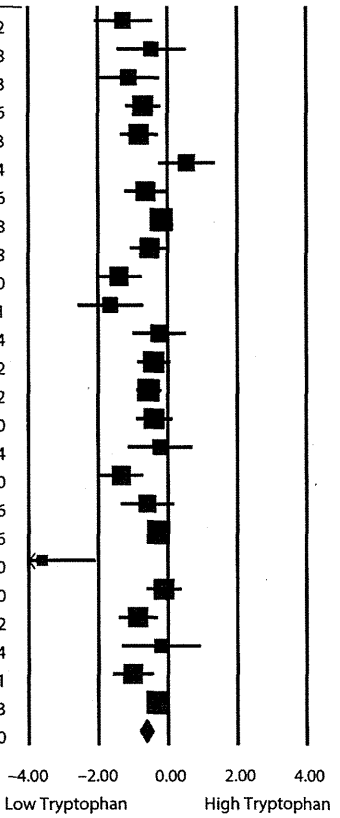
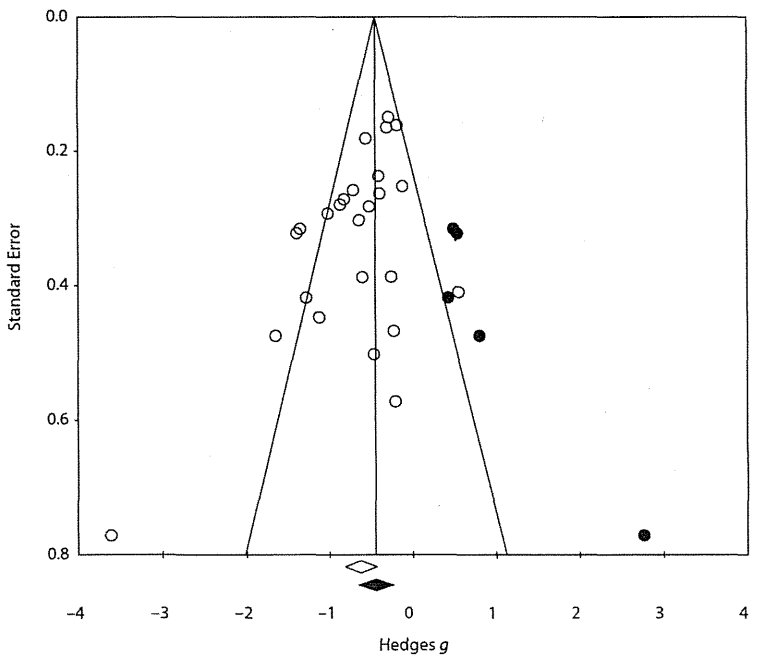
Figure 2. Forest Plots and Funnel Plots of Meta-Analysis[a]

A. Forest Plot of Meta-Analysis on 25 Comparisons

Study	Hedges *g*	Standard Error	Lower Limit	Upper Limit	*Z* Value	*P* Value
DeMyer et al, 1981[41]	−1.274	0.419	−2.095	−0.453	−3.040	.002
Menna-Perper et al, 1981[40]	−0.462	0.503	−1.449	0.524	−0.919	.358
Joseph et al, 1984[39]	−1.117	0.448	−1.995	−0.239	−2.492	.013
Anderson et al, 1990[38]	−0.705	0.259	−1.212	−0.198	−2.726	.006
Chiaroni et al, 1990[37]	−0.815	0.272	−1.348	−0.281	−2.992	.003
Russ et al, 1990[36]	0.558	0.411	−0.247	1.363	1.359	.174
Maes et al, 1990[24]	−0.635	0.304	−1.231	−0.040	−2.092	.036
Price et al, 1991[35]	−0.176	0.162	−0.493	0.142	−1.084	.278
Quintana, 1992[33]	−0.516	0.283	−1.071	0.039	−1.822	.068
Lucca et al, 1992[34]	−1.385	0.323	−2.018	−0.752	−4.289	.000
Maes et al, 1993[25]	−1.645	0.475	−2.577	−0.713	−3.461	.001
Ortiz et al, 1993[26]	−0.247	0.388	−1.008	0.513	−0.637	.524
Moller, 1993[32]	−0.400	0.238	−0.866	0.066	−1.683	.092
Maes et al, 1995[27]	−0.551	0.182	−0.907	−0.195	−3.035	.002
Mauri et al, 1998[31]	−0.389	0.264	−0.906	0.128	−1.475	.140
Song et al, 1998[30]	−0.223	0.469	−1.142	0.695	−0.476	.634
Hoekstra et al, 2001[29]	−1.341	0.316	−1.961	−0.721	−4.238	.000
Mauri et al, 2001[28]	−0.595	0.388	−1.356	0.166	−1.532	.126
Myint et al, 2007[10]	−0.276	0.150	−0.570	0.018	−1.838	.066
Manjarrez-Gutierrez et al, 2009[8]	−3.607	0.772	−5.120	−2.095	−4.675	.000
Sublette et al, 2011[12]	−0.115	0.253	−0.611	0.381	−0.453	.650
Maes and Rief, 2012[7]	−0.862	0.280	−1.411	−0.313	−3.076	.002
Pinto et al, 2012[11]	−0.202	0.573	−1.325	0.921	−0.352	.724
Xu et al, 2012[9]	−1.014	0.293	−1.589	−0.438	−3.454	.001
Present Study	−0.297	0.165	−0.621	0.027	−1.795	.073
Total	−0.627	0.098	−0.819	−0.436	−6.407	.000



B. Funnel Plot of Standard Error by Hedges *g*

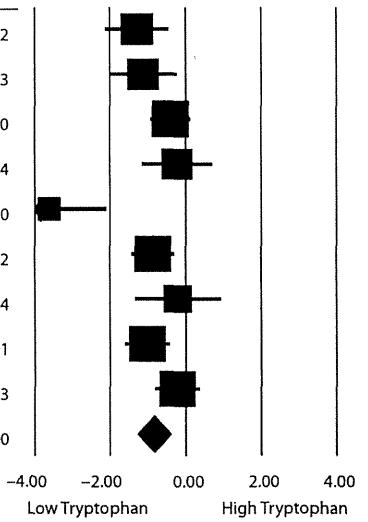


(continued)

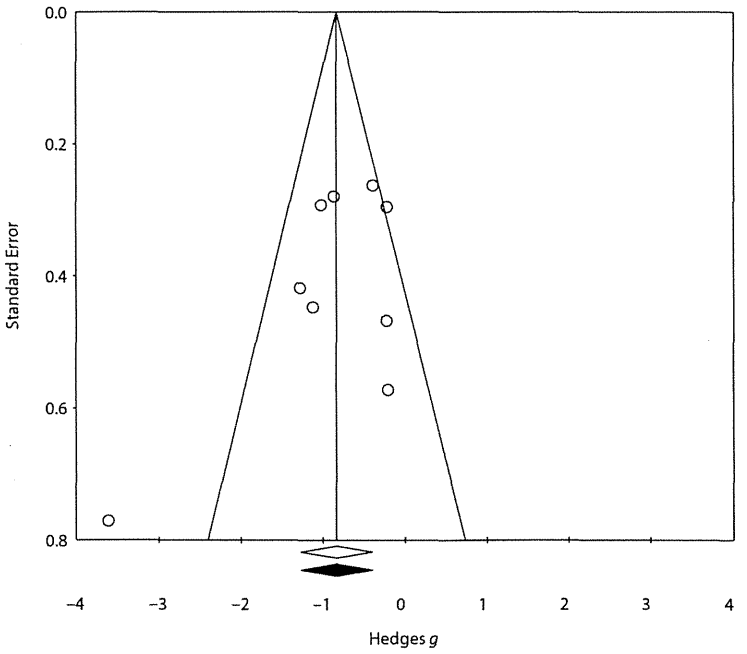
Figure 2 (continued). Forest Plots and Funnel Plots of Meta-Analysis[a]

C. Forest Plot of Meta-Analysis Using Psychotropic Drug–Free Patients of Our Subjects and Previous Studies

Study	Hedges *g*	Standard Error	Lower Limit	Upper Limit	*Z* Value	*P* Value
DeMyer et al, 1981[41]	−1.274	0.419	−2.095	−0.453	−3.040	.002
Joseph et al, 1984[39]	−1.117	0.448	−1.995	−0.239	−2.492	.013
Mauri et al, 1998[31]	−0.389	0.264	−0.906	0.128	−1.475	.140
Song et al, 1998[30]	−0.223	0.469	−1.142	0.695	−0.476	.634
Manjarrez-Gutierrez et al, 2009[8]	−3.607	0.772	−5.120	−2.095	−4.675	.000
Maes and Rief, 2012[7]	−0.862	0.280	−1.411	−0.313	−3.076	.002
Pinto et al, 2012[11]	−0.202	0.573	−1.325	0.921	−0.352	.724
Xu et al, 2012[9]	−1.014	0.293	−1.589	−0.438	−3.454	.001
Present Study	−0.213	0.297	−0.794	0.368	−0.718	.473
Total	−0.836	0.220	−1.268	−0.404	−3.790	.000



D. Funnel Plot of Standard Error by Hedges *g*

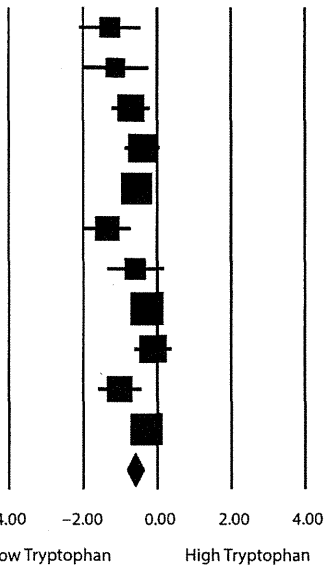


[a]The forest plots describe statistical data and effect size of each study, and the result of quantitative synthesis. Black squares depict effect size, and horizontal bars indicate 95% confidence interval. The funnel plots, which were made to examine the presence of publication bias, depict the effect size against the standard error of individual studies. Black circles represent potentially missing trials that were imputed based on the trim-and-fill method. The white rhombus represents the point estimate for plasma tryptophan concentration effect based on published trials. The black rhombus represents the new point estimate for the effect size of plasma tryptophan when publication bias was adjusted by means of the trim-and-fill method.

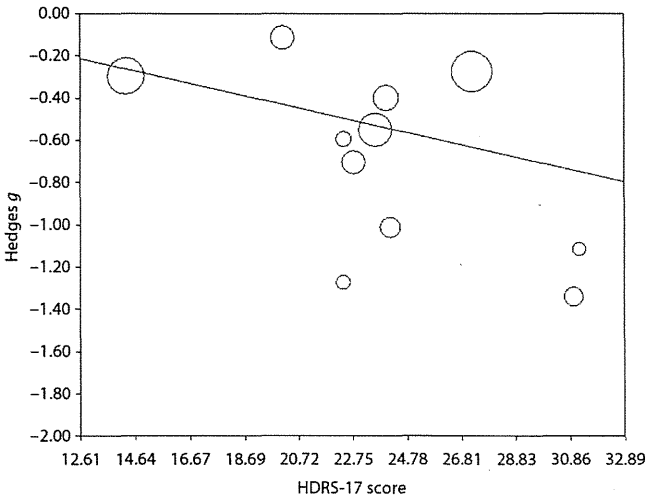
Figure 3. Forest Plot and Scatter Plot of Meta-Regression on HDRS-17 Scores and Effect Size (Hedges *g*)[a]

A.

Study	Hedges *g*	Standard Error	Lower Limit	Upper Limit	*Z* Value	*P* Value
DeMyer et al, 1981[41]	−1.274	0.419	−2.095	−0.453	−3.040	.002
Joseph et al, 1984[39]	−1.117	0.448	−1.995	−0.239	−2.492	.013
Anderson et al, 1990[38]	−0.705	0.259	−1.212	−0.198	−2.726	.006
Moller, 1993[32]	−0.400	0.238	−0.866	0.066	−1.683	.092
Maes et al, 1995[27] *	−0.551	0.182	−0.907	−0.195	−3.035	.002
Hoekstra et al, 2001[29]	−1.341	0.316	−1.961	−0.721	−4.238	.000
Mauri et al, 2001[28]	−0.595	0.388	−1.356	0.166	−1.532	.126
Myint et al, 2007[10]	−0.276	0.150	−0.570	0.018	−1.838	.066
Sublette et al, 2011[12] *	−0.115	0.253	−0.611	0.381	−0.453	.650
Xu et al, 2012[9]	−1.014	0.293	−1.589	−0.438	−3.454	.001
Present Study	−0.297	0.165	−0.621	0.027	−1.795	.073
Total	−0.603	0.114	−0.827	−0.379	−5.277	.000



B.



[a]The forest plot of 11 comparisons for meta-regression shows statistical data and effect size on each trial and result of quantitative synthesis (A). Scatter plot and regression line depict the result of meta-regression analysis. Those circles represent each trial (B). Our selected method for estimating was "method of moments," which is a mixed-effects model rather than fixed-effect model, for carrying out this meta-regression.
*As described in the Meta-Analytic Method section, values of subgroups of patients (n, SD, mean) were united into one group.
Abbreviation: HDRS-17 = Hamilton Depression Rating Scale 17-item version.

concentrations than controls, suggesting that MDD is associated with low plasma concentration in our Japanese sample, which is in accordance with the results of the meta-analysis.

The initial meta-analysis on the total subjects indicated a heterogeneity and a publication bias. The heterogeneity may have resulted from differences in demographic and clinical characteristics, including medication across the studies. After adjustment of the publication bias, the effect size became somewhat lower (Hedges *g* of −0.45).

When the meta-analysis was performed only for patients without psychotropic medication, the obtained effect size (Hedges *g* of −0.84, ie, a large effect size) became substantially higher than that in the total subjects, suggesting that the observed difference in tryptophan concentration between patients and controls is not attributable to medication.

Rather, medication may have reduced the difference between patients and controls.

With regard to the possible correlation between depression severity and plasma tryptophan levels, we obtained no evidence for such a correlation in our Japanese sample. In the meta-regression analysis, however, we found a small but significant correlation between severity and plasma tryptophan. The failure to detect the correlation in our sample might be due in part to the small effect and that the majority of our subjects were medicated.

There might be several mechanisms underlying the association between MDD and decreased plasma tryptophan levels. Recent studies have suggested the stress- and inflammation-related mechanisms. There are enzymes to degrade tryptophan to kynurenine: tryptophan 2,3-dioxygenase (TDO) and IDO. TDO is highly expressed in the liver and activated by glucocorticoids (ie, cortisol in humans).[43,44] In line, both patients and control subjects who were administered dexamethasone, a synthetic glucocorticoid, showed lower plasma tryptophan concentrations.[24] Many studies, including ours, demonstrated that patients with MDD show hypercortisolism.[45,46] Therefore, increased enzymatic activity of TDO due to hypercortisolism is a mechanism underlying the observed reduction in plasma tryptophan levels in patients with MDD.

IDO may also play a role, since proinflammatory cytokines induce IDO activation,[47,48] and cytokine levels are elevated in MDD patients.[49] In line, a drastic fall of plasma tryptophan was observed in patients with inflammatory disorder and in those patients receiving immunotherapy.[3] Indeed, the immune system activation by hepatitis C virus infection or chronic interferon-α administration increases prevalence of MDD.[50,51] Moreover, we found higher interleukin-6 levels in cerebrospinal fluid (CSF) of MDD patients compared with controls,[52] suggesting neuroinflammation in at least a portion of the patients. In the brain, IDO is expressed in astrocytes and microglial cells. In astrocytes, kynurenine is converted to kynurenic acid, which has a neuroprotective effect by antagonizing glycine coagonist site of *N*-methyl-D-aspartate (NMDA) receptor.[53] In microglial cells, by contrast, kynurenine is predominantly converted to quinolinic acid or 3-hydroxykynurenine, which have a neurotoxic effect through agonizing the NMDA receptor.[53] Therefore, inflammation-induced activation of IDO and microglial cells might be another mechanism.

Since tryptophan is an essential amino acid, it is also possible that the dietary intake of tryptophan might be decreased in patients with MDD. The tryptophan depletion procedure is known to precipitate low mood and other symptoms of depression in vulnerable subjects and there is some evidence that tryptophan loading is effective as a treatment for depression (reviewed by Parker and Brotchie[54]). However, there is little information on the dietary tryptophan intake in depressed patients. In a population-based prospective study of 29,133 men in Finland whose intake of amino acids was calculated from a diet history questionnaire, there was no significant association between reduced dietary intake of tryptophan and depressed mood.[55] However, a possibility remains that tryptophan intake may be specifically important for depressive symptoms in persons with a diagnosed depressive disorder, as opposed to depressive symptoms within a general population. Further studies are warranted to see whether the dietary intake contributes to the observed decrease in plasma tryptophan levels in MDD.

There are several limitations in the study. We measured only total tryptophan level, so we could not address whether free tryptophan levels were different between the MDD patients and controls. In our case-control study, the measurement of plasma tryptophan level was done in the "real world" setting, ie, we did not control for fasting, time of sampling, or medication. The majority of previous studies controlled fed status (ie, overnight fasting). With respect to timing of sampling, there was no significant difference in the timing of measurement between the 2 groups (data not shown). The majority of our subjects were medicated. Benzodiazepines increase free tryptophan concentration in rat serum,[56] although conflicting negative results have also been reported in humans.[57] Antidepressants such as citalopram decrease TDO activity,[58] which may have increased plasma tryptophan level in medicated MDD patients. Therefore, medication is likely to have minimized rather than exaggerated the difference in plasma tryptophan level between our patients and controls. This is consistent with the results of our meta-analysis. Our cross-sectional study precludes us from elucidating the cause-effect relationship between low plasma tryptophan and the development of MDD. In addition, plasma tryptophan concentration may not be an index for brain tryptophan level.[59] To examine brain tryptophan levels, analyses of CSF tryptophan levels in MDD patients are currently underway. In the meta-analysis, we did not search for the literature outside of the PubMed database, which may have caused us to miss some studies included in other databases.

In conclusion, in spite of these limitations, the present study clearly indicated that MDD is associated with lower plasma tryptophan levels. Although the majority of previous studies were from Western populations, results of our case-control study are in accordance with those of Western studies regardless of differential lifestyle and dietary habits. If there is any correlation between plasma tryptophan level and depression severity, the effect size would be small.

Drug names: citalopram (Celexa and others), diazepam (Diastat, Valium, and others), imipramine (Tofranil and others).

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Increased cerebrospinal fluid interleukin-6 levels in patients with schizophrenia and those with major depressive disorder

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ABSTRACT

Elevated peripheral levels of interleukin-6 (IL-6) are common findings in schizophrenia and depression. However, previous studies that measured cerebrospinal fluid (CSF) IL-6 levels in these disorders reported controversial results. The present study examined whether CSF IL-6 levels are altered in patients with schizophrenia and those with depression. Lumbar punctures were performed in 32 patients with schizophrenia, 30 with major depressive disorder (MDD), and 35 healthy controls. Serum samples were simultaneously collected from all subjects in the patient groups and from 32 of the control group. CSF and serum IL-6 levels were determined by enzyme-linked immunosorbent assay. Both the patients with schizophrenia and MDD had significantly higher CSF IL-6 levels compared to the controls (schizophrenia: $P = 0.0027$; MDD: $P = 0.012$). IL-6 levels were significantly higher in the CSF than in the serum. No significant correlation was observed between CSF and serum IL-6 levels. The present findings suggest that IL-6 of central origin is associated with the pathophysiology of schizophrenia and MDD, although confounding effect of smoking status can not be entirely excluded.

1. Introduction

Elevated serum or plasma levels of interleukin-6 (IL-6) are common findings in schizophrenia (Potvin et al., 2008; Sasayama et al., 2011) and depression (Howren et al., 2009; Liu et al., 2012). Although the source of the elevated blood IL-6 remains to be elucidated, such evidence suggests immune alterations in the peripheral tissues of these disorders.

IL-6 is not only synthesized in immune cells of the peripheral blood but is also produced in the central nervous system (CNS) by astrocytes and microglia. According to the recent microglia hypothesis of schizophrenia (Monji et al., 2009), activated microglia release pro-inflammatory cytokines and free radicals, causing neuronal degeneration, white matter abnormalities, and decreased neurogenesis associated with the pathophysiology of schizophrenia. In previous studies of patients with depression (Hamidi et al., 2004; Ongur et al., 1998), loss of glial elements in mood-relevant brain regions, such as amygdala and subgenual prefrontal cortex, has been observed. Such findings suggest that the effect of cytokines and central inflammatory processes on glia may play a role in the etiology of depression. These hypothetical models of immune pathophysiology underline the importance of the assessment of CNS levels of IL-6 in schizophrenia and depression. Some previous studies have shown that CSF IL-6 levels may not significantly correlate with peripheral IL-6 levels (Lindqvist et al., 2009; Stenlof et al., 2003). Therefore, measurement in the cerebrospinal fluid (CSF) is necessary for the direct assessment of CNS-derived IL-6.

A few studies have measured IL-6 levels in the CSF in patients with schizophrenia (Barak et al., 1995; Garver et al., 2003) and depression (Carpenter et al., 2004; Levine et al., 1999; Lindqvist et al., 2009; Martinez et al., 2012; Stubner et al., 1999). However, the findings are inconsistent across studies. Barak et al. (1995) reported no significant difference in CSF IL-6 levels between schizophrenic patients and healthy controls, while Garver et al. (2003) found significantly higher CSF IL-6 levels in a subtype of schizophrenia. As for depressed patients, CSF IL-6 levels were found to be decreased (Levine et al., 1999; Stubner et al., 1999), unaltered (Carpenter et al., 2004; Martinez et al., 2012), or elevated (Lindqvist

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et al., 2009) compared to healthy controls. However, findings among previous studies measuring CSF IL-6 levels in schizophrenia and depression should be interpreted with caution due to the small numbers of subjects.

1.1. Aims of the study

The aims of the present study were to examine whether CSF IL-6 levels were altered in patients with schizophrenia and those with depression. From the inflammatory hypotheses of these disorders (Maes, 2011; Miller et al., 2009; Monji et al., 2009), we hypothesized that the central IL-6 levels would be increased in the patient groups compared to the healthy controls.

2. Material and methods

2.1. Subjects

Lumbar punctures were performed in 32 patients with schizophrenia, 30 patients with major depressive disorder (MDD), and 35 healthy controls. The mean age and sex ratio were matched across the three groups. Most subjects of the patient groups were on antipsychotic and/or antidepressant treatment. Simultaneously with the lumbar punctures, serum samples were also collected from all subjects in the patient groups and from 32 of the control group. Table 1 shows the demographic and clinical characteristics of the participants. All subjects were biologically unrelated Japanese who were recruited from the outpatient clinic of the National Center Hospital, National Center of Neurology and Psychiatry, Kodaira, Tokyo, Japan or through advertisements in free local information magazines and by our website announcement. Consensus diagnosis by at least two 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 (M.I.N.I.) (Otsubo et al., 2005; Sheehan et al., 1998) by a research psychiatrist to rule out any axis I psychiatric disorders. Participants were excluded if they had prior medical histories of central nervous system disease or severe head injury, if they met the criteria for substance abuse or dependence, or mental retardation, if they were currently taking anti-inflammatory medication, or if they suffered from any inflammatory, infectious, or systemic immune diseases, based on self-reports, at the time of assessment. The study protocol was approved by the ethics committee at the National Center of Neurology and Psychiatry, Japan. After description of the study, written informed consent was obtained from every subject.

2.2. Laboratory methods

CSF was drawn between 1000 h and 1600 h from the L4–L5 or L3–L4 interspace, with the subject in the left decubitus position. The samples were immediately transferred on ice, centrifuged at 4000 × *g*, aliquoted and stored at −80 °C until they were assayed. Serum samples were collected immediately before the lumbar punctures. All the samples were collected during the period of 2010–2011. CSF and serum levels of IL-6 were determined by a commercially available immunoassay kit (Quantikine, R&D systems, Inc., Minneapolis) according to manufacturer's instructions. The mean minimum detectable dose of the kit was 0.039 pg/ml. The within and between-run coefficients of variance of the assay were less than 10%.

2.3. Clinical measures

Schizophrenic symptoms and depressive symptoms were assessed 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, 21-item version (HAMD-21) (Hamilton, 1967), which have both been demonstrated to show good inter-rater reliability (Igarashi et al., 1998; Tabuse et al., 2007). Daily doses of antipsychotics in patients with schizophrenia and antidepressants in patients with MDD were converted to chlorpromazine and imipramine equivalent doses, respectively, using published guidelines (Inagaki et al., 1999).

2.4. Statistical analysis

Difference in gender distribution between groups was analyzed by χ^2 analysis. Clinical characteristics between groups were compared using analysis of variance. Because CSF and serum IL-6 levels were not normally distributed, difference between diagnostic groups was assessed using Kruskal–Wallis test, and thereafter pairwise Mann–Whitney *U* tests for *post hoc* comparisons. Relationship between IL-6 levels and clinical measures were assessed using Spearman's rank correlation coefficients (ρ). Serum and CSF samples were compared using Spearman's rank correlation and Wilcoxon's signed rank test. All statistical tests were two tailed and statistical significance was considered when $P < 0.05$. Bonferroni correction was applied for the *post hoc* pairwise Mann–Whitney *U* tests between the three diagnostic groups

Table 1
Demographic and clinical characteristics.

	Controls ($N = 35$)	Schizophrenia ($N = 32$)	MDD ($N = 30$)	Analysis
Age [years]	41.3 (16.4)	40.8 (8.8)	42.7 (8.2)	$F = 0.21$, $P = 0.81$
Gender [M/F]	21/14	20/12	19/11	$\chi^2 = 0.08$, $P = 0.96$
Age at onset [years]		25.0 (8.0)	33.6 (13.3)	
Illness duration [years]		16.2 (7.9)	8.8 (8.9)	
BMI	23.4 (4.0)	24.2 (5.1)	23.1 (4.3)	$F = 0.47$, $P = 0.63$
%Smokers	11.4	50.0	46.7	$\chi^2 = 13.5$, $P < 0.01$
CP equivalent dose [mg/day]	0.0 (0.0)	803.5 (583.0)	83.7 (175.2)	$F = 50.2$, $P < 0.01$
IMI equivalent dose [mg/day]	0.0 (0.0)	15.6 (48.7)	164.3 (128.6)	$F = 43.7$, $P < 0.01$
PANSS positive scores		13.2 (5.1)		
PANSS negative scores		14.5 (5.5)		
HAMD-21 scores			13.3 (9.8)	
Time of day of sampling [h]	1340 (0139)	1327 (0129)	1309 (0141)	$F = 0.42$, $P = 0.66$
Number of days between sample collection and IL-6 assay	308 (140)	292 (144)	293 (150)	$F = 0.13$, $P = 0.88$

Values are shown as mean (standard deviation).
MDD: major depressive disorder; BMI: body mass index; CP: chlorpromazine; IMI: imipramine; PANSS: Positive and Negative Syndrome Scale; HAMD-21: 21 item Hamilton Rating Scale for Depression.

(significance criteria of $P < 0.017$). Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 11.0 (SPSS Japan, Tokyo).

3. Results

As shown in Table 1, no significant difference was found between diagnostic groups in mean age, gender distribution, or body mass index (BMI). The prevalence of smoking was higher in the patients groups compared to controls. Fig. 1 shows the CSF and serum IL-6 levels in each diagnostic group. All samples analyzed were well above the lower detection limit of 0.039 pg/ml. The difference in serum IL-6 levels between the groups was not statistically significant ($\chi^2 = 1.8$, $df = 2$, $P = 0.40$); however, CSF IL-6 levels differed significantly across the groups ($\chi^2 = 10.7$, $df = 2$, $P = 0.0049$). *Post hoc* pairwise Mann–Whitney-*U* test showed that both the patients with schizophrenia and MDD had significantly higher CSF IL-6 levels compared to the controls (schizophrenia: $U = 321$, $P = 0.0027$; MDD: $U = 334$, $P = 0.012$).

No significant correlation between CSF and serum IL-6 levels was observed for each diagnostic group. Spearman's rank correlation coefficients and the 95% confidence intervals (95% CI) were as follows: controls, $\rho = 0.18$ (95% CI: −0.18–0.55); schizophrenia, $\rho = 0.23$ (−0.13–0.59); MDD, $\rho = 0.19$ (−0.18–0.57); and all groups combined, $\rho = 0.20$ (−0.006–0.41). IL-6 levels were significantly higher in the CSF than in the serum ($Z = 4.04$, $P < 0.0001$). When analyzed separately in each diagnostic group, the difference between CSF and serum IL-6 levels reached statistical significance in only patients with schizophrenia (schizophrenia: $Z = 3.54$, $P = 0.0004$; MDD: $Z = 1.74$, $P = 0.082$; controls: $Z = 1.82$, $P = 0.068$).

Next, we examined the influence of clinical factors on CSF IL-6 levels (Table 2). CSF IL-6 levels of the schizophrenic patients did not significantly correlate with the antipsychotic dose ($\rho = 0.12$, $P > 0.1$) or with the PANSS scores (positive symptoms: $\rho = 0.065$, $P > 0.1$; negative symptoms: $\rho = 0.12$, $P > 0.1$). Similarly, CSF IL-6 levels of the patients with MDD did not significantly correlate with the antidepressant dose ($\rho = 0.044$, $P > 0.1$) or with the HAMD-21 score ($\rho = -0.036$, $P > 0.1$). Because smoking prevalence was significantly different between controls and patient groups, we also compared CSF IL-6 levels in only nonsmokers to avoid the confounding effects of smoking. When only nonsmokers were compared, patients with schizophrenia had significantly higher CSF IL-6 levels compared to the controls ($U = 158$, $P = 0.04$), but the difference between MDD patients and controls did not reach statistical significance ($U = 194$, $P = 0.22$). No significant correlation with CSF IL-6 levels was observed for time of day of sampling or number of days between sample collection and IL-6 assay. Furthermore, no significant difference in CSF IL-6 levels of those sampled before and after noon was observed for each diagnostic group.

4. Discussion

The results showed that CSF IL-6 levels were higher in patients with schizophrenia and those with MDD than in healthy controls. The present findings further support the evidence for the role of IL-6 in the pathogenesis of these disorders. No significant increase in serum IL-6 levels of patients with schizophrenia or MDD was obtained. However, this does not contradict with previous findings, because the effect size reported in previous meta-analyses (Howren et al., 2009; Potvin et al., 2008) requires a sample more than twice as large as ours to reach 80% power to detect the difference at the

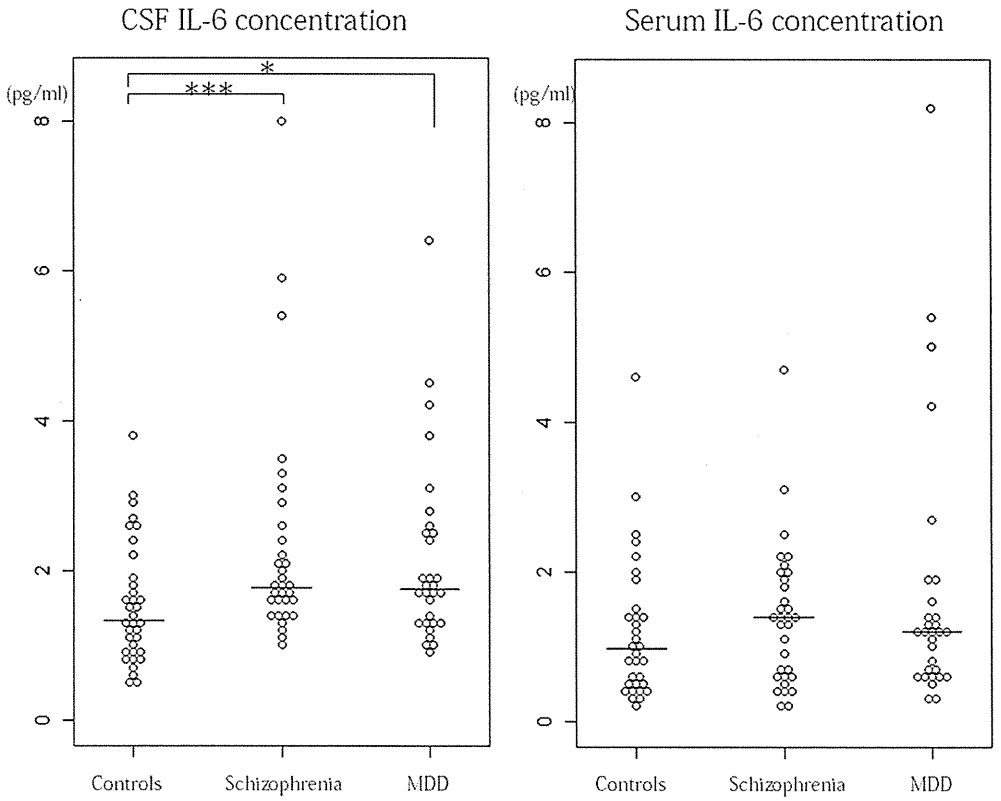

Fig. 1. CSF and serum IL-6 levels in patients with schizophrenia, those with major depressive disorder, and healthy controls CSF IL-6 levels of both the patients with schizophrenia and those with MDD were significantly higher compared to that of the healthy controls. The horizontal lines indicate the median value of each group. *$P < 0.05$, ***$P < 0.005$ (Mann–Whitney *U* test) n.s.: no significant difference; MDD: major depressive disorder; CSF: cerebrospinal fluid; IL-6: interleukin-6.

Table 2
Association between cerebrospinal fluid IL-6 levels and clinical factors.

		Controls	Schizophrenia	MDD
Spearman's correlation coefficients between CSF IL-6 levels and clinical factors				
Age [years]		$\rho = 0.18$	$\rho = 0.36$[a]	$\rho = 0.062$
Age at onset [years]			$\rho = 0.41$[a]	$\rho = -0.057$
Illness duration [years]			$\rho = 0.079$	$\rho = 0.067$
BMI		$\rho = 0.36$[a]	$\rho = 0.27$	$\rho = 0.11$
CP equivalent dose [mg/day]			$\rho = 0.12$	$\rho = -0.28$
IMI equivalent dose [mg/day]			$\rho = 0.12$	$\rho = 0.044$
PANSS positive scores			$\rho = 0.065$	
PANSS negative scores			$\rho = 0.12$	
HAMD-21 scores				$\rho = -0.036$
Time of day of sampling [h]		$\rho = 0.088$	$\rho = 0.023$	$\rho = -0.11$
Number of days between sample collection and IL-6 assay		$\rho = -0.23$	$\rho = 0.066$	$\rho = -0.17$
Mean (standard deviation) CSF IL-6 levels [pg/ml]				
Gender	Men	1.70 (0.78)	2.57 (1.61)	2.37 (1.37)
	Women	1.30 (0.78)	1.92 (1.29)	1.75 (0.83)
Smoking status	Smokers	1.44 (0.80)	2.06 (0.68)	2.60 (1.48)
	Nonsmokers	1.55 (0.81)	2.60 (2.03)	1.74 (0.80)

MDD: major depressive disorder; CSF: cerebrospinal fluid; BMI: body mass index; CP: chlorpromazine; IMI: imipramine; PANSS: Positive and Negative Syndrome Scale; HAMD-21: 21 item Hamilton Rating Scale for Depression.
[a] $P < 0.05$.

5% significance level (calculated by G*Power 3.1.3 (Faul et al., 2007)). It is of note that significant difference in CSF IL-6 levels was obtained with the present sample, suggesting that the effect size may be larger for CSF than for serum.

No significant correlation was observed between CSF and serum IL-6 levels. Although there is a possibility that a larger sample may yield a significant correlation, the correlation coefficient is likely to be lower than the upper limit of the 95% confidence interval (i.e. $\rho = 0.41$) obtained in the present study. Furthermore, IL-6 levels were higher in the CSF compared to the serum, especially for schizophrenic patients. Thus, the increased CSF IL-6 levels in patients with schizophrenia and MDD are unlikely to be explained by the diffusion from the peripheral circulation. These findings suggest that IL-6 of central origin is associated with the pathophysiology of these disorders.

Increased CSF IL-6 levels in both patients with schizophrenia and those with MDD suggest that inflammatory mediators may be commonly involved in the pathogenesis of these disorders. Although a plethora of studies examining peripheral cytokine levels also support the hypothesis that inflammation plays a role in these disorders, a unique cytokine profile capable of distinguishing these two disorders has not been described. There is a possibility that common underlying pathogenic mechanisms may be involved in schizophrenia and MDD.

A number of studies indicate involvement of abnormal neurogenesis in the pathophysiology of MDD (Leonard and Maes, 2012) as well as schizophrenia (Balu and Coyle, 2011). Monje et al. (2003) have shown that inflammation can inhibit neurogenesis and that IL-6 is implicated as a potential regulator of hippocampal neurogenesis in neuroinflammation. Therefore, increased microglial production of IL-6 may be a common etiological risk factor for schizophrenia and MDD. Another common potential etiological factor of these two disorders may be the changes in kynurenine metabolism. The increased kynurenine induces increased production of kynurenic acid in schizophrenia and quinolinic acid in depression, which may result in an imbalance in glutamatergic neurotransmission. Raison et al. (2010) have shown that the changes in kynurenine metabolism are linked to central cytokine responses. Thus, the increased central IL-6 observed in the present study is in line with the role of kynurenine pathway on the pathophysiology of schizophrenia (Muller et al., 2011) and MDD (Myint et al., 2007, 2012).

Not all individuals with depression or schizophrenia exhibit high levels of CSF IL-6 levels. Therefore, it is likely that inflammation is involved in the pathogenesis of a subgroup of patients. We could not identify any major clinical features specific to those with high CSF IL-6 levels. The positive correlation observed between CSF IL-6 levels and age at onset in patients with schizophrenia suggests that inflammatory mechanism may be more likely to be associated with late-onset schizophrenia; however, the sample size was too small to draw definitive conclusion regarding the association with particular clinical features.

Some previous studies failed to find significant change of CSF IL-6 levels in patients with schizophrenia (Barak et al., 1995) or those with MDD (Carpenter et al., 2004; Martinez et al., 2012). Because the sample sizes were smaller than that in the present study, insufficient statistical power may have precluded detection of statistically significant differences in these studies. Some other studies have yielded results consistent with the present findings. Garver et al. (2003) reported increased CSF IL-6 levels in schizophrenic patients who subsequently responded to antipsychotic treatment. Lindqvist et al. (2009) reported that CSF IL-6 levels in patients with MDD after a suicide attempt were higher compared to healthy controls. In contrast to our findings, one previous study of patients with geriatric depression (Stubner et al., 1999) and another of patients with acute severe depression (Levine et al., 1999) have shown that CSF IL-6 levels were lower in depressed subjects compared to controls. Since the majority of the patients in our study were middle-aged and were in the chronic stage of illness, the influence of the patients' age and the illness stage may have resulted in a different outcome. Further studies are necessary to clarify how the clinical characteristics of the disease affect IL-6 levels.

The major limitation of the present study was the uncontrolled medication. The results showed that neither the chlorpromazine equivalent dose in schizophrenic patients nor the imipramine equivalent dose in MDD patients significantly correlated with CSF IL-6 levels. However, the effects of medication could not be adequately assessed due to the variability in types and doses. Evidence shows that both antipsychotic and antidepressant treatment decrease peripheral IL-6 levels (Hiles et al., 2012; Miller et al., 2011). If similar effects occur in the CSF, the increase in CSF IL-6 levels would be more prominent in untreated patients than observed in the medicated patients in the present study. The present study provides evidence that IL-6 levels of central origin may be increased in patients receiving treatment in the real-world setting. However, the possible confounding effects of medications must be addressed in future studies including medication-free patients. Different smoking prevalence between patients and controls may

have also influenced the findings of the present study. The results of comparison in only non-smoking subjects indicate that CSF IL-6 levels are increased in patients with schizophrenia regardless of the smoking status. However, a larger number of non-smoking subjects are needed to confirm the results for MDD patients. Another limitation was that the time of day of sampling was not consistent across subjects. Although no significant association was found between CSF IL-6 levels and the sampling time of day, larger sample size is necessary to clarify the influence of sampling time on IL-6 levels. However, because the average time of day of sampling was similar between diagnostic groups, it is unlikely that the sampling time of day had a major impact on the overall results of the present study. Finally, the cross-sectional design of the study did not allow determination of whether the increased IL-6 levels preceded or resulted from illness onset. The lack of significant correlation with PANSS or HAMD-21 scores suggests that IL-6 levels are not greatly influenced by the severity of the symptoms. However, further studies with a longitudinal design are required to investigate how CSF IL-6 levels change during the course of the disease.

In conclusion, CSF IL-6 levels were significantly increased in patients with schizophrenia and those with MDD. No significant correlation was observed between CSF and serum IL-6 levels. The present findings suggest that IL-6 of central origin is associated with the pathophysiology of these disorders.

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Contributors

D.S., K.H., C.W., and H.K. designed the study and D.S. wrote the draft of the manuscript. D.S., H.H., T.T., K.H., M.O., S.Y., and H.K. screened the study participants using the Mini International Neuropsychiatric Interview (M.I.N.I.) and diagnosed the patients according to the DSM-IV criteria. D.S., K.H., H.H., T.T., and M.O. collected plasma and cerebrospinal fluid samples. D.S. measured the IL-6 levels and undertook the statistical analysis. H.K. supervised the data analysis and writing of the paper. K.A., T.H., and N.A. also supervised the writing of the paper and gave critical comments on the manuscript. All authors contributed to and have approved the final manuscript.

Conflict of interest

The authors report no conflicts of interest.

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Research report

Relationship of temperament and character with cortisol reactivity to the combined dexamethasone/CRH test in depressed outpatients

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ABSTRACT

Background: Evidence shows that depression is associated with hypothalamic–pituitary–adrenal (HPA) axis hyperactivation, although such findings are not entirely unequivocal. In contrast, various psychiatric conditions, including atypical depression, are associated with hypocortisolism. Another line of research has demonstrated that personality is associated with HPA axis alteration. It is thus hypothesized that different personality pathology in depression would be associated with distinct cortisol reactivity.

Methods: Eighty-seven outpatients with DSM-IV major depressive disorder were recruited. Personality was assessed by the temperament and character inventory (TCI). HPA axis reactivity was measured by the combined dexamethasone (DEX)/corticotropin-releasing hormone (CRH) test. According to our previous studies, two subgroups were considered based on their cortisol responses to the DEX/CRH test: incomplete-suppressors whose cortisol response was exaggerated and enhanced-suppressors whose cortisol response was blunted.

Results: The analysis of covariance, controlling for age, gender and symptom severity, revealed that incomplete-suppressors scored significantly higher on cooperativeness than enhanced-suppressors ($p=0.002$). A multivariate stepwise logistic regression analysis predicting the cortisol suppression pattern from the seven TCI dimensions, controlling for age, gender and symptom severity, revealed that lower cooperativeness ($p=0.001$) and higher reward dependence ($p=0.018$) were significant predictors toward enhanced suppression.

Limitations: The neuroendocrine challenge test was administered only once, based on a simple test protocol.

Conclusions: Our findings suggest that (personality-related) subtypes of depression might be differentiated based on the different pattern of cortisol reactivity. Future studies are warranted to further characterize the HPA axis alteration in relation to various subtypes of depression.

1. Introduction

Depression imposes a great burden on afflicted individuals and society, while its pathophysiology remains elusive. One of the repeatedly reported biological abnormalities in depression is the alteration in the hypothalamic–pituitary–adrenal (HPA) axis function (Holsboer, 2000; Kunugi et al., 2010). To quantify the dysregulation of HPA axis, the dexamethasone suppression test (DST) has been enthusiastically studied since Carroll et al. (1981) introduced it as a biological marker for the diagnosis of "melancholia". In serial DST studies, cortisol levels were shown to be increased in depressed patients (e.g., Carroll, 1982). However, it has subsequently become clear that its sensitivity to differentiate depressed patients from healthy controls is not very high (Arana et al., 1985; Braddock, 1986), and elevated cortisol levels were also observed in non-clinical populations under various stressful conditions (Ceulemans et al., 1985; Mellsop et al., 1985). The DST thus failed to fulfill the initial promise as a diagnostic tool for depression.

The dexamethasone (DEX)/corticotropin-releasing hormone (CRH) test, which was developed by Holsboer et al. (1987), Heuser et al. (1994a) in an attempt to enhance the sensitivity of

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the DST, is an integrated challenge test for HPA axis function that combines DEX-pretreatment with CRH administration on the following day; thus, it is essentially a DST followed by CRH challenge. The merit of this combined test is that at the moment of CRH infusion the HPA axis is downregulated due to negative feedback induced by the DEX. A number of independent studies have confirmed that sensitivity of the DEX/CRH test for depression is relatively high (Kunugi et al., 2004; Kunugi et al., 2006; Watson et al., 2006; Ising et al., 2007). However, such findings are not necessarily conclusive because other studies have reported rather low sensitivity of this test for depression, i.e., no more than around 20–30% (e.g., Ising et al., 2005; Nickel et al., 2003; Schüle et al., 2009). Moreover, recent studies using this test have shown that depressed patients exhibit similar (Oshima et al., 2000; Watson et al., 2002; Gervasoni et al., 2004; Van Den Eede et al., 2006), or even attenuated (Rydmark et al., 2006; Veen et al., 2009; Wahlberg et al., 2009) cortisol responses as compared to healthy controls. In these studies, patients had either of the following characteristics: outpatients (Oshima et al., 2000; Gervasoni et al., 2004; Van Den Eede et al., 2006; Carpenter et al., 2009), chronically depressed patients (Watson et al., 2002), depressed patients with psychiatric comorbidity (Veen et al., 2009) or long-term sick-leave patients (Rydmark et al., 2006; Wahlberg et al., 2009). Inconsistent findings across the DEX/CRH studies may therefore result from the heterogeneity of depression, rather than from the limited sensitivity of this neuroendocrine challenge test. A similar interpretation has also been proposed for the original DST (e.g., Fink, 2005).

A promising marker for such phenotypic heterogeneity of depression would be personality traits, given that personality profile of depressed patients is different from that of healthy controls (Enns and Cox, 1997; Bagby et al., 2008) and such profile varies even within depressed patients depending on diagnostic (sub)categories, e.g., melancholic vs. atypical depression (Joyce et al., 2004; Chopra et al., 2005) and bipolar vs. unipolar depression (Bagby et al., 1996; Mendlowicz et al., 2005; Akiskal et al., 2006; Sasayama et al., 2011).

Apart from depression, several lines of research have demonstrated that personality impacts on HPA axis function as measured by the DEX/CRH test. In a non-clinical population, Tyrka and her colleagues have found that low novelty seeking of the Cloninger's temperament dimension (Cloninger et al., 1991), particularly when combined with high harm avoidance, is associated with exaggerated cortisol responses to the DEX/CRH test (Tyrka et al., 2006, 2008). Using another well-established measure of personality, McCleery and Goodwin (2001) observed a relationship between higher neuroticism and blunted cortisol response to this pharmacological challenge test, whereas Zobel et al. (2004) found the opposite relation, i.e., higher neuroticism and greater cortisol response. More specific personality characteristics have also been examined in relation to HPA axis reactivity as measured by the DEX/CRH test. For instance, we reported that non-clinical schizotypal personality to be associated with blunted cortisol response to this test (Hori et al., 2011a). Furthermore, Rinne et al. (2002) observed exaggerated cortisol responses to the DEX/CRH test in female subjects with borderline personality disorder who had a history of sustained childhood abuse. These findings not only suggest that the DEX/CRH test can serve as a useful tool to probe the altered HPA axis function in relation to a wide variety of personality traits but also point to the importance of taking into account blunted cortisol reactivity as well as exaggerated reactivity. Indeed, it is now widely recognized that hypocortisolism, in addition to hypercortisolism, represents impaired HPA axis regulation (Raison and Miller, 2003), which is reflected by the fact that the former has been associated with various stress-related psychopathologies including posttraumatic stress disorder, fibromyalgia, chronic fatigue syndrome and atypical depression (Heim et al., 2000; Gold and Chrousos, 2002; Fries et al., 2005).

In this context, the present study aimed to explore the relationship between personality traits as assessed by the temperament and character inventory (TCI) (Cloninger et al., 1993) and cortisol reactivity to the DEX/CRH test in depressed outpatients. To this end, we first dimensionally examined this relationship and then compared the personality traits between patients who exhibited exaggerated cortisol reactivity and those who did blunted reactivity. We hypothesized that these two extreme ends of cortisol reactivity would be associated with different temperament/character traits.

2. Methods

2.1. Participants

Eighty-seven depressed outpatients (age range: 21–69; 46 women) were recruited from the outpatient clinic of the National Center of Neurology and Psychiatry (NCNP) Hospital, Tokyo, Japan, or through advertisements in free local magazines and our website announcement. Most of the patients recruited via advertisements or website announcement were regularly attending to a nearby hospital or clinic located in the same geographical area, i.e., the western part of Tokyo. Consensus diagnoses were made based on clinical interviews, observations and case notes by at least two experienced psychiatrists. For those patients under treatment at the NCNP Hospital, the diagnosis was confirmed using the Structured Clinical Interview for DSM-IV Axis I disorders (First et al., 1997). For the remaining patients under treatment at a nearby hospital/clinic, the diagnosis made by his/her attending doctor was confirmed by the Mini-International Neuropsychiatric Interview (Sheehan et al., 1998; Otsubo et al., 2005) by a trained research psychiatrist. All met the DSM-IV criteria (American Psychiatric Association, 1994) for major depressive disorder (MDD). Patients who were in remission, as defined by the total score on the Hamilton Depression Rating Scale 21-item version (HAMD-21) (Hamilton, 1967) of less than 8, were excluded from the study. Of the total 87 MDD patients, 13 were diagnosed as having comorbid dysthymic disorder. Patients with bipolar disorders were not enrolled as they are shown to have a different personality profile from that of MDD patients (Bagby et al., 1996; Mendlowicz et al., 2005; Akiskal et al., 2006; Sasayama et al., 2011). Patients who were taking carbamazepine were also excluded from the study since it induces dexamethasone metabolism (Privitera et al., 1982). Additional exclusion criteria for study participation were as follows: having a prior medical history of central nervous system disease or severe head injury, having a history of substance abuse/dependence, taking corticosteroids, antihypertensives or oral contraceptives, and being on hormone replacement therapy. The present experiment on our participants was conducted in accordance with the Declaration of Helsinki. After the nature of the study procedures had been fully explained, written informed consent was obtained from all participants. The study was approved by the ethics committee of the NCNP, Tokyo, Japan.

2.2. DEX/CRH test procedure and presentation for neuroendocrine data

The DEX/CRH test was administered to all participants according to a simple test protocol (Kunugi et al., 2006), which was modified from the original protocol of Heuser et al. (1994a). This simple protocol was described in our recent reports (Hori et al., 2010, 2011a, b). Briefly, participants took 1.5 mg of DEX (Banyu Pharmaceutical Corporation, Tokyo, Japan) orally at 2300 h.

On the next day, a vein was cannulated at 1430 h to collect blood at 1500 h and 1600 h. Human CRH (100 μg) (hCRH 'Mitsubishi', Mitsubishi Pharma Corporation, Tokyo, Japan) was administered intravenously at 1500 h, immediately after the first blood collection. Plasma concentrations of cortisol were measured by radioimmunoassay at SRL Corporation (Tokyo, Japan). The detection limit for cortisol was 27.59 nmol/l (=1.0 μg/dl). Cortisol values under the detection limit were treated as 0 nmol/l. Outcome measures of this neuroendocrine test were "DST-Cort" (i.e., the concentration of cortisol [nmol/l] at 1500 h) and "DEX/CRH-Cort" (i.e., the concentration of cortisol at 1600 h). To dissect the extent to which the subject's HPA axis responded to the CRH challenge, the magnitude of change from DST-Cort to DEX/CRH-Cort, namely ΔCort, was calculated for each subject.

As our hypotheses was that the two extreme ends of cortisol values (i.e., both exaggerated and blunted cortisol reactivity) would be each related to unique personality profiles, in the main analysis we adopted a categorical division of participants according to a priori defined cut-off values of cortisol. Considering the marked gender difference in cortisol reactivity to the DEX/CRH challenge, the cut-off values were separately defined for men and women, using our database of healthy controls. Recruitment methods for these control subjects were described previously (Hori et al., 2010). For men, "incomplete-suppressors" were defined as those individuals whose DEX/CRH-Cort level was equal to or more than 137.95 nmol/l (=5.0 μg/dl) and "enhanced-suppressors" were defined as those individuals whose DEX/CRH-Cort level was less than 27.59 nmol/l (=1.0 μg/dl), based on our previous studies (Hori et al., 2010, 2011a, b). These cut-off values resulted in 21.3% incomplete-suppressors and 19.7% enhanced-suppressors out of the total sample of healthy men. We then applied these percentages for incomplete- and enhanced-suppressors in healthy men to the cortisol data of healthy women in order to make the percentages identical between men and women. Consequently, cortisol levels to define incomplete- and enhanced-suppressors in women were "equal to or more than 273.14 nmol/l (=9.9 μg/dl)" and "less than 49.66 nmol/l (=1.8 μg/dl)", respectively, and these cut-off values yielded 21.0% and 19.6% healthy women who fell into the respective suppressor categories. Finally, these cut-off values separately defined for healthy men and women were applied to the present sample of 87 MDD patients, which yielded 11 incomplete- and 11 enhanced-suppressors in men and 13 incomplete- and 8 enhanced-suppressors in women. The remaining patients were considered to be "moderate-suppressors" and excluded from the categorical analyses comparing incomplete- vs. enhanced-suppressors.

2.3. Personality assessment

Personality was assessed in all subjects using the TCI. The TCI (Cloninger et al., 1993) is a 240-item (including 14 items which are not analyzed) self-report questionnaire; each item requires a true/false answer. The term temperament refers to automatic emotional reactions to subjective experiences that may be genetically transmitted and therefore stable over time. Four dimensions of temperament are distinguished: novelty seeking, harm avoidance, reward dependence, and persistence. Novelty seeking, harm avoidance, and reward dependence have been assumed to relate to dopaminergic, serotonergic, and noradrenergic neurotransmission, respectively (Cloninger, 1987). The term character refers to concepts pertaining to the individual, focusing on personal differences in intentions, decisions and values. Three dimensions of character are distinguished: self-directedness, cooperativeness, and self-transcendence. The reliability and validity of the original American version of the TCI in general community dwellers as well as in psychiatric patients have been established (Cloninger et al., 1993; Svrakic et al., 1993). The Japanese version of the TCI translated and validated by Kijima et al. (1996, 2000) was used in the present study.

2.4. Symptom assessment

Severity of the depressive symptoms was assessed with the HAMD-21 interview at the time of the neuroendocrine testing. In addition to the total score of the HAMD-21, we used the following four factors that had been identified in a previous study where a factor analytic technique (using the principal axis factoring method with oblique rotation) had been applied to the scores on the 17-item version of HAMD in depressed outpatients (Pancheri et al., 2002), in order to further examine the possible association between different symptom dimensions and cortisol reactivity. The four factors were "somatic anxiety" (consisting of early insomnia, middle insomnia, late insomnia, somatic anxiety, general somatic symptoms, and hypochondria), "psychic anxiety" (guilt, agitation, psychic anxiety, and insight), "core depressive symptoms" (depressed mood, work and interests, and retardation), and "anorexia" (gastrointestinal symptoms) (Pancheri et al., 2002).

Subjectively perceived symptoms during one week preceding the neuroendocrine test was assessed via the Hopkins Symptom Checklist (HSCL, Derogatis et al., 1974). The HSCL is a self-report questionnaire consisting of 58 (or 54) items which are scored on five underlying symptom dimensions, i.e., somatization, obsessive-compulsive, interpersonal sensitivity, anxiety, and depression symptoms. A validated Japanese version of the HSCL comprising 54 items (Nakano, 2005) was used in the present study. In this questionnaire, subjects were instructed to rate each item based on the distress perceived during the previous week, using a four-point scale of frequency, with "not-at-all" being scored 1, "occasionally", 2, "sometimes", 3, and "frequently", 4.

2.5. Statistical analyses

Averages are reported as means ± standard deviation (SD). Non-parametric tests were used to examine the association of DST-Cort with other variables, given that this cortisol index fell under the detection limit in a substantial portion of subjects and thus the data did not satisfy the assumptions for parametrical testing. In contrast, DEX/CRH-Cort and ΔCort were examined using parametric tests. The *t*-test or Mann–Whitney *U*-test was used to examine differences between two groups. Categorical variables were compared using the χ^2 test or Fisher's exact test where appropriate. Partial correlation analysis, controlling for confounders that were defined below, was performed to examine correlations of DEX/CRH-Cort and ΔCort with other variables, while Spearman's ρ was used to examine correlations for DST-Cort. The analysis of covariance (ANCOVA) was performed to examine differences between groups, controlling for confounders. Post-hoc pairwise comparisons were made with Bonferroni correction, when applicable. Since age, gender and depressive symptoms have been reported to significantly influence cortisol levels (e.g., Heuser et al., 1994b; Künzel et al., 2003; Kunugi et al., 2006) and TCI scores (e.g., Brändström et al., 2001; Miettunen et al., 2007; Spittlehouse et al., 2010), these three variables were considered as potential confounders regardless of the present data.

A multivariate forward stepwise logistic regression model based on the likelihood ratio test, with inclusion and exclusion p value thresholds of 0.05 and 0.1, respectively, was used to test the effects of TCI results, in addition to age, gender and HAMD-21 total score, on the DEX/CRH suppression pattern. Nagelkerke R^2 was used to estimate the proportion of explained variance in the

model. The Hosmer–Lemeshow goodness-of-fit test was used to evaluate the fit of the logistic model to our data, with p value greater than 0.05 indicating an acceptable fit.

Statistical significance was set at two-tailed $p < 0.05$. Analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 18.0 (SPSS Japan, Tokyo).

3. Results

3.1. Demographic/clinical characteristics of patients

Age was not significantly correlated with any of the three cortisol indices (all $p > 0.1$). Females showed significantly higher DEX/CRH-Cort ($t=2.51$, df=85, $p=0.014$) and ΔCort ($t=2.44$, df=85, $p=0.017$), but not DST-Cort (Mann–Whitney $U=901.5$, $p=0.71$), than males.

Demographic and clinical characteristics of patients stratified by the suppression status (i.e., incomplete- vs. enhanced-suppressors) are shown in Table 1. No significant differences were seen between incomplete- and enhanced-suppressors in any of the variables examined. The mean HAMD-21 total score was around 15, indicating that the subjects were mildly to moderately depressed (Table 1).

3.2. Relationships between symptoms and DEX/CRH test results

DST-Cort was not significantly correlated with depressive symptoms as indexed by the HAMD-21 total score and four factor scores (all $p > 0.2$ by Spearman's ρ) or with distress scores as indicated by the five HSCL dimensions (all $p > 0.4$ by Spearman's ρ). Similarly, DEX/CRH-Cort and ΔCort were not significantly correlated with depressive symptoms as indexed by the HAMD-21 total score and four factor scores (all $p > 0.3$ by partial correlation analysis controlling for age and gender) or with distress scores as indicated by the five HSCL dimensions (all $p > 0.1$ by partial correlation analysis controlling for age and gender).

As shown in Table 1, no significant differences were observed between incomplete- vs. enhanced-suppressors in any of the symptom dimensions as assessed by the HAM-D and the HSCL, using the ANCOVA controlling for age and gender.

3.3. Relationships between TCI scores and DEX/CRH test results

Results of the three cortisol indices for the three suppressor groups, stratified by gender, are presented in Table 2.

3.3.1. Correlations between TCI scores and cortisol measures among the patients

No significant correlations were observed between the seven TCI dimensions and DST-Cort (all $p > 0.1$ by Spearman's ρ). Partial correlation analysis, controlling for age, gender and HAMD-21 total score, revealed that cooperativeness was correlated significantly with DEX/CRH-Cort ($r=0.23$, df=82, $p=0.03$) (Fig. 1a) and marginally significantly with ΔCort ($r=0.21$, df=82, $p=0.06$) (Fig. 1b); while no significant correlations were seen between the other six TCI dimensions and these two cortisol indices.

3.3.2. Comparison of TCI scores between incomplete- vs. enhanced-suppressors

Fig. 2 shows comparisons of the seven TCI dimensions between incomplete- and enhanced-suppressors. The ANCOVA, controlling

Table 1
Demographic/clinical characteristics and symptom dimensions of the sample stratified by the suppression pattern to the DEX/CRH test.

Variable	Total sample ($n=87$)	Incomplete-suppressors[a] ($n=24$)	Enhanced-suppressors[b] ($n=19$)	Analysis (incomplete-vs. enhanced-suppressors)		
				Statistic	d.f.	p
Age, years: mean ± SD	40.1 ± 10.7	40.0 ± 9.9	37.1 ± 11.1	$t=0.92$	41	0.36
Gender, female: *n* (%)	46 (52.9)	13 (54.2)	8 (42.1)	$\chi^2=0.62$	1	0.43
Comorbid dysthymic disorder: Yes, *n* (%)	13 (14.9)	3 (12.5)	3 (15.8)	Fisher's exact test		1
Family history of any psychiatric disorder: Yes, *n* (%)	35 (40.2)	13 (54.2)	6 (31.6)	$\chi^2=2.19$	1	0.14
Lifetime hospitalization to psychiatric ward: Yes, *n* (%)	19 (21.8)	8 (33.3)	2 (10.5)	Fisher's exact test		0.14
Lifetime electroconvulsive therapy: Yes, *n* (%)	1 (1.1)	1 (4.2)	0 (0.0)	Fisher's exact test		1
Medication, *n* (%)						
Antipsychotic	27 (31.0)	11 (45.8)	4 (21.1)	Fisher's exact test		0.12
Antidepressant	75 (86.2)	22 (91.7)	17 (89.5)	Fisher's exact test		1
Lithium	9 (10.3)	5 (20.8)	0 (0.0)	Fisher's exact test		0.06
Benzodiazepine	63 (72.4)	20 (83.3)	12 (63.2)	Fisher's exact test		0.17
HAMD-21 total score: mean ± SD	15.0 ± 5.6	15.6 ± 5.9	15.2 ± 5.2	$F=0.21$[c]	1,39	0.65
Somatic anxiety: mean ± SD	4.1 ± 2.3	4.2 ± 2.8	4.2 ± 2.4	$F=0.02$[c]	1,39	0.89
Psychic anxiety: mean ± SD	1.8 ± 1.2	1.5 ± 0.9	2.1 ± 1.2	$F=1.73$[c]	1,39	0.20
Core depressive symptoms: mean ± SD	4.6 ± 1.9	4.9 ± 1.8	4.6 ± 1.9	$F=0.54$[c]	1,39	0.47
Anorexia: mean ± SD	0.8 ± 1.2	0.8 ± 1.0	0.8 ± 1.0	$F=0.01$[c]	1,39	0.94
Hopkins Symptom Checklist						
Somatization: mean ± SD	29.1 ± 7.1	29.5 ± 6.1	28.6 ± 5.9	$F=0.27$[c]	1,39	0.60
Obsessive-compulsive: mean ± SD	25.3 ± 5.6	26.3 ± 5.7	25.8 ± 5.0	$F=0.10$[c]	1,39	0.75
Interpersonal sensitivity: mean ± SD	22.8 ± 5.7	23.5 ± 5.6	23.1 ± 5.8	$F=0.18$[c]	1,39	0.67
Anxiety: mean ± SD	16.6 ± 4.7	17.1 ± 4.7	17.0 ± 4.8	$F=0.20$[c]	1,39	0.65
Depression: mean ± SD	32.6 ± 7.1	32.9 ± 6.6	33.6 ± 6.4	$F=0.09$[c]	1,39	0.77

Abbreviations: DEX/CRH test, dexamethasone/corticotropin-releasing hormone test; HAMD-21, 21-item version of the Hamilton Depression Rating Scale.
Notes:

[a] Defined as DEX/CRH-Cort ≥ 137.95 (nmol/l) for men and DEX/CRH-Cort ≥ 273.14 for women.

[b] Defined as DEX/CRH-Cort < 27.59 (i.e., under the detection limit) for men and DEX/CRH-Cort < 49.66 for women.

[c] Analysis of covariance, controlling for age and gender.

Table 2
Plasma cortisol concentrations (nmol/l) [mean ± SD (range)] for the three groups based on the suppression pattern, stratified by gender.

	Incomplete-suppressors[d]		Moderate-suppressors[e]		Enhanced-suppressors[f]	
	Men (n=11)	Women (n=13)	Men (n=19)	Women (n=25)	Men (n=11)	Women (n=8)
DST-Cort[a]	32.9 ± 24.1 (0.0–66.2)	59.0 ± 94.7 (0.0–364.2)	26.9 ± 23.4 (0.0–80.0)	19.1 ± 22.6 (0.0–80.0)	14.8 ± 17.5 (0.0–44.1)	18.3 ± 20.2 (0.0–46.9)
DEX/CRH-Cort[b]	290.4 ± 125.4 (140.7–485.6)	375.4 ± 62.4 (303.5–502.1)	63.2 ± 28.8 (30.3–118.6)	129.7 ± 60.4 (57.9–264.9)	0.0 ± 0.0 (0.0–0.0)	25.5 ± 21.3 (0.0–44.1)
ΔCort[c]	257.6 ± 128.2 (104.8–485.6)	316.4 ± 109.7 (24.8–455.2)	36.3 ± 40.9 (−13.8–118.6)	110.6 ± 58.8 (13.8–264.9)	−14.8 ± 17.5 (−44.1–0.0)	7.2 ± 13.0 (−2.8–35.9)

Notes:
[a] The concentration of cortisol (nmol/l) at 1500 h (i.e., immediately before the CRH challenge).
[b] The concentration of cortisol (nmol/l) at 1600 h (i.e., 1 h after the CRH challenge).
[c] Defined as "DEX/CRH-Cort minus DST-Cort".
[d] Defined as DEX/CRH-Cort ≥ 137.95 for men and DEX/CRH-Cort ≥ 273.14 for women.
[e] Defined as 27.59 ≤ DEX/CRH-Cort < 137.95 for men and 49.66 ≤ DEX/CRH-Cort < 273.14 for women.
[f] Defined as DEX/CRH-Cort < 27.59 (i.e., under the detection limit) for men and DEX/CRH-Cort < 49.66 for women.

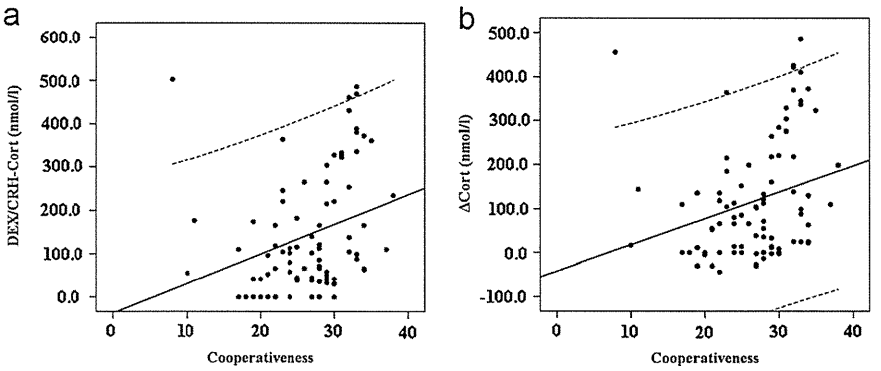

Fig. 1. Scatterplot showing the correlation between the cooperativeness score of the temperament and character inventory and cortisol measures including DEX/CRH-Cort (a) and ΔCort (b). The solid line and broken lines represent regression line and 95% prediction interval, respectively.

for age, gender and HAMD-21 total score, revealed that these two groups significantly differed on cooperativeness; Incomplete-suppressors scored higher on this dimension than enhanced-suppressors [$F(1,38)=11.7$, $p=0.002$]. No significant differences were found between the two suppressor groups in any of the other six TCI dimensions (all $p>0.2$). In order to further explore which subscales pertaining to the cooperativeness dimension contributed to this result, an additional ANCOVA was conducted on the five subscales of cooperativeness (i.e., social acceptance, empathy, helpfulness, compassion, and pure-hearted conscience), controlling for age, gender and HAM-D total score. This analysis revealed that incomplete-suppressors scored significantly higher on social acceptance [$F(1,38)=13.9$, $p<0.001$] and compassion [$F(1,38)=19.2$, $p<0.001$] than enhanced-suppressors, while no significant differences were found for the other three subscales (all $p>0.1$).

3.3.3. *Prediction of the DEX/CRH suppression pattern from TCI results*

The forward stepwise logistic regression analysis to predict the cortisol suppression pattern by the seven TCI dimensions, in addition to age, gender and HAM-D total score, revealed that cooperativeness and reward dependence were significant predictors; lower cooperativeness and higher reward dependence were associated with enhanced suppression of cortisol (Table 3). Of note, the goodness-of-fit improved considerably in the second step relative to the first step, indicating that not only cooperativeness but reward dependence played an important role in predicting the suppression pattern.

The fact that reward dependence was a significant predictor for the suppressor group, albeit not significantly different between the two suppressor groups, raised a possibility that there could be an interactive relationship between reward dependence and cooperativeness for the reactive cortisol measures. To scrutinize this interaction, a composite variable, "RD&CO", was created by dichotomizing reward dependence and cooperativeness scores based on median split. Since the incomplete suppression was associated with lower reward dependence and higher cooperativeness, three groups were considered: patients with both lower reward dependence and higher cooperativeness ($n=22$), those with both higher reward dependence and lower cooperativeness ($n=13$) and the remaining patients with either lower reward dependence or higher cooperativeness but not both ($n=52$). As illustrated in Fig. 3, patients with both low reward dependence and high cooperativeness showed the highest DEX/CRH-Cort and ΔCort values, those with both high reward dependence and low cooperativeness showed the lowest values, and in-between the remaing patients. The ANCOVA comparing DEX/CRH-Cort and ΔCort between the three RD&CO groups with age, gender and HAM-D total score as covariates revealed a significant main effect of group for both DEX/CRH-Cort [$F(2,81)=4.18$, $p=0.019$] and ΔCort [$F(2,81)=4.26$, $p=0.017$]. Post-hoc pairwise comparisons with Bonferroni correction revealed that patients with both high reward dependence and low cooperativeness showed significantly lower DEX/CRH-Cort (estimated mean difference: 135.6, 95% confidence interval: 20.8-250.5, $p=0.015$) and ΔCort (estimated mean difference: 130.0, 95% confidence interval: 20.0-240.0, $p=0.015$) than those with both low reward dependence and high cooperativeness.

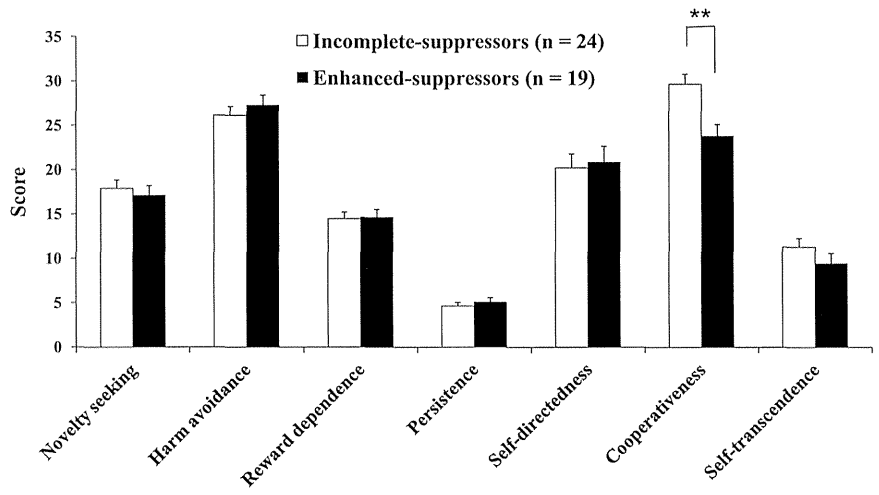

Fig. 2. Estimated mean scores of the seven dimensions of the temperament and character inventory, adjusted for age, gender and symptom severity as assessed by the Hamilton Depression Rating Scale 21-item version, for incomplete-suppressors and enhanced-suppressors. $^{**}p=0.002$ (by the analysis of covariance controlling for age, gender and symptom severity as assessed by the Hamilton Depression Rating Scale 21-item version). Error bars represent standard errors of the mean.

Table 3
Forward stepwise logistic regression analysis predicting DEX/CRH suppression pattern from the 7 dimensions of the temperament and character inventory, in addition to age, gender and depressive symptoms.

Step/variable	Nagelkerke R^2	Hosmer–Lemeshow p	B	Odds ratio	95% confidence interval	p
Step 1	**0.29**	**0.12**				
Cooperativeness			**−0.20**	**0.82**	**0.71–0.94**	**0.006**
Constant			**5.2**	**176.4**		**0.010**
Step 2	**0.46**	**0.69**				
Cooperativeness			**−0.37**	**0.69**	**0.55–0.87**	**0.001**
Reward dependence			**0.41**	**1.5**	**1.07–2.12**	**0.018**
Constant			**3.5**	**31.6**		**0.089**

Abbreviation: DEX/CRH test, dexamethasone/corticotropin-releasing hormone test.
Note: Odds ratio is the exponentiation of the B coefficient.

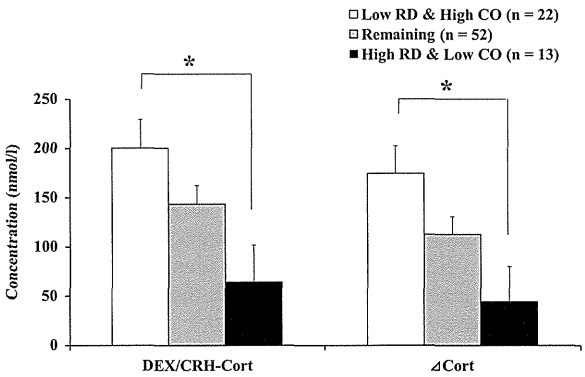

Fig. 3. Cortisol levels in response to the DEX/CRH test according to the composite RD/CO variable. RD and CO refer to reward dependence and cooperativeness, respectively. $^{*}p=0.015$ for both (by the analysis of covariance controlling for age, gender and symptom severity as assessed by the Hamilton Depression Rating Scale 21-item version, with post-hoc pairwise comparisons with Bonferroni correction). Error bars represent standard errors of the mean.

4. Discussion

The present study is the first attempt to examine the relationship between personality and cortisol reactivity as measured by the DEX/CRH test in depression, focusing on hypocortisolism. Main findings of this study were that (1) incomplete-suppressors scored significantly higher on cooperativeness than enhanced-suppressors and (2) the enhanced suppression of cortisol was significantly predicted by a unique pattern of personality traits, i.e., the combination of lower cooperativeness and higher reward dependence. These results were not confounded by age, gender or symptom severity.

To begin with, we should note that 28% (24/87) and 22% (19/87) of our patients with MDD fell into the categories of incomplete- and enhanced-suppression, respectively, by using the cutoff values for healthy adults where the highest 21% and lowest 20% were classified as the respective suppressor groups. This result indicates that the overall cortisol responses to the DEX/CRH test in patients with MDD were not very different from those in healthy controls, being in line with previous DEX/CRH studies in depressed outpatients (Oshima et al., 2000; Gervasoni et al., 2004; Van Den Eede et al., 2006; Carpenter et al., 2009), and further that cortisol reactivity varies widely within MDD, i.e., a considerable proportion of outpatients with MDD exhibit blunted cortisol reactivity. Hence, we propose that enhanced suppression, or hypocortisolism, in addition to hypercortisolism, needs to be taken into account when investigating HPA axis (dys)function in relation to depression. Supporting this, a recent study by Herbert et al. (2012) showed a significant quadratic (U-shaped) relationship between levels of morning salivary cortisol and the probability of depression onset during follow-up.

The main purpose of this study was to characterize the two extreme ends of cortisol reactivity using a set of personality dimensions. In the univariate analysis, only cooperativeness of

the seven TCI dimensions was significantly associated with cortisol reactivity; lower cooperativeness was related to more suppression of cortisol. In the multivariate analysis, however, the combination of cooperativeness and reward dependence emerged as the most powerful predictor of coritsol reactivity. The latter finding indicates that the combination of reward dependence and cooperativeness, compared to any single personality dimension, better defines a subtype of depression closely linked to altered HPA axis function. This finding also suggests that MDD as defined in the DSM-IV consists of a wide diversity of subtypes and thus the heterogeneity of depression represents a rather complex issue, as has been widely debated (e.g., Ostergaard et al., 2011).

The association between the unique personality profile and blunted cortisol reactivity observed here would be of interest in light of a number of previous studies relating a variety of psychopathologies to hypocortisolism. For example, Tops et al. (2008) found that higher rejection sensitivity was related to salivary lower cortisol awakening response. Depressed patients with long-term sick-leave are shown to display blunted cortisol responses to the DEX/CRH test (Rydmark et al., 2006; Wahlberg et al., 2009). This finding mirrors the observation of Wirtz et al. (2010) that higher overcommitment to work was associated with higher cortisol responses to the DEX/CRH test. Furthermore, O'Leary et al. (2010) reported that non-clinical students characterized by high psychopathic personality traits lacked psychosocial stress-induced cortisol increases. Concordant with this finding, several studies have found non-clinical schizotypal traits to be associated with enhanced cortisol suppression to pharmacological challenge paradigms (Schweitzer et al., 2001; Hori et al., 2011a). Concerning the natural course and meaning of hypocortisolism, however, there remains much to be elucidated. This extremely low cortisol (re)activity can represent the result of prolonged stress exposure (Heim et al., 2000; Fries et al., 2005) while it is also possible that this state could be a preexisting vulnerability to stress-related disorders (Delahanty et al., 2000; Yehuda et al., 2000; Wahlberg et al., 2009); these two possibilities are not mutually exclusive.

With regard to the different cortisol indices in the DEX/CRH test, significant relationships were observed between personality dimensions and reactive cortisol indices to the CRH challenge following the DEX administration (i.e., DEX/CRH-Cort and suppression pattern), but not the cortisol level after the DEX administration (i.e., DST-Cort). In line with the present results, previous studies have consistently found that cortisol levels after the combined DEX/CRH challenge, but not those after the administration of DEX alone, are associated with altered personality profiles (Rinne et al., 2002; Tyrka et al., 2006, 2008; Hori et al., 2011a). Although we can only speculate on the underlying mechanism, these findings suggest that the HPA axis alteration in relation to unique personality makeup would be accounted for, at least in part, by the altered regulation of HPA axis function by hypothalamic CRH (and possibly arginine vasopressin) system rather than by the glucocorticoid receptor-mediated negative feedback inhibition as measured by the DST.

The present findings may be somehow related to the following fact. In Japan, the past decade has seen a dramatic increase in a new type of depression, called modern type of depression. A shift in lifestyle caused by the rapid socioeconomic change is considered responsible for this. Corresponding to this, a survey shows that the number of depressed patients presented to psychiatric clinics/hospitals has nearly doubled during the same time period (Ministry of Health, Labour and Welfare, 2012). A recent international survey shows that this phenomenon is seen in other countries as well, particularly in urban areas (Kato et al., 2011). Although the underlying biological causes and mechanisms, including their neuroendocrine status, have not been well documented, the modern type of depression is usually characterized by their personality traits (Tarumi and Kanba, 2005). Indeed, conceptualization of modern type of depression includes a unique set of personality makeup characterized by attachment to oneself, less loyalty to rules and norms imposed by society with negative feelings toward them, and vague sense of omnipotence (Tarumi and Kanba, 2005; Kato et al., 2011). It is generally acknowledged that the modern type of depression has some phenomenological overlap with atypical depression and dysthymic disorder (Tarumi and Kanba, 2005; Kato et al., 2011). In the present study blunted cortisol reactivity was associated with lower cooperativeness, especially lower social acceptance and compassion. Since this type of personality trait corresponds to the one seen in modern type of depression, our finding points to the possibility that modern type of depression is associated with hypocortisolism, as is the case with atypical depression (Gold and Chrousos, 2002). In contrast, personality in melancholic depression has been traditionally characterized as diligent and hardworking, being loyal to the rules and orders imposed by society and community with positive feelings toward them (Shimoda, 1957; Tellenbach, 1961). Such a personality trait corresponds well to the present combination of higher cooperativeness and lower reward dependence, which in turn was associated with hypercortisolism. Since optimal treatment strategies are likely to be different between melancholic depression and modern type of depression (Tarumi and Kanba, 2005), the present findings could be of clinical importance. Future studies are warranted to define modern type of depression in terms of its underlying biology, including HPA axis function, as well as phenomenology.

The current findings should be considered in the context of a number of limitations. First, since the DEX/CRH test used here was based on a simple test protocol (i.e., measuring the cortisol level only twice), it may have provided less information on HPA axis function (e.g., lack of the ACTH data). Moreover, we did not measure baseline cortisol levels, i.e., the cortisol level before the DEX administration, which prevented us from delineating the extent to which each participant suppressed his/her cortisol in response to the 1.5 mg of DEX. Second, this cross-sectional study does not provide information as to the causality between personality traits and alteration in HPA axis function. Third, we did not collect data on the menstrual cycle or menopausal status in our female participants. While these factors may have influenced their HPA axis function, it is unlikely that the menstrual cycle had any impact on the TCI results. Finally, since most of our patients were receiving psychotropics, such medication may have influenced HPA axis function. We would like to note, however, that the two suppressor groups did not significantly differ in any classes of psychotropic medications (Table 1).

In conclusion, the present findings indicate that there could be a significant variation in cortisol reactivity within depression and thus might point to the importance of taking account of hypocortisolism as well as hypercortisolism. Our findings might also suggest the possibility of differentiating personality-related subtypes of depression based on the different pattern of cortisol reactivity. As this is the first study to examine the relationship of temperament and character with cortisol reactivity to the DEX/CRH test in depressed patients, further studies, ideally in various ethnic groups, are required to replicate these findings.

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Conflict of interest

All authors declare no conflict of interest.

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