from the statistical comparison between individuals with *CADPS2ΔExon3* positive and those with *CADPS2ΔExon3* negative. To avoid an arbitrary interpretation, we also performed statistical analyses including one-tube detection and dividing subjects into 3 groups (negative, one-tube detection, and positive).

Table 1
Demographic information and *CADPS2ΔExon3* expression levels of participants.

	N	Age (SD)	Number of tubes with *CADPS2ΔExon3* detection		
			0	1	2–3
WAIS-R					
Male	54	43.5 (15.6)	36	12	6
Female	185	45.8 (14.6)	114	52	19
Total	239	45.2 (14.8)	150	64	25
WMS-R					
Male	67	40.6 (15.5)	46	13	8
Female	199	43.9 (15.2)	122	56	21
Total	266	43.1 (15.3)	168	69	29

2.4. Neuropsychological test measures

To assess memory and intelligence, the Japanese full versions of the Wechsler Memory Scale-Revised (WMS-R) (Sugishita, 2001) and the Wechsler Adult Intelligence Scale-Revised (WAIS-R) (Shinagawa et al., 1990) respectively, were administered.

2.5. Statistical analyses

CADPS2 expression levels were converted to a -10 logarithmic scale before statistical analysis in order to obtain a normal distribution (Castensson et al., 2005) as reported previously (Hattori et al., 2011). One extremely high value of *CADPS2* expression was excluded. The relationship between *CADPS2* expression and each score was analyzed by Spearman correlation test. The effect of *CADPS2* or *CADPS2ΔExon3* expression on intelligence or memory was assessed by multiple analysis of covariance (MANCOVA), controlling for age, sex, and education years. These analyses were performed by SPSS software version 11 (SPSS Japan, Tokyo, Japan).

3. Results

First, we analyzed the association between blood *CADPS2* expression and IQ and memory indices. Spearman correlation analyses did not detect any significant correlation between blood *CADPS2* expression levels and IQ scores (Supplemental Table S1). Among WMS-R scores, verbal memory and general memory tended to correlate with *CADPS2* expression levels (Supplemental Table S1). However, no significant effect of *CADPS2* expression was detected on those scores when age, sex and education years were controlled for ($p = 0.15$ for verbal memory and $p = 0.21$ for general memory).

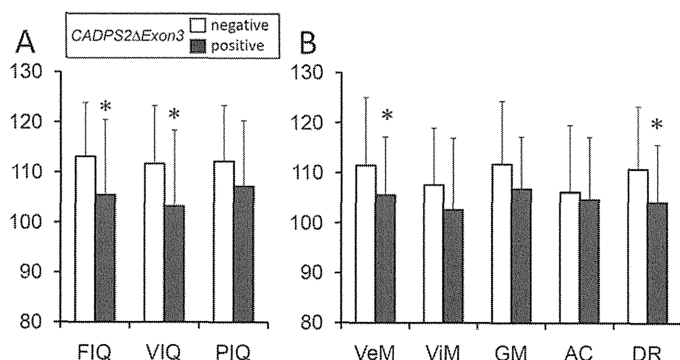

Fig. 1. Association between IQ/memory and *CADPS2 Δ Exon3* expression. WAIS-R scores and WMS-R scores were compared between those who did not (open-bar) and did (filled-bar) express *CADPS2ΔExon3* in the blood. (A) IQ and *CADPS2ΔExon3* expression. FIQ, full-scale IQ; VIQ, verbal IQ; PIQ, performance IQ. (B) Memory and *CADPS2ΔExon3* expression. VeM, verbal memory; ViM, visual memory; GM, general memory; AC, attention and concentration; DR, delayed recall. Data are mean ± SD; *$p<0.05$, MANCOVA controlled for sex, age, and education years.

Then, we analyzed the possible association of *CADPS2ΔExon3* expression with IQ and memory. As reported previously (Hattori et al., 2011), the expression level was very low and no expression was detected for the majority of samples. Thus, we counted the number of signal-detected tubes among triplicate analyses of each sample (Supplemental Fig. S1, Table 1).

With respect to WAIS-R, full-scale IQ (FIQ) was significantly lower in the *CADPS2ΔExon3* positive group, compared with that of the negative group ($F=5.3$, df = 1, $p=0.022$, $\eta_p^2=0.031$, Fig. 1A). When verbal IQ (VIQ) and performance IQ (PIQ) were examined separately, VIQ ($F=5.6$, df = 1, $p=0.019$, $\eta_p^2=0.032$) was significantly lower in the positive group.

With respect to WMS-R, verbal memory ($F=5.0$, df = 1, $p=0.026$, $\eta_p^2=0.026$) and delayed recall ($F=4.2$, df = 1, $p=0.042$, $\eta_p^2=0.021$) were significantly lower in the positive group compared with the negative group (Fig. 1B).

Even if one-tube detection was included in the analysis, the results were essentially the same. With respect to WAIS-R, there were marginal effects of *CADPS2ΔExon3* expression levels on FIQ ($F=2.33$, df = 2, $p=0.099$, $\eta_p^2=0.020$) and VIQ ($F=2.57$, df = 2, $p=0.079$, $\eta_p^2=0.022$) and the *post hoc* tests detected significant reduction of FIQ ($p=0.036$) and VIQ ($p=0.026$) in the positive group compared to negative group (Supplemental Fig. S2A). With respect to WMS-R, significant effects of expression level were detected on verbal memory ($F=4.5$, df = 2, $p=0.012$, $\eta_p^2=0.034$) and delayed recall ($F=5.8$, df = 2, $p=0.003$, $\eta_p^2=0.043$). A marginal effect on general memory ($F=3.0$, df = 2, $p=0.051$, $\eta_p^2=0.023$) was also detected. The *post hoc* tests detected significant reduction of verbal memory ($p=0.028$) and delayed recall ($p=0.001$) in the positive group compared to the negative group (Supplemental Fig. S2B).

When males and females were analyzed separately, statistically significant differences were detected only in females with respect to FIQ, VIQ, visual memory, general memory and delayed recall (Supplemental Fig. S3). Nonetheless, average scores of these tests were lower in the *CADPS2ΔExon3* positive group of the male subjects than in the negative group. The failure to reach statistical significance is likely to be ascribed to the lack of statistical power due to the small number of male subjects.

It is possible that we might have removed cognitive ability variance when education years were controlled for. To examine this possibility, we performed an additional analysis in which education was not controlled for. However, the results were essentially unchanged; *CADPS2ΔExon3* expression levels were significantly associated with FIQ ($F=6.3$, df = 1, $p=0.013$, $\eta_p^2=0.036$), VIQ ($F=6.7$, df = 1, $p=0.011$, $\eta_p^2=0.038$), verbal memory ($F=5.1$, df = 1, $p=0.025$, $\eta_p^2=0.026$) and delayed recall ($F=4.5$, df = 1, $p=0.035$, $\eta_p^2=0.023$).

4. Discussion

In the present study, we examined the possible association between the expression of *CADPS2* transcripts (*CADPS2* and *CADPS2ΔExon3*) in the peripheral blood and higher brain functions such as intelligence and memory in healthy subjects. While *CADPS2* expression levels were not associated with the scores of these measurements, *CADPS2ΔExon3* expression was significantly associated with lower IQ, lower verbal memory and delayed recall of WMS-R.

4.1. Evaluation of CADPSΔExon3 expression levels

Because there were relatively large number of 1-tube detection samples, we suppose that there are continuity between negative and positive samples, and 1-tube detection might stochastically reflects the expression levels between negative (0) and positive (>1). Since inclusion or exclusion of 1-tube detection samples in the criteria did not affect the results essentially, our conclusion; the expression of *CADPS2Δ3* was associated with cognition and memory, was supported. However, because the expression levels might continuous rather than qualitative values, future studies should improve the sensitivity of analyses, i.e. by using larger sample volume.

4.2. Did the participants include autism?

It has been reported that *CADPS2ΔExon3* was present in individuals with autism but not in controls (Sadakata et al., 2007b). In the present study, all participants were screened for current and past psychiatric histories by experienced psychiatrists using structured (M.I.N.I.) and unstructured interviews. As the M.I.N.I. is not designed to diagnose autism, there remains the possibility that some patients with mild, high functioning autism could have been included. However, interviews by experienced psychiatrists did not detect any subject who could be diagnosed as autism or other pervasive developmental disorders. Thus, our results suggest that *CADPS2ΔExon3* may be positive even in non-autistic individuals. Rather, *CADPS2ΔExon3* is likely to be present in individuals with lower VIQ and lower memory function which may be intermediate phenotypes of autism (see below).

4.3. Autism and intelligence

Approximately three-quarters of individuals with autism have low (<70) full-IQ scores (Yeargin-Allsopp et al., 2003). With respect to profiles of IQ, Lincoln et al. reported depressed verbal IQ relative to performance IQ (VIQ < PIQ) in autism (Lincoln et al., 1988), although inconsistent findings (no difference or VIQ < PIQ) have also been reported (Ehlers et al., 1997; Siegel et al., 1996; Williams et al., 2008). Thus, *CADPS2ΔExon3* positive subjects partly share similar cognitive deficits with autism.

4.4. Autism and memory

Similar to *CADPS2ΔExon3* positive subjects, adults with high functioning autism were reported to have impaired memory functions (Bennetto et al., 1996; Minshew and Goldstein, 2001; Steele et al., 2007; Williams et al., 2005b). Not all but several studies have shown that the impairments were prominent in visual memories especially for human faces rather than verbal memories (Hillier et al., 2007; Williams et al., 2005a, 2006). In the case of *CADPS2ΔExon3* positive subjects in the present study,

although visual memory also tended to be lower, the significant reduction was rather detected in verbal memory. The different memory profiles between previous findings in autistic adults and our *CADPS2ΔExon3*-positive subjects might be partly due to the reduced IQ in our *CADPS2ΔExon3* positive subjects, while the previous studies compared memory between patients with high functioning autism and IQ-matched controls.

4.5. Implications on schizophrenia susceptibility

Previously, we analyzed *CADPS2/CADPS2ΔExon3* expression both in the post-mortem brains of psychiatric patients from the Stanley neuropathology consortium, consisting of 15 patients with schizophrenia, 15 with depression, 15 with bipolar disorder and 15 control subjects and bloods of 121 schizophrenic patients and 318 controls (Hattori et al., 2011). In the brain samples, we found that not only *CADPS2* but also *CADPS2ΔExon3* was significantly increased in the schizophrenic group. These changes were not observed in other psychiatric disorders. We also found that the ratio of blood *CADPS2ΔExon3* positive subjects was higher in the schizophrenic group (21 out of 121 patients, ratio = 0.17) compared to the control group (36 out of 318, ratio= 0.11), although the difference in the ratio was not statistically significant.

Of all cognitive domains, verbal memory is one of the most frequently and severely affected in schizophrenia (Aleman et al., 1999; Heinrichs and Zakzanis, 1998; Leeson et al., 2009). Thus, *CADPS2ΔExon3* positive subjects and schizophrenic patients share a similar phenotype. On the other hand, with respect to IQ, many studies showed PIQ is lower than VIQ in schizophrenic patients (Amminger et al., 2000; Aylward et al., 1984), suggesting that *CADPS2ΔExon3* positive subjects also have a different feature to schizophrenia.

Because autism and schizophrenia are supposed to be heterogeneous disorders, *CADPS2ΔExon3* could be a susceptibility marker for a sub-type, especially the patients with affected verbal functions. Alternatively, *CADPS2ΔExon3* expression might be merely related to verbal functions or associated with verbal learning disorder.

4.6. How does blood CADPS2ΔExon3 affect brain function?

The mechanism of how peripheral *CADPS2ΔExon3* expression affects brain functions is unclear. Although *CADPS2ΔExon3* was shown to express in the human brain (Eran et al., 2009; Hattori et al., 2011), there is no evidence that blood *CADPS2ΔExon3* expression reflects its high expression in the brain. In case of autism, high *CADPS2ΔExon3* expression might have genetic basis as *CADPS2* has been suggested to be a susceptibility gene for autism (Cisternas et al., 2003). Sadakata et al. (2007b) found several non-synonymous SNPs in *CADPS2* from autistic patients but such SNPs were not detected in healthy subjects. Although CADPS2ΔExon3 retains BDNF releasing activity, it lacks ability to be transported to axons, which would result in the loss of local synaptic BDNF release (Sadakata et al., 2007b). Therefore, higher *CADPS2ΔExon3* expression in the brain, may affect BDNF release through the dominant-negative effect.

CADPS2 mediates the release of monoamine neurotransmission as well. CADPS2 promotes monoamine uptakes and storage (Brunk et al., 2009; Liu et al., 2008) and mediates priming process of monoamine-containing dense core vesicles, so that it facilitates Ca^{2+}-triggered release of neurotransmitters (Jockusch et al., 2007). Therefore, it is also plausible that *CADPS2ΔExon3* expression affects brain function through altered monoamine transmission.

Among monoamines, dopamine's roles on cognition and learning have been established by both animal and human studies (Kehagia et al., 2010). The dopamine neurotransmission in the hippocampus and the prefrontal cortex plays an essential role in working memory (Goldman-Rakic, 1998) and that in striatum also mediates reinforcement and reversal learning (Kehagia et al., 2010). Several studies suggest a link between human verbal function and dopamine D2 receptor. Striatal dopamine D2/D3 receptor availability was reported to correlate with VIQ assessed by WAIS-R (Guo et al., 2006). Hippocampal D2/D3 receptor availability was also related to verbal memory (Takahashi et al., 2007). In addition, dopamine release was enhanced in the frontal cortex, the amygdala and the hippocampus during verbal working memory task (Aalto et al., 2005). Thus, the features of *CADPS2ΔExon3* positive subjects have some similarity with deficits in the D2 receptor function. Because CADPS2 is highly expressed in the dopamine-rich brain areas such as ventral tegmental area and substantia nigra of mice brain (Sadakata et al., 2006), and it is reported to interact with dopamine D2 receptor (Binda et al., 2005), the features of *CADPS2ΔExon3* positive subjects could be ascribed, at least in part, to impaired dopamine transmission.

Although no significant association was detected between wild-type *CADPS2* expression levels and WMS-R scores, *CADPS2* expression level tended to correlate with verbal/general memory. Thus, CADPS2 and CADPS2ΔExon3 might have opposite effects on memory, which is consistent with the above discussion about CADPS2/CADPS2ΔExon3 functions. On the other hand, with respect to intelligence, the mechanism of CADPS2/CADPS2ΔExon3 effects might be more complicated or CADPS2ΔExon3 might have functions independent from wild-type CADPS2.

4.7. Limitation

A limitation is that the mean IQ of our sample was relatively high (mean full-scale IQ: 111.7 ± 12.1). This may have arisen by the geographic area of the sample (i.e. Western part of Tokyo Metropolitan) because average income in Tokyo is approximately 20% higher than national average (Ministry of Health Labour and Welfare, 2009) and income correlates with education level. Therefore, our sample was overrepresented by individuals with higher IQ relative to the Japanese population as a whole. This may have missed the possible effects of *CADPS2* and *CADPS2ΔExon3* on brain functions in people with relatively low IQ.

5. Conclusion

We found that expression of the splice variant of *CADPS2*, *CADPS2ΔExon3*, in the peripheral blood is associated with human brain functions such as intelligence and memory. Those individuals who expressed *CADPS2ΔExon3* had lower IQ and memory. Because CADPS2 mediates the release of BDNF and monoamines, impaired function of CADPS2 due to CADPS2ΔExon3 might exert detrimental effects on BDNF and monoamine functions which are crucial in the brain development and higher brain functions. Further studies to elucidate the mechanisms of production of CADPS2ΔExon3 and its effects at the molecular level are warranted.

Acknowledgements

This work was supported by a Health and Labor Science Research Grant (H21-KOKORO-WAKATE-20 and H21-KOKORO-001), CREST of JST, Grant-in-Aid for Scientific Research (KAKENHI, 22591269), Intramural Research Grant for Neurological and Psychiatric Disorders of NCNP, Takeda Science Foundation, and Mitsubishi Pharma Research Foundation.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.biopsycho.2011.09.017.

References

Aalto, S., Bruck, A., Laine, M., Nagren, K., Rinne, J.O., 2005. Frontal and temporal dopamine release during working memory and attention tasks in healthy humans: a positron emission tomography study using the high-affinity dopamine D2 receptor ligand [11C]FLB 457. Journal of Neuroscience 25, 2471–2477.

Aleman, A., Hijman, R., de Haan, E.H., Kahn, R.S., 1999. Memory impairment in schizophrenia: a meta-analysis. American Journal of Psychiatry 156, 1358–1366.

Amminger, G.P., Schlogelhofer, M., Lehner, T., Looser Ott, S., Friedrich, M.H., Aschauer, H.N., 2000. Premorbid performance IQ deficit in schizophrenia. Acta Psychiatrica Scandinavica 102, 414–422.

Aylward, E., Walker, E., Bettes, B., 1984. Intelligence in schizophrenia: meta-analysis of the research. Schizophrenia Bulletin 10, 430–459.

Bennetto, L., Pennington, B.F., Rogers, S.J., 1996. Intact and impaired memory functions in autism. Child Development 67, 1816–1835.

Binda, A.V., Kabbani, N., Levenson, R., 2005. Regulation of dense core vesicle release from PC12 cells by interaction between the D2 dopamine receptor and calcium-dependent activator protein for secretion (CAPS). Biochemical Pharmacology 69, 1451–1461.

Bishop, D.V., 1989. Autism Asperger's syndrome and semantic-pragmatic disorder: where are the boundaries? British Journal of Disorders of Communication 24, 107–121.

Brunk, I., Blex, C., Speidel, D., Brose, N., Ahnert-Hilger, G., 2009. Ca2+-dependent activator proteins of secretion promote vesicular monoamine uptake. Journal of Biological Chemistry 284, 1050–1056.

Castensson, A., Aberg, K., McCarthy, S., Saetre, P., Andersson, B., Jazin, E., 2005. Serotonin receptor 2C (HTR2C) and schizophrenia: examination of possible medication and genetic influences on expression levels. American Journal of Medical Genetics B: Neuropsychiatry and Genetics 134B, 84–89.

Cisternas, F.A., Vincent, J.B., Scherer, S.W., Ray, P.N., 2003. Cloning and characterization of human CADPS and CADPS2, new members of the Ca^{2+}-dependent activator for secretion protein family. Genomics 81, 279–291.

Egan, M.F., Kojima, M., Callicott, J.H., Goldberg, T.E., Kolachana, B.S., Bertolino, A., Zaitsev, E., Gold, B., Goldman, D., Dean, M., Lu, B., Weinberger, D.R., 2003. The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. Cell 112, 257–269.

Ehlers, S., Nyden, A., Gillberg, C., Sandberg, A.D., Dahlgren, S.O., Hjelmquist, E., Oden, A., 1997. Asperger syndrome, autism and attention disorders: a comparative study of the cognitive profiles of 120 children. Journal of Child Psychology and Psychiatry and Allied Disciplines 38, 207–217.

Eran, A., Graham, K.R., Vatalaro, K., McCarthy, J., Collins, C., Peters, H., Brewster, S.J., Hanson, E., Hundley, R., Rappaport, L., Holm, I.A., Kohane, I.S., Kunkel, L.M., 2009. Comment on "Autistic-like phenotypes in Cadps2-knockout mice and aberrant CADPS2 splicing in autistic patients". Journal of Clinical Investigation 119, 671–680, 679–680; author reply.

Fukumoto, N., Fujii, T., Combarros, O., Kamboh, M.I., Tsai, S.J., Matsushita, S., Nacmias, B., Comings, D.E., Arboleda, H., Ingelsson, M., Hyman, B.T., Akatsu, H., Grupe, A., Nishimura, A.L., Zatz, M., Mattila, K.M., Rinne, J., Goto, Y., Asada, T., Nakamura, S., Kunugi, H., 2010. Sexually dimorphic effect of the Val66Met polymorphism of BDNF on susceptibility to Alzheimer's disease: new data and meta-analysis. American Journal of Medical Genetics B: Neuropsychiatry and Genetics 153B, 235–242.

Goldman-Rakic, P.S., 1998. The cortical dopamine system: role in memory and cognition. Advances in Pharmacology 42, 707–711.

Guo, J.F., Kuang Yang, Y., Tsing Chiu, N., Lieh Yeh, T., See Chen, P., Lee, I.H., Lin Chu, C., 2006. The correlation between striatal dopamine D2/D3 receptor availability and verbal intelligence quotient in healthy volunteers. Psychological Medicine 36, 547–554.

Hattori, K., Tanaka, H., Wakabayashi, C., Yamamoto, N., Uchiyama, H., Teraishi, T., Hori, H., Arima, K., Kunugi, H., 2011. Expression of Ca(2+)-dependent activator protein for secretion 2 is increased in the brains of schizophrenic patients. Progress in Neuro-Psychopharmacology and Biological Psychiatry 35, 1738–1743.

Heinrichs, R.W., Zakzanis, K.K., 1998. Neurocognitive deficit in schizophrenia: a quantitative review of the evidence. Neuropsychology 12, 426–445.

Hillier, A., Campbell, H., Keillor, J., Phillips, N., Beversdorf, D.Q., 2007. Decreased false memory for visually presented shapes and symbols among adults on the autism spectrum. Journal of Clinical and Experimental Neuropsychology 29, 610–616.

Houlihan, L.M., Harris, S.E., Luciano, M., Gow, A.J., Starr, J.M., Visscher, P.M., Deary, I.J., 2009. Replication study of candidate genes for cognitive abilities: the Lothian Birth Cohort 1936. Genes, Brain and Behaviour 8, 238–247.

Jockusch, W.J., Speidel, D., Sigler, A., Sorensen, J.B., Varoqueaux, F., Rhee, J.S., Brose, N., 2007. CAPS-1 and CAPS-2 are essential synaptic vesicle priming proteins. Cell 131, 796–808.

Kehagia, A.A., Murray, G.K., Robbins, T.W., 2010. Learning and cognitive flexibility: frontostriatal function and monoaminergic modulation. Current Opinion in Neurobiology 20, 199–204.

Leeson, V.C., Barnes, T.R., Hutton, S.B., Ron, M.A., Joyce, E.M., 2009. IQ as a predictor of functional outcome in schizophrenia: a longitudinal, four-year study of first-episode psychosis. Schizophrenia Research 107, 55–60.

Lincoln, A.J., Courchesne, E., Kilman, B.A., Elmasian, R., Allen, M., 1988. A study of intellectual abilities in high-functioning people with autism. Journal of Autism and Developmental Disorders 18, 505–524.

Liu, Y., Schirra, C., Stevens, D.R., Matti, U., Speidel, D., Hof, D., Bruns, D., Brose, N., Rettig, J., 2008. CAPS facilitates filling of the rapidly releasable pool of large dense-core vesicles. Journal of Neuroscience 28, 5594–5601.

Ministry of Health, Labour and Welfare, 2009. Basic Survey on Wage Structure, http://www.e-stat.go.jp/SG1/estat/List.do?bid=000001025026&cycode=0.

Minshew, N.J., Goldstein, G., 2001. The pattern of intact and impaired memory functions in autism. Journal of Child Psychology and Psychiatry and Allied Disciplines 42, 1095–1101.

Mizuno, M., Yamada, K., Olariu, A., Nawa, H., Nabeshima, T., 2000. Involvement of brain-derived neurotrophic factor in spatial memory formation and maintenance in a radial arm maze test in rats. Journal of Neuroscience 20, 7116–7121.

Monteggia, L.M., Barrot, M., Powell, C.M., Berton, O., Galanis, V., Gemelli, T., Meuth, S., Nagy, A., Greene, R.W., Nestler, E.J., 2004. Essential role of brain-derived neurotrophic factor in adult hippocampal function. Proceedings of the National Academy of Sciences of the United States of America 101, 10827–10832.

Naoe, Y., Shinkai, T., Hori, H., Fukunaka, Y., Utsunomiya, K., Sakata, S., Matsumoto, C., Shimizu, K., Hwang, R., Ohmori, O., Nakamura, J., 2007. No association between the brain-derived neurotrophic factor (BDNF) Val66Met polymorphism and schizophrenia in Asian populations: evidence from a case-control study and meta-analysis. Neuroscience Letters 415, 108–112.

Otsubo, T., Miyaoka, H., Kamijima, K. (Eds.), 2005. M.I.N.I. Mini International Neuropsychiatric Interview. Seiwa Shoten Publishers, Tokyo.

Robbins, T.W., Arnsten, A.F., 2009. The neuropsychopharmacology of fronto-executive function: monoaminergic modulation. Annual Review of Neuroscience 32, 267–287.

Rybakowski, J.K., 2008. BDNF gene: functional Val66Met polymorphism in mood disorders and schizophrenia. Pharmacogenomics 9, 1589–1593.

Sadakata, T., Itakura, M., Kozaki, S., Sekine, Y., Takahashi, M., Furuichi, T., 2006. Differential distributions of the Ca^{2+}-dependent activator protein for secretion family proteins (CAPS2 and CAPS1) in the mouse brain. Journal of Comparative Neurology 495, 735–753.

Sadakata, T., Kakegawa, W., Mizoguchi, A., Washida, M., Katoh-Semba, R., Shutoh, F., Okamoto, T., Nakashima, H., Kimura, K., Tanaka, M., Sekine, Y., Itohara, S., Yuzaki, M., Nagao, S., Furuichi, T., 2007a. Impaired cerebellar development and function in mice lacking CAPS2, a protein involved in neurotrophin release. Journal of Neuroscience 27, 2472–2482.

Sadakata, T., Mizoguchi, A., Sato, Y., Katoh-Semba, R., Fukuda, M., Mikoshiba, K., Furuichi, T., 2004. The secretory granule-associated protein CAPS2 regulates neurotrophin release and cell survival. Journal of Neuroscience 24, 43–52.

Sadakata, T., Washida, M., Iwayama, Y., Shoji, S., Sato, Y., Ohkura, T., Katoh-Semba, R., Nakajima, M., Sekine, Y., Tanaka, M., Nakamura, K., Iwata, Y., Tsuchiya, K.J., Mori, N., Detera-Wadleigh, S.D., Ichikawa, H., Itohara, S., Yoshikawa, T., Furuichi, T., 2007b. Autistic-like phenotypes in Cadps2-knockout mice and aberrant CADPS2 splicing in autistic patients. Journal of Clinical Investigation 117, 931–943.

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. Journal of Clinical Psychiatry 59 (Suppl. 20), 34–57, 22–33; quiz.

Shinagawa, F., Kobayashi, S., Fujita, K., Maekawa, H., 1990. Japanese Wechsler Adult Intelligence Scale-Revised. Nihonbunkakagakusha, Tokyo.

Siegel, D.J., Minshew, N.J., Goldstein, G., 1996. Wechsler IQ profiles in diagnosis of high-functioning autism. Journal of Autism and Developmental Disorders 26, 389–406.

Steele, S.D., Minshew, N.J., Luna, B., Sweeney, J.A., 2007. Spatial working memory deficits in autism. Journal of Autism and Developmental Disorders 37, 605–612.

Stein, J.L., Hua, X., Lee, S., Ho, A.J., Leow, A.D., Toga, A.W., Saykin, A.J., Shen, L., Foroud, T., Pankratz, N., Huentelman, M.J., Craig, D.W., Gerber, J.D., Allen, A.N., Corneveaux, J.J., Dechairo, B.M., Potkin, S.G., Weiner, M.W., Thompson, P., 2010. Voxelwise genome-wide association study (vGWAS). Neuroimage 53, 1160–1174.

Strauss, J., Barr, C.L., George, C.J., Ryan, C.M., King, N., Shaikh, S., Kovacs, M., Kennedy, J.L., 2004. BDNF and COMT polymorphisms: relation to memory phenotypes in young adults with childhood-onset mood disorder. Neuromolecular Medicine 5, 181–192.

Sugishita, M., 2001. Japanese Wechsler Memory Scale-Revised. Nihonbunkakagakusha, Tokyo.

Takahashi, H., Kato, M., Hayashi, M., Okubo, Y., Takano, A., Ito, H., Suhara, T., 2007. Memory and frontal lobe functions; possible relations with dopamine D2 receptors in the hippocampus. Neuroimage 34, 1643–1649.

Tsai, S.J., Hong, C.J., Yu, Y.W., Chen, T.J., 2004. Association study of a brain-derived neurotrophic factor (BDNF) Val66Met polymorphism and personality trait and intelligence in healthy young females. Neuropsychobiology 49, 13–16.

Volkmar, F.R., Lord, C., Bailey, A., Schultz, R.T., Klin, A., 2004. Autism and pervasive developmental disorders. Journal of Child Psychology and Psychiatry and Allied Disciplines 45, 135–170.

Williams, D.L., Goldstein, G., Carpenter, P.A., Minshew, N.J., 2005a. Verbal and spatial working memory in autism. Journal of Autism and Developmental Disorders 35, 747–756.

Williams, D.L., Goldstein, G., Kojkowski, N., Minshew, N.J., 2008. Do individuals with high functioning autism have the IQ profile associated with nonverbal learning disability? Research in Autism Spectrrm Disorders 2, 353–361.

Williams, D.L., Goldstein, G., Minshew, N.J., 2005b. Impaired memory for faces and social scenes in autism: clinical implications of memory dysfunction. Archives of Clinical Neuropsychology 20, 1–15.

Williams, D.L., Goldstein, G., Minshew, N.J., 2006. The profile of memory function in children with autism. Neuropsychology 20, 21–29.

Yeargin-Allsopp, M., Rice, C., Karapurkar, T., Doernberg, N., Boyle, C., Murphy, C., 2003. Prevalence of autism in a US metropolitan area. Journal of the American Medical Association 289, 49–55.

Zhang, H., Ozbay, F., Lappalainen, J., Kranzler, H.R., van Dyck, C.H., Charney, D.S., Price, L.H., Southwick, S., Yang, B.Z., Rasmussen, A., Gelernter, J., 2006. Brain derived neurotrophic factor (BDNF) gene variants and Alzheimer's disease, affective disorders, posttraumatic stress disorder, schizophrenia, and substance dependence. American Journal of Medical Genetics B: Neuropsychiatry and Genetics 141B, 387–393.

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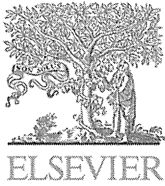

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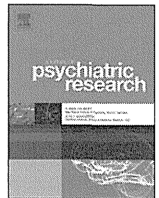

Association between the functional polymorphism (C3435T) of the gene encoding P-glycoprotein (*ABCB1*) and major depressive disorder in the Japanese population

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A R T I C L E I N F O

Article history:
Received 28 September 2011
Received in revised form
28 December 2011
Accepted 9 January 2012

Keywords:
P-glycoprotein
ABCB1
Major depressive disorder
Functional polymorphism
Genetic association

A B S T R A C T

Human P-glycoprotein (P-gp), which is encoded by *ABCB1* (ATP-binding cassette, sub-family B member 1), is expressed in the blood brain barrier and protects the brain from many kinds of drugs and toxins including glucocorticoids by acting as an efflux pump. We examined whether functional polymorphisms of *ABCB1* give susceptibility to major depressive disorder (MDD). The five functional single nucleotide polymorphisms (SNPs), A-41G (rs2188524), T-129C (rs3213619), C1236T (Gly412Gly: rs1128503), G2677A/T (Ala893Ser/Thr: rs2032582), and C3435T (Ile1145Ile: rs1045642) were genotyped in 631 MDD patients and 1100 controls in the Japanese population. A tri-allelic SNP, G2677A/T, was genotyped by pyrosequencing and the remaining SNPs were genotyped by the TaqMan 5′-exonuclease allelic discrimination assay. The minor T3435 allele was significantly increased in MDD patients than in the controls ($\chi^2 = 4.5$, df = 1, $p = 0.034$, odds ratio [OR] 1.16, 95% confidential interval [CI] 1.01–1.34). Homozygotes for the T3435 allele was significantly more common in patients than in the controls ($\chi^2 = 7.5$, df = 1, $p = 0.0062$, OR 1.43, 95%CI 1.11–1.85). With respect to the other 4 SNPs, there was no significant difference in genotype or allele distribution. In the haplotype-based analysis, the proportion of individuals with the TT1236-TT3435 haploid genotype was significantly increased in patients than in controls ($\chi^2 = 8.5$, df = 1, $p = 0.0037$, OR 1.50, 95%CI 1.14–1.98). Our results suggest that the T3435 allele or carrying two copies of this allele confers susceptibility to MDD in the Japanese population.

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1. Introduction

Major depressive disorder (MDD) is a stress-related disorder, and there is mounting evidence for an important role of hypothalamus-pituitary-adrenal axis abnormalities in the pathophysiology of MDD (Kunugi et al., 2010). In the hypothalamus-pituitary-adrenal axis, glucocorticoids are end products and central to the stress response. At the first step, stress activates the hypothalamus-pituitary-adrenal axis and increases glucocorticoid levels in blood. Next, regulated by the blood brain barrier, these hormones penetrate into the brain. Because excessive glucocorticoids are toxic to cells including neurons, high levels are considered to damage the brain and cause depression (Kunugi et al., 2010).

Human P-glycoprotein (P-gp) is encoded by *ABCB1* (ATP-binding cassette, sub-family B member 1) (Chen et al., 1986) or alternatively referred to as multidrug resistance polypeptide 1 (*MDR1*). P-gp is a 1280 amino acid transporter expressed in the blood brain barrier and protects the brain from many drugs or neurotoxic substances such as glucocorticoids and amyloid-beta as an efflux pump.

The most extensively studied *ABCB1* polymorphisms are the G2677A/T (Ala893Ser/Thr: rs2032582) and the C3435T (Ile1145Ile: rs1045642), which are located on exon 21 and exon 26, respectively. The G2677A/T polymorphism is a tri-allelic nonsynonymous polymorphism, Ala893 to Ser/Thr893. This replacement results in a change from a lipophilic residue to a hydrophilic one (Cascorbi et al., 2001). Alanine is a structurally neutral amino acid that does not introduce a constraint into the polypeptide backbone. Therefore, it is possible that the substitution of Ser or Thr for Ala would affect the geometric precision of the interaction site and the secondary structure. The G to T transversion was initially isolated from a full-length *ABCB-1* cDNA from the human adrenal gland

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doi:10.1016/j.jpsychires.2012.01.012

(Kioka et al., 1989). Although the C3435T does not result in an amino acid change, several lines of evidence have indicated that this polymorphism affects the expression and function of P-gp (Hoffmeyer et al., 2000; Goto et al., 2002; Wang et al., 2005; Kimchi-Sarfaty et al., 2007). The C1236T (rs1128503) is closely linked to G2677A/T and C3435T and the combinations of these single nucleotide polymorphisms (SNPs) were shown to be associated with P-gp activity (Wong et al., 2005).

In a previous study, genetic associations of functional polymorphisms of *ABCB1* have been reported for mood disorders including depression in a Japanese sample (62 patients and 160 controls) (Qian et al., 2006). In patients with mood disorders, the G-41 and C-129 alleles were significantly less common and the A2677 allele was more common compared with the controls (Qian et al., 2006). A-41G (rs2188524) and T-129C (rs3213619) are located in the promoter region of *ABCB1* and reported to be associated with P-gp expression levels (Tanabe et al., 2001; Koyama et al., 2006).

In the present study, we focused on the above-mentioned functional polymorphisms of *ABCB1* (A-41G, T-129C, C1236T, G2677A/T, and C3435T) and examined whether *ABCB1* is associated with MDD, using a relatively large Japanese sample. Because specific genotype combinations may play an important role, we also performed haplotype-based association analyses.

2. Methods

2.1. Subjects

A total of 631 patients with MDD (262 males and 369 females, mean age of 49.5 years [SD 15.9], range 17–89) and 1100 healthy controls (371 males and 729 females, 45.6 [SD 16.1], range 18–86) were used in the study. All participants were biologically unrelated Japanese subjects recruited from the same geographical area (Western part of Tokyo Metropolitan). Consensus diagnosis by at least two psychiatrists was made for each patient according to the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) criteria (American Psychiatric Association, 1994) on the basis of unstructured interviews and information from medical records (American Psychiatric Association, 1994). Among the total 631 patients, 49% had a single episode, 49% recurrent episodes, and the remaining patients were unclassified. With regard to history of admission, 19% had a history of admission to a psychiatric hospital and the remaining 81% did not have such a history. The controls were healthy volunteers recruited from the same geographical area. They were interviewed using the Japanese version of the Mini International Neuropsychiatric Interview (M.I.N.I.) (Sheehan et al., 1998; Otsubo et al., 2005) by a research psychiatrist to rule out any axis I psychiatric disorders, and individuals with a current or past history of psychiatric treatment were excluded. Participants were excluded from both the patient and control groups if they had a prior medical history of central nervous system disease or severe head injury, or if they met DSM-IV criteria for mental retardation, substance dependence, or substance abuse. The study protocol was approved by the ethics committee at the National Center of Neurology and Psychiatry, Japan. After describing the study, written informed consent was obtained from every subject.

2.2. Genotyping

Venous blood was drawn from subjects and genomic DNA was extracted from whole blood according to standard procedures. The SNPs, except for rs2032582, were genotyped using the TaqMan 5′-exonuclease allelic discrimination assay (Applied Biosystems, Foster City, CA); the assay ID was C__26907787_10 for rs2188524, C__27487486_10 for rs3213619, C___7586662_10 for rs1128503, and C___7586657_20 for rs1045642. Thermal cycling conditions for polymerase chain reaction (PCR) were 1 cycle at 95 °C for 10 min followed by 50 cycles at 92 °C for 15 s and 60 °C for 1 min. Genotype data were read blind to the case-control status.

Because rs2032582 is a tri-allelic SNP, we used pyrosequencing for genotyping (Supplemental Fig. S1). The region including this SNP was amplified by PCR with primers of Biotin-ABCB1_G2677TA_F137 (5′- GAATATAGCAAATCTTGGGACAGGAA-TAA-3′) and ABCB1_G2677TA_R544L (5′- AATGGCCTGAAAACT-GAAAAAGTCTGT-3′) for the reverse direction. These primers were designed with the PSQ Assay Design software ver. 1.0.6. (Biotage AB, Uppsala, Sweden). Pyrosequencing was performed with the PSQ96MA System and PSQ96 SNP Reagent Kit (Pyrosequencing, AB, Uppsala, Sweden). Sequencing primers for the reverse direction (ABCB1_G2677TA_SR333; 5′-ATCAAT-CATATTTAGTTTGACTCACCTTCC-3′) were designed with the software supplied by the PSQ Assay Design software ver. 1.0.6. (Biotage AB). For sequencing in the reverse direction, 'the sequence to analyze' and 'the dispensation order' were set as 5′-CAGA/C/TACCTTC-3′ and 5′-GCAGCTAGCT-3′, respectively. For ambiguous genotypic data, we repeated experiments and determined the genotype for every subject. Genotypes were read blind to the case-control status.

2.3. Haplotype and statistical analysis

Deviations of genotype distributions from the Hardy–Weinberg equilibrium (HWE) were assessed with the χ^2 test for goodness of fit. Genotype and allele distributions were compared between patients and controls by using the χ^2 test for independence. These tests were performed with the SPSS software ver.11 (SPSS Japan, Tokyo, Japan). Haplotype-based association analyses were performed with the SNPAlyze software ver.6.6 (http://www.dynacom.co.jp/e/products/package/snpalyze/about.html) . Measures of linkage disequilibrium (LD), denoted as D′ and r^2, were calculated from the haplotype frequency using the expectation–maximization (EM) algorithm. Haplotypes with an estimated frequency of less than 1% were considered to be rare and excluded from the analyses. All *p*-values reported are two-tailed. We performed 10,000 permutations. Odds ratios (ORs) and 95% confidence intervals (CI) were also calculated. To correct the critical *p* value for multiple testing, we used the spectral decomposition method of SNPSpD software (http://gump.qimr.edu.au/general/daleN/SNPSpD/) (Nyholt, 2004; Li and Ji, 2005), which considers marker linkage disequilibrium information and generates an experiment-wide significance threshold required to keep the type I error rate at 5%.

3. Results

Genotype and allele distributions of the examined SNPs of *ABCB1* in patients and controls are shown in Table 1. LD estimates of pairwise SNPs, expressed in D′ and r^2, are presented in Fig. 1. The genotype distributions did not significantly deviate from the HWE in patients and controls for any of the examined SNPs. For the C3435T polymorphism, the minor T allele was significantly increased in patients than in controls ($\chi^2 = 4.5$, df = 1, $p = 0.034$, odds ratio [OR] 1.16, 95% confidential interval [CI] 1.01–1.34). There was a significant difference in the genotype distribution between patients and controls ($\chi^2 = 7.5$, df = 2, $p = 0.024$) (Table 1). The proportion of individuals carrying the TT genotype was significantly increased in patients than in controls ($\chi^2 = 7.5$, df = 1, $p = 0.0062$, OR 1.43, 95% CI 1.11–1.85) (Table 2). To correct for multiple testing, we calculated the experiment-wide significance threshold required to keep the type I error rate at 5%. As a result, the corrected *p* value

Table 1
Genotype and allelic distribution of *ABCB1* SNPs in Japanese patients with MDD and controls.

SNP	position		Total N.	Genotype count (frequency)						χ2	GF-P	Allele count (frequency)			χ2	AF-P	HWE χ2	HWE-P
				A/A	G/A	G/G						A	G					
A-41G rs2188524	87230435 5'UTR	MDD	631	508 (0.81)	112 (0.18)	11 (0.02)				3.27	0.19	1128 (0.89)	134 (0.11)		2.24	0.13	2.66	0.10
		Control	1100	847 (0.77)	235 (0.21)	18 (0.02)						1929 (0.88)	271 (0.12)				0.13	0.71
				T/T	T/C	C/C						T	C					
T-129C rs3213619	87230193 5'UTR	MDD	631	549 (0.87)	78 (0.12)	4 (0.01)				1.72	0.42	1176 (0.93)	86 (0.07)		1.73	0.19	0.45	0.50
		Control	1100	934 (0.85)	155 (0.14)	11 (0.01)						2023 (0.92)	177 (0.08)				2.50	0.11
				C/C	C/T	T/T						C	T					
C1236T rs1128503	87179601 exon 12	MDD	631	93 (0.15)	304 (0.48)	234 (0.37)				0.78	0.68	490 (0.39)	772 (0.61)		0.22	0.64	0.13	0.72
		Control	1100	179 (0.16)	514 (0.47)	407 (0.37)						872 (0.40)	1328 (0.60)				0.61	0.44
				G/G	G/T	G/A	T/T	T/A	A/A			G	T	A				
G2677A/T rs2032582	87160618 exon 21	MDD	631	121(0.19)	211(0.33)	86(0.14)	110(0.17)	83(0.13)	20(0.03)	5.47	0.36	539 (0.427)	514 (0.407)	209 (0.166)	1.85	0.40	1.50	0.68
		Control	1100	193(0.18)	385(0.35)	175(0.16)	156(0.14)	158(0.14)	33(0.03)			946 (0.43)	855 (0.39)	399 (0.18)			2.37	0.50
				C/C	C/T	T/T						C	T					
C3435T rs1045642	87138645 exon 26	MDD	631	207 (0.33)	299 (0.47)	125 (0.20)				7.50	0.024	713 (0.56)	549 (0.44)		4.49	0.034	0.82	0.37
		Control	1100	386 (0.35)	552 (0.50)	162 (0.15)						1324 (0.60)	876 (0.40)				2.44	0.12

was calculated as 0.011. The recessive genotypic association (CC3435, CT3435 vs TT3435) remained significant after this correction (Table 2). However, with respect to the other 4 SNPs (A-41G, T-129C, C1236T, and G2677 A/T), there was no significant difference in genotype or allele distribution between patients and controls (Table 1).

The results of haplotype-based analyses are shown in Table 3. There was no significant haplotypic association of the SNPs in *ABCB1* when comparing the patients and controls. However, the proportion of individuals with the TT1236 - TT3435 haploid genotype was significantly increased in patients than in controls ($\chi^2 = 8.5$, df = 1, $P = 0.0037$, OR 1.50, 95% CI 1.14–1.98) (Table 2).

4. Discussion

We performed a genetic association study on 5 functional SNPs of *ABCB1* with MDD in a fairly large Japanese sample. Among the 5 SNPs, only one SNP (C3435T polymorphism) differed in genotype and allele frequencies between patients with MDD and controls. In particular, the TT genotype of this SNP was significantly more common in patients with MDD than in controls. The frequency of the TT haplotype (C1236T-C3435T) homozygote was also significantly higher in patients when compared to controls. These results suggest that the homozygosity of the T3435 allele or the T1236-T3435 haplotype is a risk factor for MDD. The C3435T may play a key role in the development of MDD. It is also possible that unknown functional polymorphisms, which are in linkage disequilibrium to the C3435T may give susceptibility to MDD. To reveal other polymorphisms responsible for the susceptibility, full sequencing of the *ABCB1* gene would be more desirable.

On the other hand, Qian et al. (2006) found no significant association between the C3435T polymorphism and mood disorders in a relatively small Japanese sample (62 cases and 160 controls) (Qian et al., 2006). This negative result may have arisen by inadequate statistical power due to the sample size. Furthermore, the subjects in the Qian et al. (2006) study were composed of patients with MDD, bipolar disorder, and dysthymia, whereas all our patients were diagnosed as MDD. This difference may explain in part the inconsistent results between the two studies. To our knowledge, there have been only two studies (Qian et al., 2006 and ours) that investigated the genetic association between the C3435T polymorphism and the development of MDD in a case-control design. Although several studies examined the C3435T polymorphism for association with response to antidepressants in MDD patients, they did not compare genotype/allele distributions between patients and controls (Kato et al., 2008; Menu et al., 2010; Mihaljevic-Peles et al., 2007; Mihaljevic Peles et al., 2008; Peters et al., 2008; Roberts et al., 2002; Uhr et al., 2008). Future replication studies in various genetic backgrounds are necessary.

Many kinds of antidepressants have been identified as P-gp substrates. For example, amitriptyline and its metabolites (including nortriptyline) are P-gp substrates and have been shown to have enhanced penetration into the brain in P-gp deficient mice compared to wild type (Uhr et al., 2000, 2007; Grauer and Uhr, 2004; Ejsing et al., 2006). Roberts et al. found an association between the C3435T polymorphism and frequency of side effects, and reported that homozygosity of the T3435 allele is a risk factor for nortriptyline-induced postural hypotension (Roberts et al., 2002). They hypothesized that this may be due to a relative increase in the accumulation of nortriptyline, or its metabolites, in the brain due to reduced P-gp function. Similarly, we could discuss cortisol, the C3435T polymorphism, and the development of MDD. However, clinical response to nortriptyline was not associated with the C3435T in their study (Roberts et al., 2002). Citalopram, paroxetine, venlafaxine, sertraline and trimipramine have also been

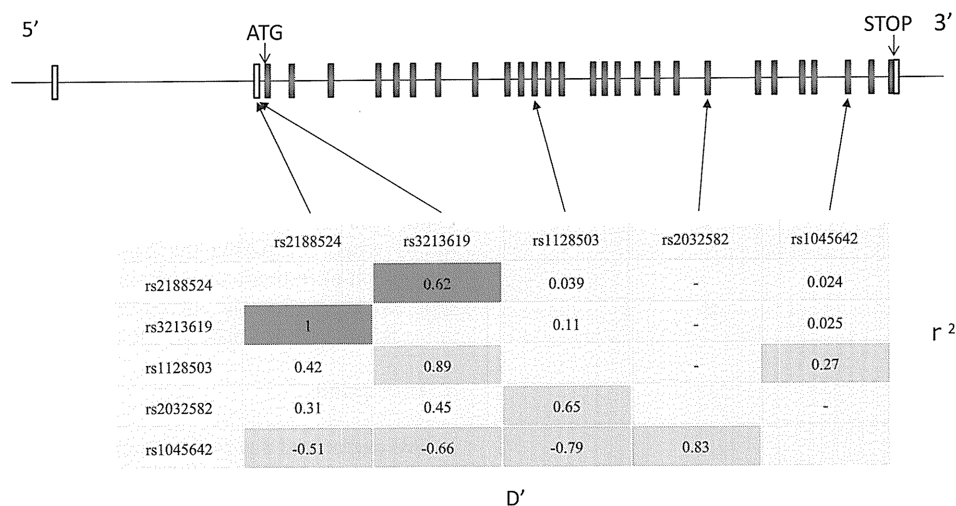

Fig. 1. The genomic structure of *ABCB1* and location of the examined SNPs. The D′ and r2 values between paired SNPs are shown in the diagram. The exons are gray squares. The intensity of the box color corresponds to the strength of D′ and r2. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Recessive model genotype distribution in Japanese patients with MDD and controls.

SNP or haplotype		Total N.	Recessive model (frequency)		$\chi 2$	*P* value
			C/C, C/T	T/T		
C3435T	MDD	631	506 (0.80)	125 (0.20)	7.5	0.0062
	Control	1100	938 (0.85)	162 (0.15)		
			non T-T/T-T	T-T/T-T		
C1236T-C3435T	MDD	631	523 (0.83)	108 (0.17)	8.5	0.0037
	Control	1100	967 (0.88)	133 (0.12)		

shown to be substrates (Ejsing et al., 2006; Uhr et al., 2003, 2000, 2008; Wang et al., 2008). However, conflicting results have raised queries as to whether the C3435T polymorphism influences the response to antidepressants in patients with MDD (Roberts et al., 2002; Peters et al., 2008; Kato et al., 2008; Mihaljevic Peles et al., 2008; Uhr et al., 2008 et al., Gex-Fabry et al., 2008). These results may be caused by the fact that P-gp is a transporter for both drugs and toxins.

We hypothesized that decreased P-gp function at the blood brain barrier may induce the increased accumulation of toxins, such as glucocorticoids, in the brain, contributing to the development of MDD. Accumulating reports support that the TT3435 genotype could be associated with the low level of expression and/or the activity of P-gp, except for some discrepancies (Hoffmeyer et al., 2000; Goto et al., 2002; Wang et al., 2005). With this in mind, the present result showing that the proportion of individuals with the TT3435 genotype was significantly increased in patients than in controls agrees with our hypothesis. Our results suggest that the C3435T polymorphism plays a role in the development of MDD.

Table 3
Pairwise linkage disequilibrium and association with MDD of the 5 SNPs and haplotypes in *ABCB1*.

dbSNP ID	Allele model *p* value	Haplotype *p*[a] 2 Locus	3 Locus	4 Locus	5 Locus
rs2188524	0.13				
		0.32			
rs3213619	0.19		0.55		
		0.64		0.51	
rs1128503	0.64		0.47		0.27
		0.54		0.30	
rs2032582	0.40		0.11		
		0.18			
rs1045642	0.034				

[a] global *p* value.

Role of funding source

This study was supported by Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (JSPS) (H.K., T.F.), Japan Human Sciences Foundation (T.F.), JST, CREST (H.K.), and the Strategic Research Program for Brain Sciences by the Ministry of Education, Culture, Sports, Science and Technology of Japan (Understanding of molecular and environmental bases for brain health) (H.K.). These agencies had no role in study design, acquisition and interpretation of data or writing the report.

Contributors

T.F. designed the study, performed genotyping of *ABCB1*, made statistical analysis, managed literature search, interpreted the data, and wrote the manuscript. N.Y. took part in genotyping. M.O., H.H., D.S., K.H., T.T., M.H., M.T., and T.H. collected samples and gave comments to the manuscript. H.K. organized recruitment and genotyping of patients and control subjects, and took part in analyzing the data and writing the manuscript.

Conflict of interest

All authors declare no conflict of interest that could influence their work.

Acknowledgements

We thank the patients and the healthy volunteers for their participation.

Appendix. Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jpsychires.2012.01.012.

References

American Psychiatric Association. DSM-IV: diagnostic and statistical manual of mental disorders. 4th ed. Washington D.C: American Psychiatric Press; 1994.

Cascorbi I, Gerloff T, Johne A, Meisel C, Hoffmeyer S, Schwab M, et al. Frequency of single nucleotide polymorphisms in the P-glycoprotein drug transporter MDR1 gene in white subjects. Clinical Pharmacology & Therapeutics 2001;69:169–74.

Chen CJ, Chin JE, Ueda K, Clark DP, Pastan I, Gottesman MM, et al. Internal duplication and homology with bacterial transport proteins in the mdr1 (P-glycoprotein) gene from multidrug-resistant human cells. Cell 1986;47:381–9.

Ejsing TB, Hasselstrom J, Linnet K. The influence of P-glycoprotein on cerebral and hepatic concentrations of nortriptyline and its metabolites. Drug Metabolism and Drug Interactions 2006;21:139–62.

Gex-Fabry M, Eap CB, Oneda B, Gervasoni N, Aubry JM, Bondolfi G, et al. CYP2D6 and ABCB1 genetic variability: influence on paroxetine plasma level and therapeutic response. Therapeutic Drug Monitoring 2008;30:474–82.

Goto M, Masuda S, Saito H, Uemoto S, Kiuchi T, Tanaka K, et al. C3435T polymorphism in the MDR1 gene affects the enterocyte expression level of CYP3A4 rather than Pgp in recipients of living-donor liver transplantation. Pharmacogenetics 2002;12:451–7.

Grauer MT, Uhr M. P-glycoprotein reduces the ability of amitriptyline metabolites to cross the blood brain barrier in mice after a 10-day administration of amitriptyline. Journal of Psychopharmacology 2004;18:66–74.

Hoffmeyer S, Burk O, von Richter O, Arnold HP, Brockmoller J, Johne A, et al. Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity in vivo. Proceedings of the National Academy of Sciences USA 2000;97:3473–8.

Kato M, Fukuda T, Serretti A, Wakeno M, Okugawa G, Ikenaga Y, et al. ABCB1 (MDR1) gene polymorphisms are associated with the clinical response to paroxetine in patients with major depressive disorder. Progress in Neuro-Psychopharmacology and Biological Psychiatry 2008;32:398–404.

Kimchi-Sarfaty C, Oh JM, Kim IW, Sauna ZE, Calcagno AM, Ambudkar SV, et al. A "silent" polymorphism in the MDR1 gene changes substrate specificity. Science 2007;315:525–8.

Kioka N, Tsubota J, Kakehi Y, Komano T, Gottesman MM, Pastan I, et al. P-glycoprotein gene (MDR1) cDNA from human adrenal: normal P-glycoprotein carries Gly185 with an altered pattern of multidrug resistance. Biochemical and Biophysical Research Communications 1989;162:224–31.

Koyama T, Nakamura T, Komoto C, Sakaeda T, Taniguchi M, Okamura N, et al. MDR1 T-129C polymorphism can be predictive of differentiation, and thereby prognosis of colorectal adenocarcinomas in Japanese. Biological & Pharmaceutical Bulletin 2006;29:1449–53.

Kunugi H, Hori H, Adachi N, Numakawa T. Interface between hypothalamic-pituitary-adrenal axis and brain-derived neurotrophic factor in depression. Psychiatry and Clinical Neurosciences 2010;64:447–59.

Li J, Ji L. Adjusting multiple testing in multilocus analyses using the eigenvalues of a correlation matrix. Heredity 2005;95:221–7.

Menu P, Gressier F, Verstuyft C, Hardy P, Becquemont L, Corruble E. Antidepressants and ABCB1 gene C3435T functional polymorphism: a naturalistic study. Neuropsychobiology 2010;62:193–7.

Mihaljevic-Peles A, Bozina N, Sagud M. Pharmacogenetics in modern psychiatry. Psychiatria Danubina 2007;19:231–3.

Mihaljevic Peles A, Bozina N, Sagud M, Rojnic Kuzman M, Lovric M. MDR1 gene polymorphism: therapeutic response to paroxetine among patients with major depression. Progress in Neuro-Psychopharmacology and Biological Psychiatry 2008;32:1439–44.

Nyholt DR. A simple correction for multiple testing for single-nucleotide polymorphisms in linkage disequilibrium with each other. American Journal of Human Genetics 2004;74:765–9.

Otsubo T, Tanaka K, Koda R, Shinoda J, Sano N, Tanaka S, et al. Reliability and validity of Japanese version of the mini-international neuropsychiatric Interview. Psychiatry and Clinical Neurosciences 2005;59:517–26.

Peters EJ, Slager SL, Kraft JB, Jenkins GD, Reinalda MS, McGrath PJ, et al. Pharmacokinetic genes do not influence response or tolerance to citalopram in the STAR*D sample. PLoS One 2008;3:e1872.

Qian W, Homma M, Itagaki F, Tachikawa H, Kawanishi Y, Mizukami K, et al. MDR1 gene polymorphism in Japanese patients with schizophrenia and mood disorders including depression. Biological & Pharmaceutical Bulletin 2006;29:2446–50.

Roberts RL, Joyce PR, Mulder RT, Begg EJ, Kennedy MA. A common P-glycoprotein polymorphism is associated with nortriptyline-induced postural hypotension in patients treated for major depression. Pharmacogenomics Journal 2002;2:191–6.

Sheehan DV, Lecrubier Y, Sheehan KH, Amorim P, Janavs J, Weiller E, et al. 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. Journal of Clinical Psychiatry 1998;59(Suppl. 20):22–33. quiz 34–57.

Tanabe M, Ieiri I, Nagata N, Inoue K, Ito S, Kanamori Y, et al. Expression of P-glycoprotein in human placenta: relation to genetic polymorphism of the multidrug resistance (MDR)-1 gene. Journal of Pharmacology and Experimental Therapeutics 2001;297:1137–43.

Uhr M, Grauer MT, Holsboer F. Differential enhancement of antidepressant penetration into the brain in mice with abcb1ab (mdr1ab) P-glycoprotein gene disruption. Biological Psychiatry 2003;54:840–6.

Uhr M, Grauer MT, Yassouridis A, Ebinger M. Blood-brain barrier penetration and pharmacokinetics of amitriptyline and its metabolites in p-glycoprotein (abcb1ab) knock-out mice and controls. J Psychiatry Research 2007;41:179–88.

Uhr M, Steckler T, Yassouridis A, Holsboer F. Penetration of amitriptyline, but not of fluoxetine, into brain is enhanced in mice with blood-brain barrier deficiency due to mdr1a P-glycoprotein gene disruption. Neuropsychopharmacology 2000;22:380–7.

Uhr M, Tontsch A, Namendorf C, Ripke S, Lucae S, Ising M, et al. Polymorphisms in the drug transporter gene ABCB1 predict antidepressant treatment response in depression. Neuron 2008;57:203–9.

Wang D, Johnson AD, Papp AC, Kroetz DL, Sadee W. Multidrug resistance polypeptide 1 (MDR1, ABCB1) variant 3435Cgt;T affects mRNA stability. Pharmacogenet Genomics 2005;15:693–704.

Wang JS, Zhu HJ, Gibson BB, Markowitz JS, Donovan JL, DeVane CL. Sertraline and its metabolite desmethylsertraline, but not bupropion or its three major metabolites, have high affinity for P-glycoprotein. Biological & Pharmaceutical Bulletin 2008;31:231–4.

Wong M, Evans S, Rivory LP, Hoskins JM, Mann GJ, Farlow D, et al. Hepatic technetium Tc 99m-labeled sestamibi elimination rate and ABCB1 (MDR1) genotype as indicators of ABCB1 (P-glycoprotein) activity in patients with cancer. Clinical Pharmacology & Therapeutics 2005;77:33–42.

Journal of Affective Disorders 136 (2012) 1047–1052

Contents lists available at SciVerse ScienceDirect

Journal of Affective Disorders

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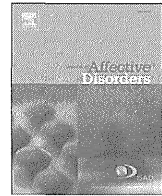

Preliminary communication

More severe impairment of manual dexterity in bipolar disorder compared to unipolar major depression

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ARTICLE INFO

Article history:
Received 22 September 2011
Received in revised form 26 October 2011
Accepted 14 November 2011
Available online 12 December 2011

Keywords:
Unipolar depression
Bipolar disorder
Dexterity
Lithium
Antipsychotics

ABSTRACT

Background: Mood disorders are associated with various neurocognitive deficits. However, few studies have reported the impairment of motor dexterity in unipolar depression and bipolar disorder. In the present study, manual dexterity was compared between unipolar major depression, bipolar disorder, and healthy controls.
Methods: Manual dexterity was assessed by the Purdue pegboard test in 98 patients with unipolar major depression, 48 euthymic or depressed patients with bipolar disorder, and 158 healthy controls, matched for age and gender.
Results: Compared to healthy controls, sum of the scores of right, left, and both hands subtests (R + L + B) was significantly lower in both patients with unipolar depression and bipolar disorder ($P = 0.0034$ and $P < 0.0001$, respectively). Furthermore, R + L + B was significantly lower in bipolar disorder compared to unipolar depression ($P = 0.0016$). Lithium dose and chlorpromazine equivalent dose of antipsychotics were significantly negatively correlated with some of the subtest scores. On the other hand, depression severity did not significantly correlate with any of the subtest scores. Difference in R + L + B between unipolar depression and bipolar disorder remained statistically significant even after controlling for gender, age, lithium dose, and chlorpromazine equivalent dose ($P = 0.0028$).
Limitations
Bipolar patients during manic episode were not included in the study.
Conclusions: Gross movement dexterity was impaired in both patients with unipolar depression and bipolar disorder. The severity of impairment was significantly greater in patients with bipolar disorder. The functional difference between unipolar and bipolar patients may suggest different pathological conditions between the two depressive disorders.
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1. Introduction

Classifications of mood disorders are based on the polarity of episodes. However, similar depressive symptomatology between unipolar and bipolar disorders makes the differentiation difficult in depressed patients without a history of manic episode. Although several clinical characteristics such

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doi:10.1016/j.jad.2011.11.031

as hypersomnia and psychotic symptoms have been suggested to be helpful in distinguishing bipolar depression from unipolar depression (Forty et al., 2008; Mitchell et al., 2001), the lack of clear-cut clinical features distinguishing the two disorders has prompted researchers to seek genetic markers and endophenotypes. A few studies have found differences in personality profiles between unipolar and bipolar depression (Akiskal et al., 2006; Mendlowicz et al., 2005; Nowakowska et al., 2005; Sasayama et al., 2011). Recent evidence suggests that several neurocognitive deficits may also serve as endophenotypes of bipolar disorder (Bora et al., 2009; Langenecker et al., 2010). However, neurocognitive impairment is observed in unipolar depression as well (Han et al., in press; Schrijvers et al., 2009), and thus, whether there are characteristic neurocognitive deficits in bipolar disorder needs to be investigated.

Impaired dexterity is one of the neurocognitive phenotypes reported in patients with bipolar disorder (Langenecker et al., 2010; Wilder-Willis et al., 2001). On the other hand, some studies have also reported impaired fine motor movement in unipolar depression compared to healthy controls (Pier et al., 2004b; Swann et al., 1999). However, it remains to be elucidated whether the factors affecting dexterity and the severity of the impairment are similar in unipolar and bipolar disorder. In the present study, the Purdue pegboard test (Tiffin and Asher, 1948) was used to assess the manual dexterity in unipolar depression and bipolar disorder. The influence of depression severity and antipsychotics and lithium medications on dexterity was also examined.

2. Methods

2.1. Subjects

Subjects were 98 patients with unipolar major depressive disorder (50 patients with recurrent depression), 48 euthymic or depressive patients with bipolar disorder (8 patients with bipolar I and 40 with bipolar II disorder), and 158 healthy volunteers, matched for gender and age distributions. Participants were recruited from the outpatient clinic of the National Center of Neurology and Psychiatry Hospital, Tokyo, Japan or through advertisements in local free magazines, website announcement, notices posted in the hospital, flyers, and word of mouth. Only self-reported right-handed subjects were included in the study. Consensus diagnoses by at least two research psychiatrists were made according to the DSM-IV criteria (American Psychiatric Association, 1994) for unipolar major depressive disorder and bipolar disorder for enrollment in the study. Healthy participants were interviewed using the Japanese version of the Mini-International Neuropsychiatric Interview (Otsubo et al., 2005; Sheehan et al., 1998) by a research psychiatrist, and only those who demonstrated no history of psychiatric illness or contact to psychiatric services were enrolled as healthy controls. Participants were excluded from both the patient and control groups if they had a prior medical history of central nervous system disease or severe head injury, or if they met DSM-IV criteria for mental retardation, substance dependence, or substance abuse. All subjects were biologically unrelated Japanese individuals who resided in the Western part of Tokyo. Written informed consent was obtained from all subjects prior to their inclusion in the study and the study was approved by the ethics committee of the National Center of Neurology and Psychiatry, Japan.

2.2. Measures

2.2.1. Purdue pegboard test

All participants were administered the Purdue pegboard test (Model 32030, manufactured by Lafayette Instrument Company USA) for evaluation of manual dexterity. The pegboard contains two vertical arrays of 25 holes in which pegs are placed one hand at a time and then with both hands simultaneously under timed conditions (30 s per trial). Scores for these measures (right, left, and both hands subtests) were derived for each trial according to how many pegs were placed within the time limit. The sum of the right, left, and both hands subtest scores ($R+L+B$) was used as the representation of gross dexterity of the fingers, hands, and arms. Fine fingertip dexterity was assessed by the assembly subtest, which involves using both hands alternately to construct assemblies consisting of a pin, a washer, a collar and another washer. This subtest requires participants to complete as many assemblies as possible within 60 s. The total number of pieces assembled was recorded as the score of the assembly subtest.

2.2.2. Handgrip force

Handgrip force was measured using a digital handgrip dynamometer (T.K.K.5401; Takei Co., Tokyo, Japan) to record the muscle strength of each hand. Participants were instructed to exert maximum grip force while standing upright, keeping their active arm stretched down vertically close to the body. The average of the two trials for each hand was defined as the maximal handgrip force.

2.2.3. Hamilton Depression Rating Scale

Depressive symptoms were assessed by an experienced research psychiatrist using the Japanese version of the GRID Hamilton Depression Rating Scale, 17-item version (HDRS) (Hamilton, 1967), which has been demonstrated to show excellent inter-rater reliability (Tabuse et al., 2007).

2.3. Statistical analyses

Statistical differences of demographic data among groups were evaluated by the chi-squared test for categorical variables and one-way analysis of variance (ANOVA) for continuous variables. Student t-test was used for the post hoc analysis and for the comparisons of clinical variables between unipolar and bipolar patients. Due to the non-normal distribution of the Purdue pegboard scores and handgrip force, these data were compared between the three diagnostic groups using Kruskal–Wallis test, and thereafter, pairwise comparisons between two groups were done using Mann–Whitney test. Because antipsychotics and lithium, which are often prescribed for bipolar disorder, could cause tremors that may impair manual dexterity, correlations of the pegboard scores with the dose of these medications as well as with age, gender, antidepressant and anxiolytic prescription status, and HDRS scores were assessed using stepwise linear regression analysis (entry criteria $P<0.05$, removal criteria