Sasayama et al., 2011b) and in depression (Dowlati et al., 2010). Increased levels of IL-1β in the cerebrospinal fluid of patients with first-episode schizophrenia (Soderlund et al., 2009) and depression (Levine et al., 1999) have also been reported. Furthermore, some studies reported significant associations of *IL-1β* polymorphisms with schizophrenia (Hanninen et al., 2008; Papiol et al., 2004; Sasayama et al., 2011a; Zanardini et al., 2003) and with depression (Borkowska et al., 2011).

The incidence of overweight, as well as the risks of type 2 diabetes and cardiovascular disease, is increased in major depressive disorder (MDD) and schizophrenia (De Hert et al., 2009). A recent meta-analysis showed that depression was found to be predictive of developing obesity and that obesity also increases the risk of depression (Luppino et al., 2010). The activation of inflammatory factors related to obesity may be one of the possible explanations for the relationship between obesity and psychiatric illnesses. Based on these findings, we examined whether the Trp64Arg polymorphism in *ADRB3* confers susceptibility to developing schizophrenia and depression. Furthermore, the association of the Trp64Arg polymorphism with being overweight in these disorders was examined.

2. Materials and methods

2.1. Subjects

Trp64Arg was genotyped in 504 patients with schizophrenia (274 men and 230 women; mean age (standard deviation: *SD*): 43.1 (14.0) years), 650 patients with MDD (309 men and 341 women; 45.1 (14.5) years), and 1170 healthy controls (395 men and 775 women; 46.0 (16.2) years). Self-reported body weight and height were obtained from a portion of the participants. Thus, body mass index (BMI) data were available for 125 patients with schizophrenia (74 men and 51 women; mean age: 39.8 (11.7) years), 219 patients with major depressive disorder (MDD) (97 men and 122 women; 42.0 (12.4) years), and 261 healthy controls (71 men and 190 women; 48.5 (15.4) years). Most of the patients with schizophrenia were on chronic treatment of antipsychotic medication; the average (*SD*) chlorpromazine equivalent converted from daily doses of antipsychotics (American Psychiatric Association, 1997; Inagaki et al., 1999) was 574.5 (509.9) mg/day, and the average duration of treatment was 14.1 (10.7) years. All subjects were biologically unrelated Japanese and were 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. 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 histories of psychiatric treatment and were screened using the Japanese version of the Mini International Neuropsychiatric Interview (Otsubo et al., 2005; Sheehan et al., 1998) 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. None of the participants were under treatment for cardiovascular diseases or diabetes at the time of assessment. 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

Genomic DNA was prepared from venous blood according to standard procedures. The Trp64Arg polymorphism was genotyped using the TaqMan 5′-exonuclease allelic discrimination assay (assay ID: C___2215549_20). The thermal cycling conditions for polymerase chain reaction were as follows: 1 cycle at 95 °C for 10 min followed by 50 cycles of 92 °C for 15 s and 60 °C for 1 min. The allele-specific fluorescence was measured with ABI PRISM 7900 Sequence Detection Systems (Applied Biosystems, Foster City, CA). Ambiguous genotype data were not included in the analysis.

2.3. Statistical analysis

Deviations of genotype distributions from Hardy–Weinberg equilibrium (HWE) were assessed using 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. Comparison of BMI between genotypes was analyzed using two-way analysis of covariance (ANCOVA) with genotype and diagnosis as independent variables and age and gender as covariates. For patients with schizophrenia, ANCOVA was also performed adding the chlorpromazine equivalent dose as a covariate to control for use of antipsychotics. Because the frequency of Arg/Arg homozygotes in the general population is low, we combined the heterozygotes and variant homozygotes into one group to assess the effect of the polymorphism on the degree of obesity, as in previous studies (Clement et al., 1995; Kurokawa et al., 2003; Widen et al., 1995). 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.

Power calculation for the genetic association analysis was performed using the power calculator for genetic studies (http://www.sph.umich.edu/csg/abecasis/CaTS/). Assuming a genotype relative risk of 1.3 under an additive model, a disease prevalence of 1% for schizophrenia and 10% for MDD, and a minor allele frequency of 20%, our sample size had 79% and 91% power, respectively, to detect disease associations with an alpha of 0.05. Similarly, assuming a relative risk of 1.3 under a multiplicative model, the power to detect disease associations was 83% and 94% for schizophrenia and depression, respectively.

Power calculation for ANCOVA in subjects with BMI data was performed using G*Power 3.1.3 (Faul et al., 2007). Assuming that frequency of Arg/Arg homozygotes is 0.671 as in the HapMap data (http://www.hapjmap.org/), the present study provided a power of greater than 0.80 to detect an effect size of 0.38, 0.41, and 0.55 for healthy controls, MDD patients, and schizophrenic patients, respectively.

3. Results

The genotype and allele distributions of the Trp64Arg are shown in Table 1. The genotype and allele distributions did not significantly deviate from the HWE. No significant difference in genotype or allele distribution was found across the three diagnostic groups.

BMI was compared between Arg carriers and non-carriers using ANCOVA with Arg allele carrier status and diagnosis as independent variables and age and gender as covariates. The results showed a significant interaction effect between genotype and diagnosis ($F(2,597)=5.34$, $P=0.005$). Therefore, we further compared the BMI between Arg carriers and non-carriers in each diagnostic group separately, with Arg allele carrier status as the independent variable and age and gender as covariates. No significant difference in BMI was observed between the Arg carriers and the non-carriers in the MDD and the control groups. However, patients with schizophrenia carrying the Arg allele had significantly higher BMI compared to their Trp/Trp homozygous counterparts (mean (*SD*): Arg carriers: 26.5 (6.9), Arg non-carriers: 23.8 (4.3); $F(1,121)=5.69$, $P=0.019$). The difference remained significant even after including the chlorpromazine equivalent dose as a covariate ($F(1,120)=4.97$, $P=0.028$). The categorical analysis also showed that schizophrenic patients carrying the Arg allele were more likely to be

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Table 1
The results of the association analysis of theTrp64Arg polymorphism.

Subjects	N	Genotype			Allele		χ^2 test		HWE *P*-value	Allelic OR versus controls (95% CI)
		Arg/Arg	Trp/Arg	Trp/Trp	Arg	Trp	Genotype	Allele		
Schizophrenia	504	21 (0.04)	148 (0.29)	335 (0.66)	190 (0.19)	818 (0.81)	$\chi^2=1.89$ $df=4$ $P=0.76$	$\chi^2=0.87$ $df=2$ $P=0.65$	0.37	1.06 (0.87–1.28)
MDD	650	26 (0.04)	198 (0.30)	426 (0.66)	250 (0.19)	1050 (0.81)			0.62	1.08 (0.91–1.29)
Controls	1170	36 (0.03)	350 (0.30)	784 (0.67)	422 (0.18)	1918 (0.82)			0.68	

HWE: Hardy–Weinberg equilibrium; OR: odds ratio; CI: confidence interval; MDD: major depressive disorder; *df*: degree of freedom. Numbers in parentheses in the genotype and the allele columns represent the frequencies of genotypes and alleles.

overweight (BMI of 25 or more) than their Trp/Trp homozygous counterparts (% overweight (standard error: *SE*): Arg carriers: 52.3 (7.5), Arg non-carriers: 32.1 (5.2); $\chi^2=4.87$, $df=1$, $P=0.027$; odds ratio = 2.32 (95% confidence interval: 1.09 to 4.92); sensitivity of 0.47 and specificity of 0.72). As shown in Table 2, no significant difference between Arg carriers and non-carriers in age, gender rate, antipsychotic equivalent dose, or treatment duration was observed in patients with schizophrenia. Fig. 1 shows the rate of being overweight for the Arg allele carriers and the non-carriers in each diagnostic group.

4. Discussion

The present study had sufficient power to detect a relatively modest effect of the *ADRB3* gene Trp64Arg on the development of schizophrenia and MDD. Thus, our findings suggest that the Trp64Arg polymorphism is unlikely to have a major role in the development of schizophrenia or MDD. The hypothesized effect of Trp64Arg variant on the development of schizophrenia and MDD, however, was due to an indirect action, mediated by the inflammatory process. Therefore, the association may have been too weak to be detected by the sample size used in this study. The observed effect of the Trp64Arg on BMI was not significant in healthy controls and patients with MDD. In patients with schizophrenia, however, the Arg allele was associated with higher BMI, which was in line with the evidence that Arg allele is associated with lower lipolytic activities (Umekawa et al., 1999).

Previous studies carried out in Japanese (Oizumi et al., 2001) and in Finnish subjects (Widen et al., 1995) reported that the Arg allele of the Trp53Arg polymorphism was associated with obesity. However, some studies failed to find such an association (Buettner et al., 1998; Gagnon et al., 1996; Gjesing et al., 2008; Oeveren van-Dybicz et al., 2001). The inconsistency between studies may be partially explained by the population differences between samples. A meta-analysis suggests that the effect of this polymorphism on BMI is greater in East Asians than in Europeans (Kurokawa et al., 2008). The Trp64Arg may play a particularly important role in the Japanese population, since the minor allele frequency is higher in Japanese than in other populations in the HapMap data (http://www.hapjmap.org/).

Table 2
Demographic and clinical characteristics of Arg carriers and non-carriers in patients with schizophrenia.

	Arg carriers	Arg non-carriers	Analysis
Age (years)	39.9 (11.4)	39.8 (11.9)	ANOVA: $F(1,123)=0.0$, $P=0.98$
Gender (M/F)	31/13	43/38	χ^2 test: $\chi^2=3.56$, $df=1$, $P=0.06$
CP equivalent dose (mg/day)	663.2 (613.2)	526.3 (440.7)	ANOVA: $F(1,123)=2.1$, $P=0.15$
Treatment duration (years)	15.4 (11.2)	13.2 (10.5)	ANOVA: $F(1,123)=1.2$, $P=0.27$

Values are shown as mean (standard deviation). BMI: body mass index; CP: chlorpromazine; ANOVA: analysis of variance; *df*: degree of freedom.

The genetic homogeneity of the Japanese population was a major strength of the present study. However, contrary to the results of the meta-analysis in Japanese subjects (Kurokawa et al., 2008), our results showed no significant association between the Trp64Arg and the BMI in healthy subjects. These negative results may have arisen by the small number of subjects in the present study. Intriguingly, however, the patients with schizophrenia carrying the Arg allele had significantly higher BMI compared to their Trp/Trp homozygous counterparts. Obesity is highly prevalent in patients with schizophrenia due to illness-related factors and use of antipsychotic medications (Kolotkin et al., 2008). Our results suggest that schizophrenic patients carrying the Arg allele especially have a greater tendency to gain weight. Clement et al. (1995) demonstrated that although the frequency of the Arg allele was similar in the morbidly obese patients and the normal subjects, the obese patients with Arg allele had higher capacity to gain weight. Taken together, the Trp64Arg variant may enhance weight gain in individuals already at risk for obesity.

The major limitation of this study was that the effects of medication could not be fully controlled due to the variability in types and doses. Particularly, antipsychotic medications are known to induce metabolic abnormalities such as obesity, hyperglycemia, and metabolic syndrome (De Hert et al., 2011). Therefore, the use of antipsychotics in patients with schizophrenia may have confounded the results. However, the chlorpromazine equivalent dose did not differ between Arg carriers and non-carriers in patients with schizophrenia. Furthermore, using the chlorpromazine equivalent dose as a covariate in an ANCOVA still resulted in significantly higher BMI in Arg carriers of schizophrenic patients. Thus, controlling for total chlorpromazine equivalent dose did not affect the findings of the present study. Nevertheless, the influence of the Trp64Arg on BMI may differ in non-medicated patients or may depend on the type of antipsychotics used. Further investigations are required to elucidate the effects of

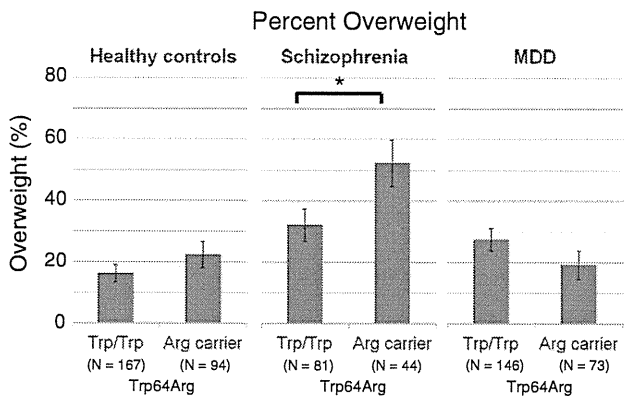

Fig. 1. Percentage overweight in Arg carriers and non-carriers of the Trp358Ala. The rate of being overweight (BMI of 25 or more) is shown for the Arg carriers and the non-carriers in healthy controls and in patients with schizophrenia and MDD. Error bars indicate 1 standard error. In patients with schizophrenia, Arg carriers were significantly more likely to be overweight than the non-carriers. * $P<0.05$.

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antipsychotics. Another limitation of the study is that BMI data relied on self-reports of the participants. However, previous studies show that self-reported BMI is satisfactorily accurate for the assessment of the prevalence of overweight (Craig and Adams, 2009; Dekkers et al., 2008).

In conclusion, we obtained no evidence for the association of *ADRB3* Trp64Arg with the development of MDD or schizophrenia. However, the Arg allele of the Trp64Arg polymorphism was found to be associated with higher BMI in patients with schizophrenia. This may imply that genotyping *ADRB3* is of clinical use to detect schizophrenic individuals at risk for developing obesity, which is an important issue in the antipsychotic medication. Further studies are warranted to elucidate the influence of the *ADRB3* gene variation on the development of psychiatric disorders and also to understand the factors that contribute to the risk of obesity in patients with psychiatric disorders.

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Schizotypal Personality in Healthy Adults Is Related to Blunted Cortisol Responses to the Combined Dexamethasone/ Corticotropin-Releasing Hormone Test

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Key Words
Cortisol · Dexamethasone/corticotropin-releasing hormone test · Hypothalamic-pituitary-adrenal axis · Schizotypal personality

Abstract
Background/Aims: Schizotypy is viewed as a dimensional trait ranging from healthy people to schizophrenic spectrum patients. Stress activates the hypothalamic-pituitary-adrenal (HPA) axis, and accumulated evidence suggests that schizophrenia is associated with altered HPA axis function; however, HPA axis function in relation to schizotypal personality has not been well documented. ***Methods:*** We examined the relationship between schizotypal traits as assessed with the Schizotypal Personality Questionnaire (SPQ) and cortisol responses to the combined dexamethasone/corticotropin-releasing hormone test in 141 healthy volunteers. Subjects were divided into three groups based on their cortisol responses to the dexamethasone/corticotropin-releasing hormone test: incomplete suppressors, moderate suppressors, and enhanced suppressors. SPQ scores were compared between these three groups using the analysis of covariance, controlling for age and sex. ***Results:*** The analysis of covariance showed significant main effects of the suppressor status on the ideas of reference and suspiciousness/paranoid ideation subscales and cognitive-perceptual factor. Post-hoc analyses with Bonferroni correction revealed that the enhanced suppressors scored significantly higher than the moderate suppressors on these SPQ indices. ***Conclusion:*** These results indicate that nonclinical schizotypal traits in healthy adults are associated with blunted cortisol reactivity, potentially suggesting a shared neuroendocrinological mechanism across schizophrenia spectrum pathology.

Introduction

Research into schizotypal personality is important as it provides key insights into our understanding of schizophrenia [1]. The dimensional model of schizotypal personality posits that the degree of schizotypal traits varies on a continuum where normality lies on one extreme,

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nonclinical schizotypy in the middle, and clinically expressed schizophrenia on the opposite extreme [2, 3]. The putative distinction between schizotypal personality disorder (SPD) and nonclinical schizotype is that the former, like schizophrenia, is considered as an outgrowth of the latter, with additional modifying influences such as environmental stressors that cause frank psychotic symptoms. Although some investigators have argued for the competing theory, namely the categorical approach to schizotypy [4, 5], a large number of schizophrenia/schizotypy researchers have substantiated the dimensional model of schizotypy [6, 7]. Supporting this notion, a growing body of evidence indicates that schizotypal traits at a nonclinical level are associated with a range of endophenotypic characteristics of schizophrenia. More specifically, nonclinical schizotypes have been shown to display neurophysiological [8, 9], neurocognitive [10–13] and neuroimaging [14–16] abnormalities that are intrinsically similar to, albeit less severe than, those in schizophrenia patients. There is also some evidence that SPD may represent a risk factor for developing schizophrenia [17].

Hypothalamic-pituitary-adrenal (HPA) axis is activated by all sorts of stressors, and this fact constitutes the foundation of investigations into HPA axis function in schizophrenia, a disorder where stress could play a pivotal role in its onset and precipitation. A large number of earlier studies have used the dexamethasone (DEX) suppression test (DST) to investigate negative feedback inhibition of cortisol in schizophrenia patients, yielding controversial findings; the rate of nonsuppression to the DST varies from 0 to 73% [18]. A meta-analysis of 26 DST studies [19] revealed that nonsuppression rates of schizophrenia patients (19%) were significantly lower than those of patients with major depression (51%) and significantly higher than those of healthy controls (7%). Subsequently, Ismail et al. [20] found that less than 2% of their schizophrenia patients were nonsuppressors. On the other hand, studies employing psychosocial challenge paradigms have, rather consistently, reported blunted cortisol responses in schizophrenia patients [21–24]. All these findings indicate that schizophrenia would be associated with alteration in HPA axis function, with some suggesting hyperactive HPA axis function while others the opposite, i.e. hypoactive one.

In patients with SPD or nonclinical schizotypes, two studies have been conducted to examine the cortisol reactivity to pharmacological challenge paradigms; Schweitzer et al. [25] found schizotypal traits to be associated with enhanced suppression to DST, and Mitropoulou et al. [26] observed blunted cortisol responses to an acute metabolic stressor (i.e. 2-deoxyglucose) in patients with SPD. Findings from these studies were consistent with each other in that they have demonstrated blunted cortisol reactivity in schizotypal subjects. Paucity of research, however, has precluded any definitive conclusions to be drawn concerning the relation between schizotypy and cortisol activity/reactivity. Given that findings on cortisol levels of schizophrenia patients are not unequivocal, nonclinical schizotypes, who are free of medication, hospitalization, and psychosocial consequences of psychiatric diagnoses [27], would be a suitable target for investigating the HPA axis function in schizophrenia spectrum disorders. Indeed, a number of studies have suggested that cortisol levels are considerably affected by antipsychotic drugs [28–30].

Apart from the viewpoint of dimensional model of schizophrenia, it has been suggested that personality itself would confer sensitivity to stressors [31, 32]. Several lines of research in the nonclinical population have demonstrated that a variety of personality traits are related to altered HPA axis function. While there exist several (challenge) paradigms to assess HPA axis function, an increasing number of studies have used the DEX/corticotropin-releasing hormone (CRH) test [33, 34], a sensitive test for HPA axis function that combines DEX pretreatment with CRH challenge on the following day, to examine the association of personality traits with HPA axis reactivity. For example, using a questionnaire that measures Cloninger's temperamental dimension, Tyrka et al. [35] have shown that low novelty seeking, particularly when combined with high harm avoidance [36], is associated with exaggerated cortisol responses to the DEX/CRH test. Using other personality measures, McCleery and Goodwin [37] observed a relationship between lower neuroticism and greater cortisol responses to this pharmacological challenge test, whereas Zobel et al. [38] found the opposite relation, i.e. higher neuroticism and greater cortisol response. Rinne et al. [39] observed exaggerated cortisol responses to the DEX/CRH test in female subjects with borderline personality disorder who had a history of sustained childhood abuse. It would thus be intriguing to examine the association between schizotypy and cortisol reactivity by using the DEX/CRH test.

In this context, we aimed to explore the possible relationship between schizotypal traits at a nonclinical level and cortisol responses to the DEX/CRH test. To disentangle potential confounding effects of other personality dimensions, we also examined the association of temper-

ament/character with schizotypal personality and with cortisol responses. We predicted that nonclinical schizotypy on its own would be related to either of the two extremes of cortisol responses (i.e. exaggerated or blunted) to the DEX/CRH test.

Methods

Participants

One hundred and forty-one volunteers (35 males and 106 females; age range: 20–70 years) participated in this study, which was conducted in the western part of Tokyo between 2006 and 2010. They were recruited from the community through advertisements in free local magazines and our website announcement. At the first visit, participants were interviewed using the Japanese version of the Mini-International Neuropsychiatric Interview [40, 41] by a research psychiatrist, and only those who demonstrated no current axis I psychiatric disorders were enrolled in this study. In addition, those who demonstrated one or more of the following conditions during a non-structured interview performed by an experienced psychiatrist were excluded from this study: past or current contact to psychiatric services, taking psychotropic drugs, having a history of regular use of psychotropics or substance abuse/dependence, and other obvious self-reported signs of past primary psychotic and mood disorders as well as posttraumatic stress disorder. Additional exclusion criteria were: having a prior medical history of central nervous system disease or severe head injury, having major systemic medical illnesses, or taking corticosteroids, antihypertensive medications, oral contraceptives or estrogen replacement therapies. The present experiments on our subjects were 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 subjects. The study was approved by the ethics committee of the National Center of Neurology and Psychiatry, Japan.

DEX/CRH Test Procedure and Presentation for Neuroendocrine Data

The DEX/CRH test was administered to all subjects according to a protocol proposed in our previous report [42], and this protocol was identical to that employed in our recent report [43]. First, they took 1.5 mg of DEX (Banyu Pharmaceutical Corporation, Tokyo, Japan) orally at 23:00 h. On the next day, they attended our laboratory and sat on a comfortable couch in a calm room. A vein was cannulated at 14:30 h to collect blood at 15:00 h and 16:00 h via an intravenous catheter. Human CRH (100 μg; hCRH 'Mitsubishi', Mitsubishi Pharma Corporation, Tokyo, Japan) was administered intravenously at 15:00 h, immediately after the first blood collection. Subjects fasted and rested semi-supine throughout the testing. Blood samples were immediately centrifuged and stored at –20°C. Plasma concentrations of cortisol were measured by radioimmunoassay at SRL Corporation (Tokyo, Japan). The detection limit for cortisol was 1.0 μg/dl. Cortisol values under the detection limit were treated as 0 μg/dl. The intra-assay coefficients of variation at 2.37, 13.02, and 36.73 μg/dl were 6.90, 4.94, and 5.78%, respectively. The interassay coefficients of variation at 2.55, 13.04, and 34.17 μg/dl were 8.91, 6.03, and 6.44%, respectively (SRL Corporation). Outcome measures of this neuroendocrine test were the DST-cortisol (i.e. the concentration of cortisol [μg/dl] at 15:00 h) and DEX/CRH-cortisol (i.e. the concentration of cortisol at 16:00 h). To dissect the extent to which the subject's HPA axis responded to the CRH challenge, the magnitude of change from DST-cortisol to DEX/CRH-cortisol, namely Δcortisol, was calculated for each subject.

As our hypothesis was that the two extreme ends of cortisol values (i.e. both exaggerated and blunted cortisol reactivity) would be related to greater schizotypal traits, in the main analysis we adopted the categorical division of participants based on two cutoff values of cortisol (i.e. 1 and 5 μg/dl), by referring to several previous studies [42, 44, 45]. The cutoff criterion employed here was identical to that used in our recent report [43]. Briefly, incomplete suppressors were defined to be individuals where either or both of DST- and DEX/CRH-cortisols were ≥5 μg/dl. Enhanced suppressors were defined as those individuals whose DST-cortisol was <5 μg/dl and DEX/CRH-cortisol was <1 μg/dl, because this DEX/CRH-cortisol value corresponded to its detection limit and can therefore be regarded as an extremely low cortisol level. The remaining individuals were considered to be moderate suppressors.

Schizotypal Personality Questionnaire

The Schizotypal Personality Questionnaire (SPQ) [46] is a 74-item validated self-report questionnaire with a 'yes/no' response format that incorporates DSM-III-R [47] criteria for a diagnosis of SPD. The questionnaire consists of nine subscales, which have been found to load onto three factors: cognitive-perceptual, interpersonal, and disorganized factors [48]. In the present study the Japanese version of the SPQ translated by Fujiwara [49] was used. This questionnaire had been administered to 258 Japanese college students in a validation study [50], and the reliability and validity of this Japanese version of SPQ were demonstrated to be similar to those of the original version of Raine [46].

Temperament and Character Inventory

Temperament and Character Inventory (TCI) [51] is a 240-item (including 14 items which are not analyzed) self-report questionnaire; each item requires a true/false answer. Four dimensions of temperament (i.e. novelty seeking, harm avoidance, reward dependence, and persistence) and three dimensions of character (i.e. self-directedness, cooperativeness, and self-transcendence) are distinguished. The Japanese version of the TCI translated and validated by Kijima et al. [52, 53] was used in the present study.

Statistical Analysis

Averages are reported as means ± SD (standard deviation). The analysis of variance was used to examine differences between three groups. Pearson's r or Spearman's ρ was used to examine correlations. Partial correlation analysis, controlling for confounding variables, was used to examine correlations among psychological measures. The analysis of covariance (ANCOVA), controlling for confounders, was performed to compare scores of the two questionnaires between the three participant groups. Since age and sex have been shown to significantly influence cortisol levels [42, 54, 55] and schizotypal personality [56], these two vari-

Table 1. Demographics, schizotypal personality, and temperament/character dimensions for the three groups based on the suppression pattern

	Incomplete suppressors (n = 69)[a]	Moderate suppressors (n = 61)[b]	Enhanced suppressors (n = 11)[c]	Statistics	p
Age, years	49.9 ± 12.7	44.4 ± 14.9	41.9 ± 14.9	F(2, 138) = 3.29	0.04
Sex, male/female	10/59	20/41	5/6	$\chi^2(2) = 8.53$	0.014
Temperament and character inventory[d]					
Novelty seeking	20.4 ± 4.2	20.5 ± 4.3	21.9 ± 4.0	F(2, 136) = 0.52	0.59
Harm avoidance	18.3 ± 5.8	19.1 ± 6.0	20.6 ± 7.5	F(2, 136) = 0.51	0.60
Reward dependence	15.0 ± 3.4	15.3 ± 3.6	16.4 ± 2.7	F(2, 136) = 2.25	0.11
Persistence	4.4 ± 1.8	4.1 ± 1.8	4.0 ± 1.4	F(2, 136) = 1.89	0.15
Self-directedness	29.2 ± 6.3	28.4 ± 6.4	27.1 ± 9.5	F(2, 136) = 0.15	0.86
Cooperativeness	29.2 ± 5.6	29.1 ± 4.9	27.8 ± 5.9	F(2, 136) = 0.21	0.81
Self-transcendence	11.3 ± 6.0	9.9 ± 6.0	12.7 ± 8.1	F(2, 136) = 1.40	0.25
Schizotypal personality questionnaire[d]					
Total score	13.7 ± 11.0	12.4 ± 9.3	19.5 ± 17.4	F(2, 136) = 1.89	0.16
Cognitive-perceptual factor	4.6 ± 4.5	3.1 ± 3.3	7.5 ± 7.4	F(2, 136) = 5.48	0.005
Interpersonal factor	6.5 ± 5.8	7.0 ± 5.8	9.0 ± 8.8	F(2, 136) = 0.31	0.74
Disorganized factor	3.8 ± 3.7	3.3 ± 3.2	5.5 ± 4.7	F(2, 136) = 2.02	0.14

[a] Defined as DST-cortisol ≥5 or DEX/CRH-cortisol ≥5. [b] Defined as DST-cortisol <5 and 1 ≤ DEX/CRH-cortisol <5. [c] Defined as DST-cortisol <5 and DEX/CRH-cortisol <1. [d] Scores of the three suppressor groups were compared using the ANCOVA with age and sex as covariates.

ables were considered as potential confounders regardless of the present data. To confirm the results obtained by the ANCOVA, Kruskal-Wallis test was used to examine differences in SPQ scores between the three groups. Statistical significance was set at two-tailed $p < 0.05$ unless otherwise specified. Analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 18.0 (SPSS Japan, Tokyo).

Results

Demographic Characteristics and SPQ Scores of the Subjects

Scores on the total SPQ, cognitive-perceptual factor, interpersonal factor, and disorganized factor of the total 141 participants were 13.6 ± 11.0 (range: 0–57, median: 11), 4.2 ± 4.4 (range: 0–24, median: 3), 6.9 ± 6.0 (range: 0–25, median: 5), and 3.7 ± 3.6 (range: 0–16, median: 3), respectively. The numbers of incomplete, moderate, and enhanced suppressors were 69, 61, and 11, respectively. These three groups significantly differed in age and sex; suppression status tended to become incomplete with advancing age, and females demonstrated less suppression than males (table 1). Age was significantly negatively correlated with eccentric/odd behavior and appearance ($r = -0.23$, $p = 0.006$) and odd speech ($r = -0.18$, $p = 0.03$) subscales and their resulting disorganized factor ($r = -0.23$, $p = 0.006$). Males and females were significantly different in social anxiety ($t = 3.27$, $p = 0.001$), no close friends ($t = 2.46$, $p = 0.02$) and constricted affect ($t = 2.76$, $p = 0.01$) subscales and their resulting interpersonal factor ($t = 3.19$, $p = 0.002$); males scored significantly higher than females in all of these four indices.

Correlations between SPQ and TCI Scores

Table 2 shows the partial correlations, controlling for age and sex, between nine subscales of the SPQ and seven dimensions of the TCI. In general, those subscales which load onto the cognitive-perceptual factor of SPQ were significantly negatively correlated with self-directedness and significantly positively correlated with self-transcendence. Those subscales which load onto the interpersonal factor were significantly positively correlated with harm avoidance and significantly negatively correlated with reward dependence, self-directedness and cooperativeness. Similarly to the subscales belonging to the cognitive-perceptual factor, those subscales which belong to the disorganized factor were significantly negatively correlated with self-directedness and significantly positively correlated with self-transcendence.

Table 2. Partial correlations between schizotypal personality and temperament/character dimensions, controlling for age and sex (n = 141)

	Novelty seeking	Harm avoidance	Reward dependence	Persistence	Self-directedness	Cooperativeness	Self-transcendence
Ideas of reference (C-P)	0.03	0.12	0.03	0.07	−0.35***	0.01	0.31***
Social anxiety (I)	−0.14	0.50***	−0.004	0.01	−0.37***	−0.06	0.01
Odd beliefs/magical thinking (C-P)	−0.03	−0.09	0.10	0.26**	−0.10	0.14	0.50***
Unusual perceptual experiences (C-P)	−0.05	0.10	0.01	0.14	−0.22**	0.13	0.38***
Eccentric/odd behavior and appearance (D)	−0.06	0.05	−0.18*	0.10	−0.20*	−0.12	0.26**
No close friends (I)	−0.24**	0.31***	−0.44***	−0.06	−0.31***	−0.30***	−0.01
Odd speech (D)	−0.03	0.25**	−0.09	0.04	−0.52***	−0.08	0.19*
Constricted affect (I)	−0.09	0.34***	−0.21*	−0.01	−0.51***	−0.15	0.06
Suspiciousness/paranoid ideation (C-P, I)	−0.13	0.18*	−0.27**	0.03	−0.39***	−0.26**	0.17*

Each figure represents partial correlation coefficient (d.f. = 137). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. C-P = Cognitive-perceptual factor; I = interpersonal factor; D = disorganized factor.

Table 3. Plasma cortisol concentrations [mean ± SD (range)] for the three groups based on the suppression pattern

	Incomplete suppressors (n = 69)[a]	Moderate suppressors (n = 61)[b]	Enhanced suppressors (n = 11)[c]
DST-cortisol[d]	1.3 ± 1.4 (0–5.8)	0.3 ± 0.6 (0–1.9)	0.1 ± 0.3 (0–1.1)
DEX/CRH-cortisol[e]	10.3 ± 4.8 (5.0–25.1)	2.5 ± 1.1 (1.1–4.9)	0 ± 0
Δcortisol[f]	9.0 ± 4.6 (2.3–20.2)	2.2 ± 1.1 (−0.3 to 4.8)	−0.1 ± 0.3 (−1.1 to 0)

[a] Defined as DST-cortisol ≥5 or DEX/CRH-cortisol ≥5. [b] Defined as DST-cortisol <5 and 1 ≤ DEX/CRH-cortisol <5. [c] Defined as DST-cortisol <5 and DEX/CRH-cortisol <1. [d] The concentration of cortisol (μg/dl) at 15:00 h (i.e. immediately before the CRH challenge). [e] The concentration of cortisol (μg/dl) at 16:00 h (i.e. 1 h after the CRH challenge). [f] Defined as DEX/CRH-cortisol minus DST-cortisol.

Relationships between TCI Scores and DEX/CRH Outcomes

Results of the three cortisol indices for the three suppressor groups are provided in table 3.

No significant correlations were seen between the seven dimensions of TCI and the three cortisol indices (all $p > 0.05$ by Spearman's ρ). As shown in table 1, the ANCOVA controlling for age and sex showed no significant main effect of the suppressor group on any of the seven dimensions of TCI. These results indicated that personality dimensions as assessed with the TCI did not significantly affect the cortisol responses to the DEX/CRH test. Therefore, we decided not to control for this personality dimension when examining the association between schizotypal personality and cortisol responses.

Relationships between SPQ Scores and DEX/CRH Outcomes

No significant correlations were seen between the SPQ scores (including nine subscales, three factors and the total score) and the three cortisol indices (all $p > 0.05$ by Spearman's ρ), indicating that there was no simple linear correlational relationship between SPQ scores and cortisol levels.

Figure 1 illustrates the relationships between nine subscales of the SPQ and DEX/CRH suppression status. The ANCOVA on the nine subscales, controlling for age and sex, showed a significant main effect of the suppressor group on ideas of reference [$F(2, 136) = 5.92$, $p = 0.003$] and suspiciousness/paranoid ideation [$F(2, 136) = 4.50$, $p = 0.013$] subscales. Post-hoc analyses with Bonferroni correction revealed that the enhanced suppressors scored

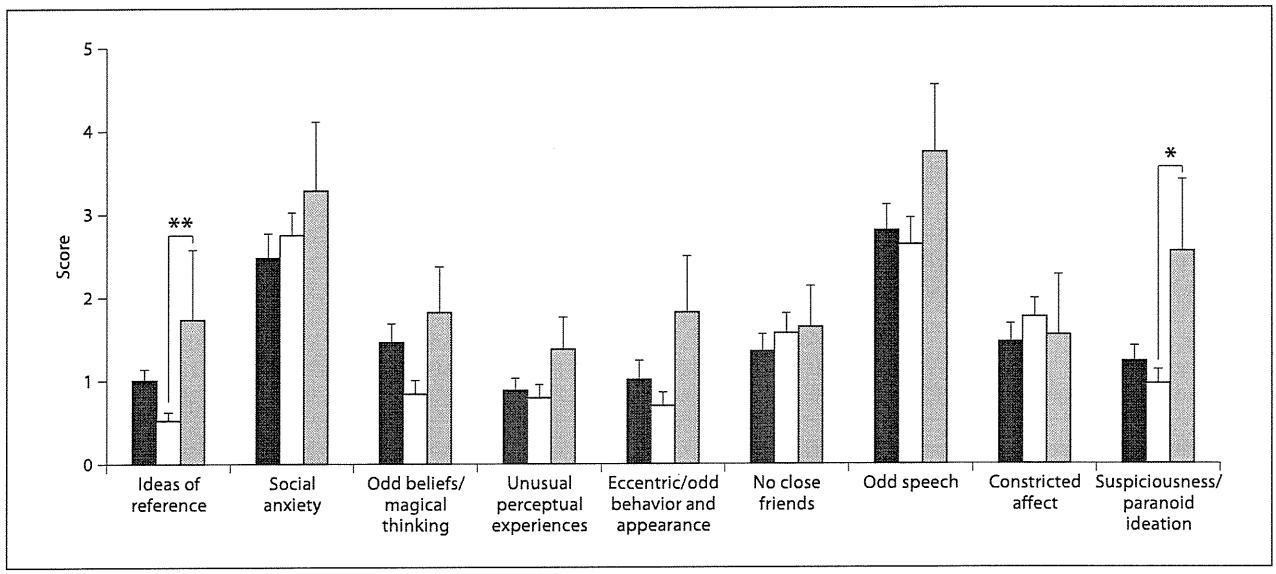

Fig. 1. Comparisons of scores on the nine subscales of the SPQ between the three suppressor groups. Black, white, and gray bars are incomplete suppressors (defined as DST-cortisol ≥5 or DEX/CRH-cortisol ≥5; n = 69), moderate suppressors (defined as DST-cortisol <5 and 1 ≤ DEX/CRH-cortisol <5; n = 61), and enhanced suppressors (defined as DST-cortisol <5 and DEX/CRH-cortisol <1; n = 11), respectively. * $p < 0.05$; ** $p < 0.01$. Error bars represent standard errors of the mean.

significantly higher than the moderate suppressors on these two subscales (fig. 1). Relationships of the SPQ total score and its three factors with DEX/CRH suppression status are provided in table 1. The ANCOVA on the SPQ total score and the three factors, controlling for age and sex, showed a significant main effect of the suppressor group on the cognitive-perceptual factor. Post-hoc analyses with Bonferroni correction revealed that the enhanced suppressors scored significantly higher than the moderate suppressors on this factor. To confirm the results obtained by the ANCOVA, we further performed Kruskal-Wallis test between the three suppressor groups. Similarly to the ANCOVA results, the Kruskal-Wallis test revealed that the three groups differed significantly on ideas of reference ($p = 0.012$) and odd beliefs/magical thinking ($p = 0.049$) subscales and the cognitive-perceptual factor ($p = 0.033$).

Discussion

We examined the relationship between schizotypal personality as assessed with the SPQ and cortisol reactivity to the DEX/CRH test in healthy adults. To our knowledge, this is the first report where HPA axis function in relation to schizotypal personality was investigated by using the DEX/CRH test. The main finding was the significant association between greater schizotypal traits, in particular the cognitive-perceptual factor, and the blunted cortisol response to CRH administration.

Although only two of nine subscales of SPQ were significantly different between the three suppressor groups (i.e. higher scores in the enhanced suppressor group), it is visually clear from figure 1 that most of the other subscales of SPQ were also higher in the enhanced suppressor group than in the other two groups. Consistent with this finding, previous studies have found schizotypal traits or SPD to be associated with enhanced suppression to DST [25] and with blunted cortisol responses to an acute metabolic stressor [26]. Studies on basal cortisol levels, by contrast, have indicated HPA axis hyperactivity in individuals with similar characteristics, such as elevated baseline cortisol levels in individuals with SPD [57] and a positive correlation between cortisol levels and schizotypal symptoms in adolescents at risk for developing schizophrenia [58]. Taken together, baseline studies and challenge studies may reveal different aspects of HPA axis alteration in relation to schizotypal traits, with the former representing the amount of biosynthesis/release of glucocorticoids while the latter reflecting sensitivity to

negative feedback (due to altered glucocorticoid receptor function) and/or downregulation of target receptors for HPA axis secretagogues. It might be of importance that, among the three factors of SPQ, the cognitive-perceptual factor, which is considered as an attenuated equivalent of positive symptoms of schizophrenia, was most strongly associated with the blunted cortisol response. Since this issue has been little studied, the direct comparison of the present findings with previous data cannot be made.

As for schizophrenia patients, findings on HPA axis alteration have not been uniform, and HPA axis dysfunction is less consistently reported in schizophrenia than in depression [59, 60]. In addition to the aforementioned controversies from DST studies, findings on basal HPA activity in schizophrenia patients have been mixed such that some investigators reported elevated baseline cortisol levels [61, 62], whereas others did not confirm such findings [22, 63, 64]. Findings on cortisol levels in first-episode psychosis patients are also inconsistent; Ryan et al. [65] reported elevated basal cortisol levels in drug-naïve first-episode patients with schizophrenia, while McGorry et al. [66], using a DST with 0.25 mg of DEX, showed that cortisol levels of drug-naïve or minimally treated patients with first episode psychosis were significantly lower than those of healthy controls. A study that administered the DEX/CRH test to schizophrenia patients found their cortisol responses to be exaggerated compared with those of healthy controls, albeit to a lesser extent compared to what has been reported in melancholic depression [28]. In contrast, Thompson et al. [67] showed that, among young people at ultra-high risk for developing psychosis, cortisol response to the DEX/CRH test of those who made the transition were lower than that of their counterparts who did not make the transition. Overall, it may be that cortisol responses of individuals with schizophrenia and/or those with high schizotypal traits depend on types of the cortisol measurements (e.g. basal cortisol vs. provoked cortisol) or sample characteristics (e.g. at risk/recent-onset vs. chronic), and our results together with previous data point to the importance of taking account of hypocortisolism as well as hypercortisolism in investigating HPA axis function in relation to schizophrenia-spectrum pathology. Still, the present finding could be considered as supporting the dimensional model of schizophrenia, in that schizotypal traits, like schizophrenia, are associated with alterations in HPA axis function, be they hyperactive or hypoactive.

A plausible explanation for the relationship between schizotypy and blunted cortisol reactivity observed here would be that greater schizotypal traits, its cognitive-perceptual factor in particular, are likely to lead to chronic stress and thus result in the hypoactive HPA axis function, given the well-established association between chronic stress and blunted HPA axis responsiveness [68, 69]. The alternative possibility of this causation (i.e. hypoactive HPA axis causes schizotypal personality) is unlikely partly because this personality trait is demonstrated to have genetic and neurodevelopmental bases [1]. Indeed, preclinical studies have found blunted cortisol reactivity in primates [70] and rodents [71] exposed to chronic social stress. Furthermore, recent clinical studies have shown that blunted cortisol responses to the combined DEX/CRH challenge are associated with stressful conditions that persist [43, 72–74]. As it is now clear that the ability to mobilize cortisol adequately in response to stressors is crucial for coping with stressful conditions [75], the blunted cortisol response observed in the present study may somehow be related to the increased sensitivity to stress in schizophrenia spectrum disorders. In the present study, the significant association between the enhanced suppression to the DEX/CRH test and greater schizotypal traits, coupled with the absence of significant correlations between DST-cortisol and SPQ scores, suggests that hyporeactive HPA axis in relation to nonclinical schizotypy might be accounted for, at least in part, by the downregulation of CRH receptors on the level of the pituitary (thereby leading to the failure to mount an adequate cortisol response to the CRH administration). It should be noted here that blunted cortisol responses to the DEX/CRH test have also been shown in nonclinical adults who have a history of childhood trauma or maltreatment [73, 76, 77]. Therefore, although we have excluded individuals with posttraumatic stress disorder from our subjects, the possibility cannot be ruled out that the association of schizotypal traits with blunted cortisol reactivity observed here might be confounded by such a history of early life adversity.

The present study may also be of relevance to previous studies that have linked nonclinical personality traits to altered HPA axis function. The impacts of neuroticism [37, 38] and novelty seeking [35, 36] on HPA axis function have been well documented, although findings on the association between neuroticism and cortisol responses to the DEX/CRH test are mixed, as described earlier [37, 38]. Interestingly, O'Leary et al. [78] have recently reported that nonclinical students characterized by high psychopathic personality traits lacked psychosocial stress-induced cortisol increases. Thus, the present finding of an association between schizotypal traits and the blunted cortisol response lends support to the evidence that per-

sonality, irrespective of whether or not it is related to schizophrenia-spectrum pathology, can influence HPA axis function. These psychopathic and schizotypal personality traits, like other personality traits, might explain some part of the HPA axis hypoactivity that has been observed in various chronic stress conditions [79, 80]; however, the issue as to what personality trait is related to hypercortisolism (or hypocortisolism) requires further investigations.

Concerning the association of schizotypal personality with temperament and character dimensions in the nonclinical population, two previous studies investigated this association by simultaneously using SPQ and TCI [81, 82]. Findings from these studies have been fairly consistent: cognitive-perceptual and disorganized factors of SPQ correlated negatively with self-directedness and positively with self-transcendence, and interpersonal factor correlated positively with harm avoidance and negatively with self-directedness [81, 82]. All of these significant correlations have been replicated in the present study (see table 2). We further examined the association between the TCI and DEX/CRH outcomes, and found no significant relationships between these outcomes, which was not in line with the finding of Tyrka et al. [35, 36]. This inconsistency might have resulted from different sample characteristics between the present and the previous two studies (e.g. Japanese vs. American or mean age of mid-40s vs. mid-20s), given that age and culture are shown to influence TCI scores [83, 84]. Nevertheless, the absence of significant association between TCI and DEX/CRH outcomes indicates that the relationship between schizotypy and blunted cortisol response observed here was not significantly confounded by temperament/character dimensions.

Several limitations to this study should be acknowledged. First, since the DEX/CRH test used here was based on a simple test protocol (i.e. measuring hormones only twice and omitting the ACTH measures), it may have provided less information on HPA axis function (e.g. inability to calculate the AUC). Moreover, we did not measure baseline cortisol levels, i.e. the cortisol level before the DEX administration, which hindered us from knowing the extent to which each participant suppressed his/her cortisol in response to the 1.5 mg of DEX. Second, this cross-sectional study cannot address the natural history of the alteration in HPA axis function. Third, we did not collect data on menstrual cycle in the female participants, which may have affected HPA axis function. Finally, as the participants were predominantly women, it is possible that the current findings are applicable only to women.

In summary, the present study found that greater schizotypal traits in healthy adults were significantly associated with the blunted cortisol response to the DEX/CRH test, indicating that schizotypy itself could affect HPA axis function. This finding, coupled with findings from previous studies in nonclinical schizotypes as well as schizophrenia patients, supports the possibility that schizophrenia-spectrum pathology would be related to altered HPA axis function.

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Expression of Ca^{2+}-dependent activator protein for secretion 2 is increased in the brains of schizophrenic patients

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ABSTRACT

Ca^{2+}-dependent activator protein for secretion 2 (CADPS2), a secretory granule associate protein, mediates monoamine transmission and the release of neurotrophins including brain-derived neurotrophic factor (BDNF) which have been implicated in psychiatric disorders. Furthermore, the expression of CADPS2deltaExon3, a defective splice variant of CADPS2, has been reported to be associated with autism. Based on these observations, we examined whether expression levels of CADPS2 and CADPS2deltaExon3 are altered in psychiatric disorders. Quantitative polymerase chain reaction analysis was performed for postmortem frontal cortex tissues (BA6) from 15 individuals with schizophrenia, 15 with bipolar disorder, 15 with major depression, and 15 controls (Stanley neuropathology consortium). The mean CADPS2 expression levels normalized to human glyceraldehyde-3phosphate dehydrogenase (GAPDH) or TATA-box binding protein levels was found to be significantly increased in the brains of the schizophrenia group, compared to the control group. On the other hand, the ratio of CADPS2deltaExon3 to total CADPS2 was similar in the 4 diagnostic groups. We then analyzed CADPS2 expression in blood samples from 121 patients with schizophrenia and 318 healthy controls; however, there was no significant difference between the two groups. Chronic risperidone treatment did not alter the expression of CADPS2 in frontal cortex of mice. The observed increase in the expression of CADPS2 may be related to the impaired synaptic function in schizophrenia.

1. Introduction

Ca^{2+}-dependent activator protein for secretion (CADPS) family, which consists of two members, CADPS1 and CADPS2, is a secretory granule-associated proteins involved in Ca^{2+}-dependent exocytosis of large dense-core vesicles containing diverse array of modulators including neurotrophines, monoamines and neuropeptides (Liu et al., 2008; Sadakata et al., 2004). CADPS2 mediates the release of neurotrophins such as brain-derived neurotrophic factor (BDNF) and neurotrophin-3. Mouse CADPS2 protein is associated with BDNF-containing secretory vesicles and promotes activity-dependent release of BDNF (Sadakata et al., 2004). BDNF release is significantly reduced in cultured neurons prepared from the brain of CADPS2 deficient mice (Sadakata et al., 2007a,b).

A number of findings suggest that BDNF action is impaired in psychiatric disorders including schizophrenia, bipolar disorder and depression. Several studies have shown decreased levels of BDNF or its receptor, TrkB, in the postmortem brains of patients with schizophrenia (Hashimoto et al., 2005; Iritani et al., 2003; Weickert et al., 2003), although there are contradictive reports (Chen et al., 2001; Dunham et al., 2009; Durany et al., 2001; Takahashi et al., 2000). The contribution of BDNF in depression has been suggested from animal studies that demonstrated stressful environments decrease, and antidepressive treatments increase BDNF levels in the brain (Duman and Monteggia, 2006; Martinowich et al., 2007). Also, centrally administered BDNF has an antidepressant-like effect in rat models (Siuciak et al., 1997). Thus, the molecules that contribute to the trafficking and release of BDNF may be a culprit of these disorders.

CADPS family also mediate monoamine transmission. Both CADPS1 and CADPS2 mediate 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 CADPS family are involved in monoamine storage as antibodies against CADPS1 or 2 inhibit monoamine

Abbreviations: ANCOVA, Analysis of covariance; BDNF, Brain-derived neurotrophic factor; CADPS2, Ca^{2+}-dependent activator protein for secretion 2; CCK, Cholecystokinin; DSM—IV, Diagnostic and Statistical Manual of Mental Disorders, 4th edition; FST, Freezer storage time; M.I.N.I., Mini-International Neuropsychiatric Interview; NT, Neurotensin; PCR, Polymerase chain reaction; PMI, Postmortem interval; SD, Standard deviation; TBP, TATA-box binding protein.

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sequestration by synaptic vesicles from mice brain (Brunk et al., 2009).

Dysregulation of monoamine neurotransmission has been hypothesized to play a central role in the etiology of psychiatric disorders including schizophrenia and mood disorders. In schizophrenia, not only classical evidence that dopamine agonists induce and dopamine D2 receptor antagonists ameliorate psychoses but also brain imaging studies on drug naïve patients have suggested that dopamine transmission is affected in this disorder (Lyon et al., 2011). In major depression, reduced monoamine transmission hypothesis was derived from the finding that most anti-depressants increase monoamine levels in the synaptic cleft and that reserpine, a monoamine-depleting drug, worsen depressive symptoms in a subset of patients with mood disorder (Krishnan and Nestler, 2008), although imaging, postmortem, or cerebrospinal fluid studies have yet to find the definitive evidence for altered monoamine neurotransmission in this disorder (Belmaker and Agam, 2008; Nikolaus et al., 2009).

While, to our knowledge, CADPS2 expression in schizophrenia or mood disorders have not yet been examined, aberrant splicing of CADPS2 mRNA was reported in autism (Sadakata et al., 2007b). In this study, an exon-3 skipped isoform, CADPS2ΔExon3, was detected in the bloods of several autistic patients but not in those of healthy controls. They also showed that CADPS2ΔExon3 was deficient in proper axonal transport, which results in the loss of local synaptic BDNF release. Though the CADPS2ΔExon3 expression in the brains of patients with autism is unclear, the aberrant splicing of CADPS2 could contribute to susceptibility to autism by affecting neurotrophin release.

Based on above findings, the present study was aimed to examine whether the expression of CADPS2 transcripts is altered in the frontal cortex of patients with psychiatric disorders including schizophrenia, major depression and bipolar disorder. The CADPS2 expression levels in the blood of schizophrenia were also examined.

2. Materials and methods

2.1. Brain samples

Frozen postmortem samples of frontal cortex (BA6) were obtained from the Stanley Foundation Neuropathology Consortium (Torrey et al., 2000). The collection consists of 60 subjects: 15 with schizophrenia, 15 bipolar disorder, 15 major depression and 15 unaffected controls. All groups were matched for age, sex, race, pH and hemispheric side (Table 1), although postmortem interval (PMI) and freezer storage time differed across the groups. The brain tissues obtained were coded. Once our blind study was complete, we sent the data to the Stanley Foundation who then returned the codes, demographic and clinical data. In a cold-room, each frozen brain tissue was broken into powder in the plastic bag using dry-ice block and dry-ice-cold hammer. The powder was then transferred and kept in dry-ice-cold tubes. Temperature of the tubes and instruments that directly contacted to the samples was frequently measured by infrared-thermometer (AD-5613A, A&D Company, Japan) and kept under −20 °C. Then, 30 to 40 mg of brain powder was used for cDNA synthesis. RNA was extracted using RNAqueous (Applied biosystems, Foster City, CA) according to manufacturer's instructions with a slight modification, i.e., after homogenization, samples were washed twice with 500 μl of chloroform, and then applied to the spin-column. Extracted RNA was quantified by optical density reading at 260 nm using NanoDrop ND-1000 (Thermo Scientific, Rockford, IL). Then, the obtained RNA (14 μl) was used for cDNA synthesis using SuperScript VILO cDNA Synthesis Kit (Invitrogen, Carlsbad, CA).

Table 1
Demographic information on brain specimens of Stanley Neuropathology Consortium.

	Control	Schizophrenia	Bipolar disorder	Major depression
Age (years)	48.1 (29–68)	44.2 (25–62)	42.3 (25–61)	46.4 (30–65)
Gender (M/F)	9/6	9/6	9/6	9/6
Race	14 C, 1 AA	13 C, 2 A	14 C, 1 AA	15 C
PMI (hours)	23.7 (8–42)	33.7 (12–61)	32.5 (13–62)	27.5 (7–47)
pH	6.3 (5.8–6.6)	6.1 (5.8–6.6)	6.2 (5.8–6.5)	6.2 (5.6–6.5)
Side of brain frozen (R/L)	7/8	6/9	8/7	6/9
Freezer storage time (months)	11.3 (1–26)	20.7 (2–31)	20.7 (7–28)	14.5 (3–31)

AA, African American; A, Asian; C, Caucasian; F, female; M, male; and PMI, postmortem interval.

2.2. Blood samples

Subjects were 121 patients with schizophrenia (84 males and 37 females; age 44.1 ± 13.7 (mean ± SD) years) and 318 controls (90 males and 228 females; age 43.1 ± 15.3 years). All subjects were biologically unrelated Japanese and 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. The controls were healthy volunteers recruited from the community, through advertisements in free local magazines and our website announcement. Control individuals were interviewed by the Japanese version of the Mini-International Neuropsychiatric Interview (M.I.N.I.) (Otsubo et al., 2005; Sheehan et al., 1998) and those who had a current or past history of psychiatric treatment were not enrolled in the study. After the nature of the study procedures had been fully explained, written informed consent was obtained from all subjects. 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.

Blood collection and RNA isolation were performed using the PAXgene blood RNA system (Qiagen, Valencia, CA). Blood samples were collected around 11 A.M. Extracted RNA was quantified as described above. 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. Chronic risperidone treatment to mice

C57BL/6J male mice aged 10 weeks were purchased from Crea Japan. Chronic oral risperidone treatment was performed according to Belforte et al., (Belforte et al., 2010). In brief, 2.5 mg/kg/day of risperidone (Rispadal liquid, Janssen Pharmaceutical, Tokyo, Japan) in drinking water freshly made every 72 h had been administered continuously for 3 weeks. Control mice received solvent (1.4 mM tartaric acid neutralized to pH 6–7). All experimental procedures were in accordance with the guidelines of the United State's National Institutes of Health (1996) and were approved by the Animal Care Committee of the National Institute of Neuroscience, NCNP.

2.4. 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). Each reaction contained 0.28 μl of cDNA sample, qPCR QuickGoldStar Mastermix Plus (Eurogentec, Seraing, Belgium) and a primer of the target, i.e. human CADPS2 (Hs01095968_m1 at Exon 4–5 on NM_017954.9), mouse CADPS2 (Mm00462577_m1), human CADPS2ΔExon3 (Forward primer: GTAGCTGACGAAGCATTTTGCA,

Reverse Primer: TGATCTGGGCTGCTTGTTCAT, Reporter: CTGCGTTATC-CAGCTCAT) and a primer of the housekeeping gene human glyceraldehyde-3phosphate dehydrogenase (GAPDH, 4326317E), mouse GAPDH (4352339E) and human TATA-box binding protein (TBP, Hs99999910_ml) all purchased from Applied Biosystems (Foster City, CA). Negative control reactions were carried out with "no RNA" samples. The real time PCR reactions ran at 50 °C for 2 min, at 95 °C for 10 min and in 40 or 45 cycles changing between 95 °C for 15 s and 60 °C for 1 min. 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 and CADPS2ΔExon3 from each sample were normalized to those of GAPDH.

2.5. Statistical analyses

Data analyses were performed with SPSS software (Version 11, SPSS Japan, Tokyo, Japan). Effect of age, brain pH, postmortem interval (PMI), and freezer storage time on each brain analysis was assessed by Pearson's correlations (Table 2). Variables showing significant correlations were included as covariates in the main analysis. Levene's test was used to assess the equality of variances across diagnostic group. Analysis of covariance (ANCOVA) was used to identify overall effects of diagnosis and significant main effects of diagnosis were investigated by planned post hoc contrasts. In the blood sample analyses, CADPS2 expression levels were converted to 10-log scale before statistical analysis in order to obtain a normal distribution (Castensson et al., 2005). The effect of diagnosis on blood CADPS2 expression was assessed by ANCOVA with sex and age as covariates after Levene's test. The effect of diagnosis on blood CADPS2ΔExon3 expression was assessed by logistic regression, controlling for sex and age as covariates. The effect of risperidone on CADPS2 expression in mice brain was assessed by student's *t*-test after F-test.

3. Results

3.1. CADPS2 expression levels in the postmortem brain (BA6)

We first analyzed the effects of age, brain pH, postmortem interval (PMI), and freezer storage time (FST) on each expression analysis (Table 2). Brain pH was significantly correlated with GAPDH expression levels or raw CADPS2 expression levels. PMI also tended to be correlated with GAPDH expression levels or raw CADPS2 expression levels. If the effects were analyzed separately within each diagnostic group, no significant correlation was detected.

CADPS2 expression levels normalized to GAPDH expression levels (CADPS2/GAPDH) in each sample are shown in Fig. 1A. ANCOVA with brain pH as covariates detected a significant effect of diagnosis on CADPS2/GAPDH levels ($F=3.4$, $df=3$, $p=0.025$) and post hoc test detected a significant difference between schizophrenia and control groups ($p=0.03$). Even if PMI was added as another covariate, the difference was significant ($p=0.002$). There was no significant difference between bipolar disorder and controls or between depression and controls. There was no significant correlation between CADPS2/GAPDH levels and lifetime dose of antipsychotic drugs (data not shown). There was a significant effect of diagnosis on GAPDH expression levels ($F=3.4$, $df=3$, $p=0.023$, Fig. 1B). GAPDH levels in the control group was significantly higher than that of schizophrenia ($p=0.012$), bipolar disorder ($p=0.009$) or major depression group ($p=0.013$). Raw CADPS2 levels did not differ among the diagnostic groups ($F=1.0$, $df=3$, $p=0.38$, Fig. 1C). There was a significant correlation between GAPDH expression levels and raw CADPS2 expression levels (Pearson's correlation 0.69, $p<0.001$, Fig. 1D).

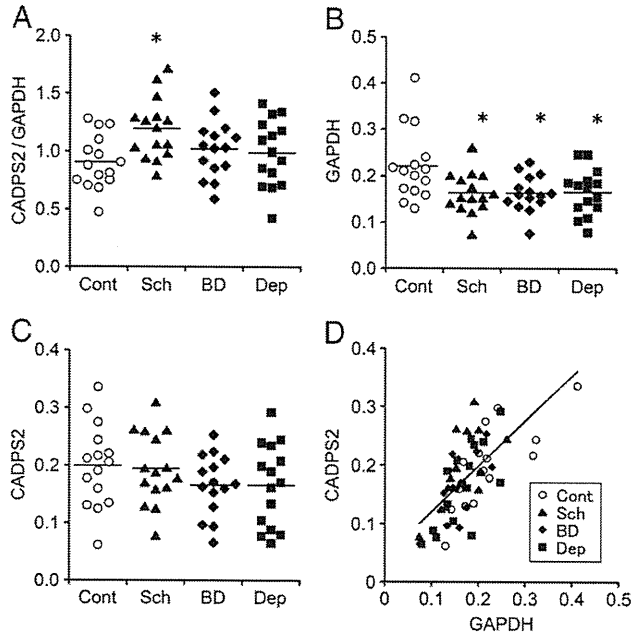

Fig. 1. CADPS2 expression levels in the postmortem brains of psychiatric disorder. (A) CADPS2 expression levels normalized by GAPDH levels. Scatter plots display the variability and differences in the CADPS2 mRNA expression levels normalized by each GAPDH expression levels. A crossbar on each scatter plot represents mean expression levels for each group. (B) GAPDH expression levels (C) Raw CADPS2 expression levels. (D) Correlation between GAPDH levels and raw CADPS2 levels. Cont, control; Sch, schizophrenia; BD, Bipolar Disorder; and Dep, Depression. *, statistically significant difference ($p<0.05$).

We compared relative CADPS2 expression levels among diagnostic groups using another endogenous control, TATA-box binding protein (TBP), and obtained similar result (Fig. S1, this experiment was done after uncode the sample). ANCOVA with brain pH as covariates detected a significant effect of diagnosis on CADPS2/TBPlevels ($F=3.3$, $df=3$, $p=0.027$) and post hoc test detected a significant

Table 2
The effect of age, pH, postmortem interval, and freezer storage time on each brain expression analysis.

		GAPDH	CADPS2	ΔExon3	CADPS2/GAPDH	ΔExon3/G APDH	ΔExon3/C ADPS2
Age	Pearson's	0.013	−0.13	0.19	−0.18	0.088	0.27
	P	0.92	0.34	0.37	0.16	0.51	0.041
pH	Pearson's	0.36	0.26	0.25	0.031	0.12	0.090
	p	0.005	0.048	0.058	0.81	0.38	0.50
Post mortem Pearson's interval (hours)	−0.23	−0.22	−0.13	−0.040	0.039	0.15	
	P	0.076	0.098	0.30	0.76	0.77	0.25
Freezer storage time (months)	Pearson's	−0.22	−0.034	−0.041	0.21	0.12	0.052
	P	0.092	0.80	0.75	0.11	0.36	0.69

ΔExon3, CADPS2ΔExon3; and Pearson's, Pearson's correlation.

difference between schizophrenia and control groups ($p=0.019$). Even if PMI was added as another covariate, the difference was significant ($p=0.012$).

With respect to CADPS2ΔExon3/GAPDH level (Fig. 2A), the effect of age was detected in the control group (Pearson's correlation 0.58, $p=0.023$) and the effect of pH was detected in the bipolar disorder group (Pearson's correlation 0.60, $p=0.018$). ANCOVA with age and brain pH as covariates detected the marginal effect of diagnosis ($F=2.8$, $df=3$, $p=0.050$) and the mean expression level was significantly increased in the schizophrenia group, compared to the control group ($p=0.030$). When the ratio of CADPS2ΔExon3 to raw (total) CADPS2 expression levels was compared, the ratio was similar in the 4 diagnostic groups ($F=1.1$, $df=3$, $p=0.36$, Fig. 2B). Neither the effect of diagnosis on raw CADPS2ΔExon3 levels was observed ($F=1.9$, $df=3$, $p=0.15$, Fig. 2C). There was a significant correlation between GAPDH expression levels and raw CADPS2ΔExon3 expression levels (Pearson's correlation 0.66, $p<0.001$, Fig. 2D).

3.2. Cortical CADPS2 expression after chronic antipsychotic treatment in mice

To see whether antipsychotics alter the mRNA expression of CADPS2, we measured the CADPS2 levels in the frontal cortex of mice, following chronic treatment with an antipsychotic risperidone. Oral administration of risperidone (2.5 mg/kg, $n=15$ for the controls and 16 for the risperidone group) for 3 weeks did not alter CADPS2 expression ($F=1.5$, $df=29$, $p=0.61$).

3.3. CADPS2 expression in blood sample

Since we observed increased expression of CADPS2 in postmortem brains of schizophrenia patients, we then examined whether such an alteration exists in peripheral blood samples. The CADPS2/GAPDH expression levels were converted to 10-logarism before statistical analyses to obtain normal distribution. The mean (Standard deviation) CADPS2 expression level was 0.17 (1.29) in the control group and 0.32 (1.46) in the schizophrenia group. ANCOVA controlling for age and sex did not detect the significant effect of diagnosis on CADPS2/GAPDH level ($F=1.67$, $df=1$, $p=0.20$). We also measured CADPS2ΔExon3 levels in the blood samples. Compared to brain samples, the expression levels were quite low and could not detect in the majority of samples. Thus, we defined "expressed" when at least 2 tubes in triplet analyses of each sample were detected until 45 cycles. CADPS2ΔExon3 expression was detected in 36 of 318 control samples (ratio = 0.11), and 21 of 121 schizophrenia samples (ratio = 0.17). There was no significant effect of diagnosis on CADPS2ΔExon3 expression by the logistic regression analysis controlling for age and sex (odds ratio 1.51, [95% CI 0.80–2.86], $p=0.21$). Even when men and women were examined separately, there was no significant difference between the patients and controls for each sex (data not shown).

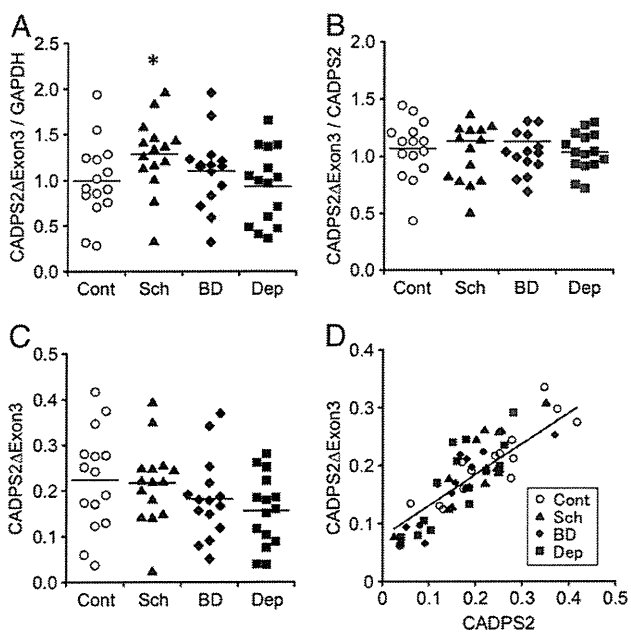

Fig. 2. CADPS2ΔExon3 expression levels in the postmortem brains of psychiatric disorder. (A) CADPS2ΔExon3 expression levels normalized by GAPDH levels. Scatter plots display the variability and differences in the CADPS2ΔExon3 mRNA expression levels normalized by each GAPDH expression levels. A crossbar on each scatter plot represents mean expression levels for each group. (B) CADPS2ΔExon3 levels normalized to each total CADPS2 expression levels. (C) Raw CADPS2ΔExon3 expression levels. (D) Correlation between GAPDH expression levels and raw CADPS2ΔExon3 expression levels. Cont, control; Sch, schizophrenia; BD, Bipolar Disorder; and Dep, Depression. *, statistically significant difference ($p<0.05$).

4. Discussion

4.1. Main findings

In the present study, we analyzed the expression of CADPS2 mRNA in the postmortem brains (BA6) of psychiatric patients (schizophrenia, major depression and bipolar disorder) and controls. A significant increase in the CADPS2 expression was detected in the brains of the schizophrenia group, compared to the control group. No change was detected in other disease groups. While a CADPS2 splice variant, CADPS2ΔExon3 showed a non-significant increase in the schizophrenia group, its ratio to the total CADPS2 levels was not different from the control group. Chronic risperidone treatment did not alter the CADPS2 levels in mice brain. We also analyzed CADPS2 or CADPS2-ΔExon3 expression levels in the blood samples of schizophrenia and control subjects; however, the levels were not significantly different between the two groups.

4.2. Brain analysis

4.2.1. Drug effect

A large number of gene expressions in the brain are affected by antipsychotic treatments (Girgenti et al.,; Mehler-Wex et al., 2006; Thomas, 2006). Therefore, the observed increase in CADPS2 mRNA in the schizophrenia group could be the result of antipsychotic treatment. However, our results did not support this assumption because the CADPS2 levels did not correlate to life-time antipsychotic dose and chronic risperidone treatment in mice did not alter CADPS2 expression on their cortices, although caution is required for the interpretation of those results because we don't have data for the latest dose before death and other drugs such as chlorpromazine, haloperidol and clozapine might be used in the patients.

4.2.2. Possible relevance to BDNF secretion, dopamine transmission, and neuropeptide release

Considering that defective BDNF signaling has been suggested in schizophrenia and mood disorders (Angelucci et al., 2005) and that CADPS2 mediates BDNF release in neurons (Sadakata et al., 2004), we initially expected that CADPS2 levels would be decreased in frontal cortex in patients with these psychiatric disorders. However, in our results, CADPS2 levels were not altered in mood disorders but increased in schizophrenia. In addition, the relative levels of defective CADPS2 isoform, CADPS2ΔExon3 were not altered in those disorders. Thus, it is unlikely that altered CADPS2 expression might be a cause of BDNF deficits in schizophrenia. It may be rather a compensatory consequence of reduced BDNF signaling.

CADPS2 also promotes monoamine storage in neurons (Brunk et al., 2009; Liu et al., 2008). 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). Growing evidence has demonstrated increased presynaptic dopamine levels in the striatum of schizophrenia patients (Lyon et al., 2009). If the observed increase in the expression of CADPS2 occurs in the subcortical regions including striatum and midbrain as well as frontal cortex, it might be the cause of hyper-dopamine transmission that reflects psychotic state (Howes et al., 2009).

Furthermore, large dense-core vesicles contain not only neurotrophins and monoamines but also neuropeptides (Salio et al., 2006). Neuropeptides such as endorphins, cholecystokinin (CCK), neurotensin (NT), somatostatin, Neuropeptide Y and neuregulin 1 have been implicated in schizophrenia (Caceda et al., 2007). Especially reduced levels of CCK and NT have been repeatedly reported in the disorder (Caceda et al., 2007), which may have caused compensatory increase in the CADPS2 expression in schizophrenia.

4.3. CADPS2 expression in the blood

4.3.1. CADPS2 expression and diagnosis

Following the report that 4 of 16 patients with autism expressed CADPS2ΔExon3 in peripheral bloods but none in 24 normal subjects (Sadakata et al., 2007b), another group reported that they detected CADPS2ΔExon3 in some control subjects (Eran et al., 2009). Thus we assumed that the ratio of CADPS2ΔExon3 to total CADPS2 rather than whether CADPS2ΔExon3 exists or not is important and therefore we applied quantitative real-time PCR to measure their expression. The pilot experiment in the present study indicated that our quantification method using SuperScript VILO and random-hexamer, was 4 to 8 fold more sensitive than one step real-time PCR using gene specific primers and could detect l0 to 100 clones of CADPS2 or CADPS2-ΔExon3 sequence-containing vector. Compared with the brains, CADPS2 expression was 32 to 128 fold lower in the blood. Unlike in the brain, CADPS2ΔExon3 could not be detected in most blood samples. So we performed qualitative analysis for each subject. As a result, we didn't detect any significant difference in the expression of CADPS2ΔExon3 in the blood between patients with schizophrenia and controls. The CADPS2ΔExon3 was abundantly expressed in the brain and the levels were unchanged across the diagnostic groups. Thus, it is unlikely that the expression or the splicing balance should relate to diseases we analyzed.

5. Conclusion

In conclusion, we found increased mRNA expression of CADPS2 in the postmortem frontal cortex of schizophrenia patients which might have some relevance to dysregulation in the release of dopamine, neurotrophins, and/or neuropeptides in the disorder. This increase was unlikely to be attributable to antipsychotic medication. We also analyzed the CADPS2ΔExon3 in human brains and found that it is abundantly present in the frontal cortex in any diagnostic group. We obtained no evidence for the specific role of the splice variant in schizophrenia or mood disorders. Future research should include the evaluation of CADPS2 expression in other brain areas, and basic studies on the cause and consequence of increased CADPS2 expression.

Supplementary materials related to this article can be found online at doi:10.1016/j.pnpbp.2011.05.004.

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Association of plasma IL-6 and soluble IL-6 receptor levels with the Asp358Ala polymorphism of the IL-6 receptor gene in schizophrenic patients

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ABSTRACT

Recent studies indicate a role of excessive interleukin-6 (IL-6) signaling in the pathogenesis of schizophrenia. A previous study reported a significant association of schizophrenia with the IL-6 receptor (IL-6R) gene Asp358Ala polymorphism, which is known to regulate circulating IL-6 and soluble IL-6R (sIL-6R) levels in healthy subjects. To further examine the influence of the polymorphism in schizophrenic patients, we compared the plasma levels of IL-6 and sIL-6R between schizophrenic patients and healthy controls for each genotype of the Asp358Ala polymorphism. Asp358Ala genotyping and plasma IL-6 level measurements were performed in 104 patients with schizophrenia and 112 healthy controls. Of these participants, 53 schizophrenic patients and 49 controls were selected for the measurement of plasma sIL-6R levels. A two-way factorial analysis of covariance was performed with the transformed plasma levels as the dependent variable, diagnosis and genotype as independent variables, and sex and age as covariates. No significant diagnosis × genotype interaction was observed for IL-6 and sIL-6R levels. The Ala allele of Asp358Ala was significantly associated with higher levels of both IL-6 and sIL-6R. IL-6 levels were significantly elevated in schizophrenic patients compared to those in controls, whereas no significant difference in sIL-6R levels was observed between schizophrenic patients and controls. Our findings suggest that the presence of schizophrenia is associated with elevated IL-6 levels, whereas sIL-6R levels are mainly predetermined by the Asp358Ala genotype and are not associated with the disease status. Increased IL-6 levels without alterations in sIL-6R levels may result in excessive IL-6 signaling in schizophrenia.

1. Introduction

Inflammatory immune processes have been strongly implicated in the etiology of schizophrenia (Watanabe et al., 2010). Elevated serum or plasma levels of interleukin-6 (IL-6) is observed in patients with schizophrenia (Potvin et al., 2008), suggesting a role of excessive IL-6 signaling in the pathogenesis of this disorder. IL-6 binds to the soluble IL-6 receptor (sIL-6R) to form an IL-6/sIL-6R complex that is capable of binding to gp130 in the cellular membrane to mediate intracellular signaling. As membrane-bound IL-6R is expressed selectively on monocytes, neutrophils, T and B lymphocytes, and hepatocytes, other cells require the IL-6/sIL-6R complex for IL-6 signaling. Therefore, it could be inferred that sIL-6R plays an important part in the pathogenesis of schizophrenia.

An increased IL-6 level is one of the most robust findings in the study of inflammatory markers in schizophrenia, as evidenced by a meta-analysis of 19 studies comprising 1219 subjects (Potvin et al., 2008). Furthermore, one study showed a positive correlation between the severity of symptoms and plasma IL-6 levels in antipsychotic-free schizophrenic patients (Pae et al., 2006). However, findings regarding changes in the circulating levels of sIL-6R in patients with schizophrenia have been equivocal. Some studies reported increased sIL-6R levels in patients with schizophrenia (Lin et al., 1998; Maes et al., 1997), whereas one study reported lower sIL-6R levels (Maes et al., 1994). Others reported no significant differences in sIL-6R levels between patients and controls (Maes et al., 1995; Muller et al., 1997; O'Brien et al., 2008). Non-significant effect size estimates were obtained for sIL-6R in a meta-analysis of 7 studies (Potvin et al., 2008).

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