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SUBJECTIVE AND OBJECTIVE MEASURES OF QUALITY OF LIFE HAVE DIFFERENT PREDICTORS FOR PEOPLE WITH SCHIZOPHRENIA[1,2]

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Summary.—This study investigated the relationship between subjective and objective quality of life and assessed predictors in people with schizophrenia. The study population consisted of 99 stabilized outpatients with schizophrenia (DSM–IV) who had been regularly receiving outpatient treatment at the Department of Psychiatry, The Tokushima University Hospital. Subjective and objective quality of life were estimated using the Schizophrenia Quality of Life Scale and the Quality of Life Scale, respectively. Psychiatric symptoms were also measured with the Brief Psychiatric Rating Scale and the Calgary Depression Scale for Schizophrenia. Scores on the Schizophrenia Quality of Life Scale Motivation and Energy scales significantly correlated with the Quality of Life Scale total scores –.40 ($p < .001$), and with the scores on Interpersonal Relations subscale –.42 ($p < .001$), Instrumental Role subscale –.28 ($p = .005$), Intrapsychic Foundations subscale –.39 ($p < .001$), and Common Objects and Activities subscale –.25 ($p = .014$). The Schizophrenia Quality of Life Scale Psychosocial scale significantly correlated with only the Quality of Life Scale total score –.20 ($p = .05$), and there was no significant correlation between the scores on the Schizophrenia Quality of Life Scale Symptoms and Side-effects scales and the Quality of Life Scale. Stepwise regression analyses showed that the Calgary Depression Scale for Schizophrenia score was the most important predictor of each scale of the Schizophrenia Quality of Life Scale, and the Brief Psychiatric Rating Scale Negative Symptoms score was the most important predictor of the Quality of Life Scale total score and each subscale. These results suggest that subjective and objective quality of life have different predictors and should be considered as separate and complementary outcome variables.

People with schizophrenia suffer distress, reduced productivity, and lowered quality of life (20). Over the past two decades, the concept of quality of life has become an important attribute in patient care and clinical research (5, 19, 21, 29). Although there seems to be no unanimous definition of qual-

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ity of life at the moment, there is general agreement that quality of life consists of access to resources and opportunities, fulfillment of life's roles, level of functioning, and a sense of well-being or life satisfaction (3, 10). Quality of life has been measured from two different viewpoints (10). One is self-rated quality of life and the other is interviewer-rated quality of life. In the present study, we assume that self-rated quality of life is subjective and interviewer-rated quality of life is objective. Subjective quality of life includes life satisfaction in general and within different life domains and objective quality of life includes health and living conditions, sociodemographic items, and role functioning (10). As patients with schizophrenia have been thought to be unable to assess their own quality of life by themselves because of their cognitive deficit, objective quality of life measures have been used in many studies. However, now there is general agreement that stabilized patients could assess their quality of life by themselves (30).

For the clinical factors associated with subjective quality of life, Dickerson, *et al.* (9) found that patients' subjective quality of life measured by the Quality of Life Interview was related to the depression factor in the Positive and Negative Syndrome Scale. Huppert, *et al.* (13) reported that more severe depression as rated on the Brief Psychiatric Rating Scale (26) was associated with lower subjective quality of life measured by the Quality of Life Interview. Other similar studies also support the association of depressive symptoms with subjective quality of life (10, 27). Regarding subjective well-being, which is the main component of subjective quality of life, Norman, *et al.* (25) found that the General Well-being Scale score was related to positive symptoms, particularly reality distortion. These previous findings suggest that depressive or positive symptoms might be important factors influencing subjective quality of life. Moreover, other factors such as anxiety, extrapyramidal adverse effects, and patients' subjective responses and attitudes towards antipsychotic treatment have been reported to be associated with subjective quality of life (4, 13, 27), while a few studies did not support the findings (18, 23).

Clinical factors associated with objective quality of life also have been investigated, and several research groups have reported that negative symptoms were much more closely related to objective quality of life than were positive symptoms (10, 25). These studies have used the Quality of Life Scale (11), which was originally designed to assess deficit symptoms. Therefore, it may stand to reason that fewer negative symptoms were associated with better quality of life measured by the Quality of Life Scale; however, some studies showed the significant relationships of positive symptoms and other clinical factors (6, 7, 8, 21).

For the relationship between subjective and objective quality of life measures, there are only a few related studies. Using the Quality of Life

Interview which contains subjective and objective measures within each domain, Dickerson, *et al.* (9) studied 72 outpatients with schizophrenia and reported few significant correlations between subjective and objective quality of life measures of specific life areas. Fitzgerald, *et al.* (10) also reported that subjective quality of life and objective quality of life were not closely related in patients with schizophrenia. Although many studies have used only a subjective or objective quality of life measure, if subjective and objective quality of life measures reflect different aspects of quality of life, using only one measure may introduce bias in the results. This important issue was studied using the Schizophrenia Quality of Life Scale (31), a newly developed subjective quality of life measure specific to people with schizophrenia, and the Quality of Life Scale, an objective quality of life measure for schizophrenia which has been the most frequently used scale in previous quality of life studies.

The purposes of the current, cross-sectional study were to investigate the relationship between subjective quality of life as measured by the Schizophrenia Quality of Life Scale and objective quality of life as assessed by the Quality of Life Scale and to assess which clinical variables predict the scores in the two scales. It was hypothesized that correlations would not be significant between the two scales, and they had different predictors.

Method

The subjects for the current study were ninety-nine stabilized outpatients with a DSM–IV diagnosis of schizophrenia (2). All subjects were Japanese and had been regularly receiving outpatient treatment at the Department of Psychiatry, Tokushima University Hospital. Their mean age was 38.5 yr. (SD = 12.4 yr.); 51 were men and 48 were women. During the 2-mo. period for recruitment, treating psychiatrists randomly asked stabilized outpatients to participate in this study. Informed consent was obtained from all subjects for the research involved in the study. Subjects were excluded if they presented with any organic central nervous system disorder, epilepsy, mental retardation, or severe somatic disorder.

Subjective quality of life was evaluated using the Schizophrenia Quality of Life Scale (17, 31). This scale is a 30-item self-report questionnaire specific to patients with schizophrenia. It is composed of three scales of Psychosocial, Motivation and Energy, and Symptoms and Side-effects. Each item has a 5-point scale using anchors of 0: Never and 4: Always; lower scores indicate higher quality of life. It was reported that there was no difference in factorial structure of the Schizophrenia Quality of Life Scale between Anglo-Saxon and Japanese populations, and the Japanese version of the scale had high internal consistency (Cronbach coefficients alpha ranged from .73 to .93) and significant correlations with other quality of life measures (17).

Objective quality of life was rated by experienced psychiatrists using the Quality of Life Scale (11, 12), which is a semistructured, interviewer-admin-

istered rating scale. This scale incorporates four major subscales measured by a total of 21 items. Each item is rated from 0 to 6. Specific anchors are provided for each item, with a score of 0 representing severely impaired quality of life and a score of 6 indicating high quality of life with regard to the specific item. The four subscales are Interpersonal Relations, Instrumental Role, Intrapsychic Foundations, and Common Objects and Activities. Higher scores reflect less impaired functioning. It was reported that there was no difference in factorial structure of the Quality of Life Scale between Anglo-Saxon and Japanese populations, and the Japanese version had a good interrater reliability (intraclass correlation coefficients ranged from .75 to .98) (10).

Psychotic symptoms were evaluated using the Brief Psychiatric Rating Scale (22, 26). This rating utilizes a 7-point scale with anchors of 1: Not present and 7: Extremely severe; higher scores indicate higher psychopathological disturbance. Based on previous groupings (21, 24), the sum of four symptoms of suspiciousness, hallucinatory behavior, conceptual disorganization, and unusual thought content were considered the positive symptoms score, and the sum of another four symptoms of emotional withdrawal, motor retardation, blunted affect, and disorientation were the negative symptoms score. It was reported that the Japanese version of the scale had an adequate interrater reliability (14 items of the scale showed an intraclass correlation coefficient greater than .70) (22).

Specifically, depressive symptoms were assessed using the Calgary Depression Scale for Schizophrenia (1, 16). This scale is a 9-item questionnaire (depression, hopelessness, self-deprecation, pathological guilt, guilty ideas of reference, morning depression, early awakening, suicidality, and observed depression). Each item is rated using anchors of 0: Absent and 3: Severe; higher scores indicate greater depression. It was reported that the Japanese version of the scale had high internal consistency (Cronbach coefficient alpha was .82), interrater agreement (kappa coefficients ranged from .72 to 1.00), and test-retest reliability (test-retest coefficient was .86), and significant correlation with other depression measures (16).

Extrapyramidal symptoms were assessed using the Drug-induced Extrapyramidal Symptoms Scale (14). This scale is composed of eight individual parameters (gait, bradykinesia, sialorrhoea, muscle rigidity, tremor, akathisia, dystonia, and dyskinesia) and one global assessment constructed to assess extrapyramidal adverse effects, rated on a 5-point scale anchored by 0: None and 4: Severe. In the current study, the ratings of overall severity were considered the extrapyramidal symptoms scores. The scale has adequate interrater reliability (intraclass correlation coefficients ranged from .89 to .99 for a pair of experienced psychiatrists) and a significant correlation score on another drug-induced extrapyramidal symptoms scale (14).

All diagnoses and interviews for the scales were done by experienced psychiatrists who had been treating the subjects. All the clinical evaluations were performed on the same day for each patient. One hundred and one outpatients participated in this study, but two did not complete the Schizophrenia Quality of Life Scale so data from 99 were analyzed.

Pearson product-moment correlations were calculated to examine the relationships between the Schizophrenia Quality of Life Scale and the Quality of Life Scale, and the relationships among clinical variables (Duration of Illness, Number of Hospitalizations, Dose of Neuroleptics, the Brief Psychiatric Rating Scale Positive Symptoms score, the Brief Psychiatric Rating Scale Negative Symptoms score, the Drug-induced Extrapyramidal Symptoms Scale score, and the Calgary Depression Scale for Schizophrenia score). The scores of the Schizophrenia Quality of Life Scale scales and the scores of the Quality of Life Scale total score and subscales were chosen as dependent variables. Forward stepwise regression analyses were performed to specify which clinical variables were the best predictors of each dependent variable. Statistical analyses were done using SPSS 11.5 J statistical software (28). A p value $< .05$ indicated statistical significance.

Results

Sample characteristics and means and standard deviations of the clinical indices are presented in Table 1.

TABLE 1
Sample Characteristics and Clinical Evaluations ($N = 99$)

Subjects		Age (yr.)		Duration of Illness (yr.)		No. of Hospitalizations		Dose of Neuroleptics (mg/day)*	
Men	Women	*M*	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*
51	48	38.5	12.4	12.3	8.7	1.4	1.5	519.7	531.0

Brief Psychiatric Rating Scale (Symptoms)						DIEPSS		CDSS	
Total		Positive		Negative					
M	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*
34.7	10.1	9.2	4.1	9.0	2.9	0.6	0.6	3.6	3.8

Schizophrenia Quality of Life Scale					
Psychosocial		Motivation and Energy		Symptoms and Side-effects	
M	*SD*	*M*	*SD*	*M*	*SD*
40.3	19.2	48.2	17.1	24.6	16.7

Quality of Life Scale									
Total		Interpersonal Relations		Instrumental Role		Intrapsychic Foundations		Common Objects and Activities	
M	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*
58.2	26.0	19.2	11.1	10.7	6.5	21.2	8.5	7.0	2.6

Note.—DIEPSS = Drug-Induced Extrapyramidal Symptoms Scale; CDSS = Calgary Depression Scale for Schizophrenia. *Dose of neuroleptics is chlorpromazine equivalence.

The correlations between the scores on scales of the Schizophrenia Quality of Life Scale and the Quality of Life Scale total score and subscale scores are shown in Table 2. Scores of the Motivation and Energy subscale correlated significantly with the Quality of Life Scale total scores ($r = -.40$, $p < .001$), Interpersonal Relations ($r = -.42$, $p < .001$), Instrumental Role ($r = -.28$, $p = .005$), Intrapsychic Foundations ($r = -.39$, $p < .001$), and Common Objects and Activities ($r = -.25$, $p = .01$) subscales. The scores of the Psychosocial scale gave a significant but weak correlation with the Quality of Life Scale total score ($r = -.20$, $p = .05$), however, there was no significant correlation between scores on the Psychosocial scale and the Quality of Life subscales. The score of the Symptoms and Side-effects subscales did not correlate significantly with the Quality of Life Scale scores.

TABLE 2
PEARSON CORRELATION COEFFICIENTS BETWEEN SCHIZOPHRENIA QUALITY OF LIFE SCALE AND QUALITY OF LIFE SCALE ($N = 99$)

Quality of Life Scale	Schizophrenia Quality of Life Scale		
	Psychosocial	Motivation and Energy	Symptoms and Side-effects
Total	–.20*	–.40‡	–.16
Interpersonal Relations	–.19	–.42‡	–.16
Instrumental Role	–.19	–.28†	–.14
Intrapsychic Foundations	–.19	–.39‡	–.14
Common Objects and Activities	–.10	–.25*	–.14

*$p < .05$. †$p < .01$. ‡$p < .001$.

Pearson correlations among the seven clinical variables are shown in Table 3. The Brief Psychiatric Rating Scale Positive Symptoms scores were significantly correlated with Number of Hospitalizations ($r = .33$, $p = .001$), Dose of Neuroleptics ($r = .43$, $p < .001$), the Brief Psychiatric Rating Scale Negative Symptoms score ($r = .50$, $p < .001$), the Drug-induced Extrapyramidal Symp-

TABLE 3
PEARSON CORRELATION COEFFICIENTS AMONG CLINICAL VARIABLES ($N = 99$)

	Duration of Illness	1	2	3	4	5
1. No. of Hospitalizations	.24*					
2. Dose of Neuroleptics	.11	.43‡				
Brief Psychiatric Rating Scale						
3. Positive Symptoms	.13	.33†	.43‡			
4. Negative Symptoms	–.10	.13	.20*	.50‡		
5. DIEPSS	.06	.18	.17	.29†	.30†	
6. CDSS	–.07	.10	.11	.27†	.27†	.31†

Note.—DIEPSS = Drug-Induced Extrapyramidal Symptoms Scale; CDSS = Calgary Depression Scale for Schizophrenia. *$p < .05$. †$p < .01$. ‡$p < .001$.

toms Scale scores ($r = .29$, $p = .004$), and the Calgary Depression Scale for Schizophrenia scores ($r = .27$, $p = .006$). In addition, the Brief Psychiatric Rating Scale Negative Symptoms scores were significantly correlated with Dose of Neuroleptics ($r = .20$, $p = .05$), the Drug-induced Extrapyramidal Symptoms Scale scores ($r = .30$, $p = .002$), and the Calgary Depression Scale for Schizophrenia scores ($r = .27$, $p = .007$). Moreover, the Calgary Depression Scale for Schizophrenia scores correlated with the Drug-induced Extrapyramidal Symptoms Scale scores ($r = .31$, $p = .002$), and Number of Hospitalizations was significantly correlated with Duration of Illness ($r = .24$, $p = .02$) and Dose of Neuroleptics ($r = .43$, $p < .001$).

Table 4 shows the results of stepwise regression analyses on the Schizophrenia Quality of Life Scale and the Quality of Life Scale. There was no sign of multicollinearity for the analyses. The Psychosocial scale score was predicted independently by the Calgary Depression Scale for Schizophrenia score ($\beta = .58$, $p < .001$), the Brief Psychiatric Rating Scale Positive Symptoms score ($\beta = .42$, $p < .001$), Dose of Neuroleptics ($\beta = -.22$, $p = .007$), and the Brief Psychiatric Rating Scale Negative Symptoms score ($\beta = -.18$, $p = .03$). The Calgary Depression Scale for Schizophrenia score contributed signifi-

TABLE 4
SUMMARY OF STEPWISE REGRESSION ANALYSES ON SCHIZOPHRENIA QUALITY OF LIFE SCALE AND QUALITY OF LIFE SCALE ($N = 99$)

Dependent Variable	Independent Variable	Adjusted R^2	β
Schizophrenia Quality of Life Scale			
Psychosocial	CDSS	.48‡	.58‡
	BPRS Positive Symptoms		.42‡
	Dose of Neuroleptics		−.22†
	BPRS Negative Symptoms		−.18*
Motivation and Energy	CDSS	.23‡	.48‡
Symptoms and Side-effects	BPRS Positive Symptoms	.21‡	.37†
	CDSS		.27†
	Dose of Neuroleptics		−.20*
Quality of Life Scale			
Total	BPRS Negative Symptoms	.46‡	−.53‡
	BPRS Positive Symptoms		−.24†
Interpersonal Relations	BPRS Negative Symptoms	.36‡	−.60‡
	Duration of Illness		−.21*
Instrumental Role	BPRS Negative Symptoms	.28‡	−.33†
	BPRS Positive Symptoms		−.31†
Intrapsychic Foundations	BPRS Negative Symptoms	.53‡	−.59‡
	BPRS Positive Symptoms		−.24†
Common Objects and Activities	BPRS Negative Symptoms	.33‡	−.58‡
	Duration of Illness		−.19*

Note.—CDSS = Calgary Depression Scale for Schizophrenia; BPRS = Brief Psychiatric Rating Scale. *$p < .05$. †$p < .01$. ‡$p < .001$.

cantly to the prediction of the Motivation and Energy scale score ($\beta = .48$, $p < .001$). The Symptoms and Side-effects scale score was predicted independently by the Brief Psychiatric Rating Scale Positive Symptoms score ($\beta = .37$, $p = .001$), the Calgary Depression Scale for Schizophrenia score ($\beta = .27$, $p = .004$), and Dose of Neuroleptics ($\beta = -.20$, $p = .05$). The Quality of Life Scale total score was predicted independently by the Brief Psychiatric Rating Scale Negative Symptoms score ($\beta = -.53$, $p < .001$) and the Brief Psychiatric Rating Scale Positive Symptoms score ($\beta = -.24$, $p = .006$). The Brief Psychiatric Rating Scale Negative Symptoms score ($\beta = -.60$, $p < .001$) and Duration of Illness ($\beta = -.21$, $p = .010$) contributed independently to the prediction of the Interpersonal Relations subscale. The Instrumental Role subscale was predicted independently by the Brief Psychiatric Rating Scale Negative Symptoms score ($\beta = -.33$, $p = .001$) and the Brief Psychiatric Rating Scale Positive Symptoms score ($\beta = -.31$, $p = .002$). The Intrapsychic Foundations was also predicted by the Brief Psychiatric Rating Scale Negative Symptoms score ($\beta = -.59$, $p < .001$) and the Brief Psychiatric Rating Scale Positive Symptoms score ($\beta = -.24$, $p = .003$). The Brief Psychiatric Rating Scale Negative Symptoms score ($\beta = -.58$, $p < .001$) and Duration of Illness ($\beta = -.19$, $p = .027$) contributed independently to the prediction of the Common Objects and Activities subscale.

Discussion

In general, for the relationship between subjective and objective quality of life, the results are consistent with those of Fitzgerald, *et al.* (10). The Psychosocial scale scores of the Schizophrenia Quality of Life Scale did not significantly correlate with any subscale of the Quality of Life Scale, but did with the total score. The Symptoms and Side-effects scores of the Schizophrenia Quality of Life Scale did not significantly correlate with either the total score or any of the subscales of the Quality of Life Scale. On the other hand, the score of the Motivation and Energy scale of the Schizophrenia Quality of Life Scale was significantly correlated with the Quality of Life Scale total scores and all its subscale scores. Since motivation and activity level are generally considered to be related to deficit symptom which is a core part of the Quality of Life Scale, the results may reflect it. These results indicate that there are some significant correlations between these subjective and objective quality of life measures even using schizophrenia-specific quality of life scales. The results did not support the hypothesis. However, that correlation coefficients were not very high indicates that these two measures were not related closely.

In the current study, although there were some significant correlations among the clinical variables, stepwise regression analyses indicated that depressive and positive symptoms were significant and independent predictors

of subjective quality of life. Considering the beta coefficients of the two predictors, it is obvious that the depressive symptom was the most important predictor of subjective quality of life. Kaneda (15) studied the relationship between daily neuroleptic dose and the Schizophrenia Quality of Life Scale scores in 42 male inpatients with schizophrenia. He found that higher doses of antipsychotics were significantly correlated with higher scores on the Psychosocial or Symptoms and Side-effects scales in the Schizophrenia Quality of Life Scale. However, the current study did not support these findings. The reason for the inconsistency is not clear but may reflect differences in the samples. The study sample in Kaneda's study consisted of inpatients on a long-term unit who had been receiving high doses of antipsychotics (an average dose of haloperidol equivalent 16.0 mg/day which is equal to chlorpromazine equivalent 800.0 mg/day). Moreover, being on a long-term inpatient unit might have influenced their subjective quality of life because their Schizophrenia Quality of Life Scale scores were rather higher than those of our subjects.

In the current study, the score for negative symptoms was a significant and independent predictor of the Quality of Life Scale score, and the results support findings of previous studies (10, 25). The score for positive symptoms also predicted the Quality of Life Scale total score and some subscales. However, beta coefficients of the regression analyses indicated that the Brief Psychiatric Rating Scale Negative Symptoms score was the most important predictor of the Quality of Life Scale. Although there was a significant correlation between the Calgary Depression Scale for Schizophrenia score and the Brief Psychiatric Rating Scale Negative Symptoms score in the current study, the correlation was weak ($r = .27$). Therefore, in general, the results indicate that subjective and objective quality of life have different predictors. The results supported the hypothesis.

A limitation of this study is that it is cross-sectional so interpreting regression analyses cannot be based on causality. A longitudinal study will be needed for that.

In conclusion, the current results indicate that subjective quality of life measured by the Schizophrenia Quality of Life Scale and objective quality of life assessed by the Quality of Life Scale do not correlate closely and have different predictors. Data suggest that subjective and objective quality of life should be considered separate and complementary outcome variables. Treatment efforts should be directed towards not only effective control of positive symptoms but also toward depressive and negative symptoms to improve patients' quality of life.

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Interaction between catechol-O-methyltransferase (COMT) Val108/158Met and brain-derived neurotrophic factor (BDNF) Val66Met polymorphisms in age at onset and clinical symptoms in schizophrenia

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Summary Catechol-O-methyltransferase (COMT) gene is one of the candidate genes for schizophrenia because it codes an enzyme that participates in the metabolic inactivation of dopamine and noradrenaline and a limiting factor of dopamine metabolism in the prefrontal cortex. COMT gene lies on chromosome 22q11.2, which has been associated with schizophrenia susceptibility. A single-nucleotide polymorphism of COMT gene at position 108/158 results in an amino acid substitution from valine (val) to methionine (met), which modifies its enzymatic activity and may change the brain morphology and expressional behaviors. On the other hand, brain-derived neurotrophic factor (BDNF) plays a critical role in the development of mesolimbic dopaminergic-related systems. BDNF also contains a functional single-nucleotide polymorphism at codon 66 (Val66Met) of its prodomain and this polymorphism is responsible for schizophrenia susceptibility. In this study, we first investigated the relationship between COMT Val108/158Met polymorphism and age at onset as well as levels of clinical symptoms in 158 of chronic schizophrenia inpatients and then we investigated the gene-by-gene interaction between COMT Val108/158Met polymorphism and BDNF Val66Met polymorphism with age- and sex-matched control subjects ($n = 318$). We concluded that the COMT Val108/158Met polymorphism was not related to either the onset at age or the levels of clinical symptoms after long-term antipsychotic treatment in schizophrenia.

Keywords: Schizophrenia, catechol-O-methyltransferase, brain-derived neurotrophic factor, polymorphism, brief psychiatric rating scale, age at onset

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Introduction

Catechol-O-methyltransferase (COMT) gene has been considered to be one of the candidate genes for schizophrenia because it is an important enzyme that participates in the metabolic inactivation of dopamine and norepinephrine and it lies on chromosome 22q11 which has been associated with schizophrenia susceptibility (Owen et al., 2004). COMT gene contains a functional polymorphism, a single-nucleotide polymorphism at position 108/158 that results in change from valine (val) to methionine (met) and the COMT activity with Val108/158 has one fourth lower than that with Met108/158 (Lachman et al., 1996). In contrast to the striatum, dopamine transporters in the prefrontal cortex are expressed in low abundance and the variation in COMT activity has a neurobiological effect in that region (Bertolino et al., 2004). Some genetic studies have demonstrated the possible correlation between COMT Val158/108Met gene polymorphism and schizophrenia (Shifman et al., 2002; Chen et al., 2004), although some studies have failed to find any correlation (Munafo et al., 2005; Williams et al., 2005). Recently, it is reported that COMT Val108/158Met polymorphism is linked to the morphological changes in schizophrenia (Ohnishi et al., 2006).

Brain-derived neurotrophic factor (BDNF) belongs to the neurotrophic factor family that promotes the development,

regeneration, survival and maintenance of neurons (Maisonpierre et al., 1991). BDNF has also been demonstrated to modulate neurotransmitter syntheses, metabolism and release, postsynaptic ion channel fluxes, neuronal activities and long-term potentiation (Altar et al., 1997). A single-nucleotide polymorphism that results in valine (val) to methionine (met) substituition at codon 66 (Val66Met) in the prodomain of the BDNF gene was reported and 66Met BDNF has been shown to affect intracellular trafficking and activity-dependent secretion of BDNF (Egan et al., 2001). 66Met BDNF homozygotes had smaller hippocampal volume and lower scores in the Wechsler Memory Scale compared with 66Val homozygotes in normal control subjects (Egan et al., 2003). Although small size of hippocampus is reported in schizophrenia (Callicot, 1998), the results of genetic studies on the association between BDNF Val66Met polymorphism and schizophrenia have been controversial (Chen et al., 2006; Rosa et al., 2006). Recently, we reported that schizophrenic patients with Met66 BDNF had earlier onset and this BDNF polymorphism is associated with clinical symptoms in schizophrenia (Numata et al., 2006).

In this study, we performed a case-control study with COMT Val108/158Met polymorphism, and then examined whether any association exists between this polymorphism and clinical symptoms or onset age in schizophrenic patients. We also investigated the gene-by-gene interaction between COMT Val108/158Met polymorphism and BDNF Val66Met polymorphism.

Materials and methods

We used the same DNA samples as our previous study (Numata et al., 2006) from 159 inpatients (115 male and 44 female; mean age: 53.9 ± 12.8 years, mean duration of hospitalization: 12.1 years) with schizophrenia from nine psychiatric hospitals in the neighboring area of Tokushima Prefecture in Japan (population: about 820,000). All patients were Japanese and biologically unrelated. The diagnosis of schizophrenia was made by at least two experienced psychiatrists according to DSM-IV criteria (American Psychiatric Association, 1994). Clinical symptoms and antipsychotic- induced adverse effects were evaluated when blood samples were taken by the Brief Psychiatric Rating Scale (BPRS) scores (Overall and Goham, 1962) and the Drug Induced Extra-Pyramidal Symptoms Scale (DIEPSS) (Inada et al., 2002). The age at first psychotic episode was used as age at onset (mean ± SD: 25.9 ± 8.3 years) by referring to the patient's medical records. Inter- rater reliability for the BPRS and the DIEPSS was $r = 0.81$ ($p < 0.01$). If the first degree relatives of the patient were diagnosed as schizophrenia, we considered the patient as family history plus. In our samples, 72 patients received atypical and 44 patients received typical and others received both types of antipsychotics. Age- and sex-matched controls were selected from volunteers after assessing the psychiatric problems. All control subjects were Japanese, unrelated to each other, and living in Japan. All subjects signed written informed consent to participate in this study approved by the institutional ethics committees.

Genomic DNA was extracted according to standard procedures. Genotyping of COMT Val108/158Met polymorphism was performed with taqman probe according to the manufacture's instruction with ABI 7500 (Applied Biosystems, Tokyo, Japan).

Hardy-Weinberg equilibrium was tested with HWDIAG (Rogatko et al., 2002). Frequency analysis was performed with Fisher's exact test. To evaluate associations between the genotypes and age at onset, Kaplan-Meier analyses were used for survival curves. Spearman correlation coefficients (two-tailed) were used to evaluate whether clinical symptoms of schizophrenia was correlated with Met allele dose-dependency of COMT Val108/158Met polymorphism. Group mean comparisons of the BPRS among genotypic COMT Val108/158Met polymorphism were performed with the Kruskal-Wallis statistic. Multiple linear regression was performed to explore determinants of age at onset. To determine the independent and combined effects of COMT and BDNF genotypes to the BPRS scores in schizophrenia, comparisons between groups were performed by two-way ANOVA followed by multiple comparison testing using the Tamhane correction. The criterion for significance was set at $p < 0.05$ for all of the tests. Data are presented as mean ± standard deviation.

Results

The effect of COMT Val108/158Met polymorphism in schizophrenia

COMT data were available for 158 subjects (114 males and 44 females, genotyping was failed in one subject) and 318 controls (230 males and 88 females). Genotype and allele

Table 1. *Frequency of catechol-O-methltransferase and brain-derived neurotrophic factor genotypes and alleles in patients with schizophrenia and in healthy comparison subjects*

Snp	Group	Genotype			Hardy-Weinberg equilibrium	*p*-Value	Allele		*p*-Value
		Val/Val	Val/Met	Met/Met			Val	Met	
COMT Val106/156Met									
	sch ($n = 158$) (%)	59 (37.3)	85 (53.8)	14 (8.9)	0.092	0.057	203 (64.2)	113 (35.8)	0.16
	cont ($n = 317$) (%)	151 (47.6)	134 (42.3)	32 (10.1)	0.970		436 (68.8)	198 (31.2)	
BDNF Val66Met									
	sch ($n = 159$) (%)	65 (40.9)	68 (42.8)	26 (16.3)	0.528	0.40	198 (62.3)	120 (37.7)	0.16
	cont ($n = 317$) (%)	113 (35.7)	138 (43.5)	66 (20.8)	0.950		364 (57.4)	270 (42.6)	

sch Schizophrenia; *cont* control subjects. Hardy-Weinberg equilibriums are estimated by HWDIAG. *p*-values are calculated by Fisher's exact test.

Table 2. *Genotypes of catechol-O-methl transferase Val108/158Met polymorphism and clinical symptoms of patients with chronic schizophrenia* ($n = 158$)

COMT genotypes	Val/Val	Val/Met	Met/Met
Age	53.3 ± 12.4	53.7 ± 13.4	57.4 ± 10.0
Age at onset	26.3 ± 8.1	25.8 ± 8.9	25.2 ± 4.8
Duration of disease (years)	27.0 ± 12.7	27.9 ± 14.1	32.1 ± 9.1
BPRS-total	40.2 ± 10.0	40.1 ± 9.6	41.8 ± 11.1
BPRS-positive	10.5 ± 4.2	10.1 ± 3.6	11.0 ± 4.6
BPRS-negative	11.4 ± 4.0	11.0 ± 4.3	11.3 ± 3.8
DIEPS	4.6 ± 3.9	4.2 ± 4.4	4.8 ± 2.9
Daily neuroleptic dosage (mg/day)	755.4 ± 534.1	729.2 ± 530.7	594.8 ± 377.4
Duration of hospitalization (years)	14.0 ± 13.5	11.0 ± 11.2	11.4 ± 11.9
Positive first-degree family history ($n = 33$)	48.5%	48.5%	3.0%

BPRS Brief Psychiatric Rating Scales; *DIEPSS* Drug Induced Extra-Pyramidal Symptoms Scale.

distributions of COMT Val108/158Met polymorphism are shown in Table 1. The genotypic distributions did not deviate from the Hardy-Weinberg equilibrium at this polymorphism in both groups. No association between schizophrenia and control subjects was found in genotype or allele frequencies. The mean onset ages were 26.3 ± 8.1 for COMT Val/Val, 25.8 ± 8.9 for COMT Val/Met and 25.2 ± 4.8 for COMT Met/Met. No significant differences were observed among genotypes (log rank statistic: 0.200, $p = 0.904$). No significant sex effect was observed in the effect of the COMT polymorphism on age at onset. The mean BPRS total scores were 40.2 ± 10.0 for COMT Val/Val and 39.9 ± 9.6 for COMT Val/Met, 41.7 ± 11.1 for COMT Met/Met and were not significantly different comparing these three genotypic groups with Kruskal-Wallis comparison ($p = 0.838$). No significant differences were demonstrated in the COMT genotype distributions between patients with positive and negative family history ($p = 0.186$). Neither chlorpromazine-equivalent dose nor the scores of the side effect scale, DIEPSS, showed significant Spearman's rank correlation with Met allele dose-dependency of COMT polymorphism ($p = 0.476$ and $p = 0.689$, respectively). No significant effects of sex or duration of illness were observed in the effect of the COMT polymorphism on BPRS, chlorpromazine-equivalent dose or DIEPSS.

Interaction between COMT Val108/158Met polymorphism and BDNF Val66Met polymorphism

We have previously reported Met66 BDNF homozygotes patients showed significantly earlier age at onset compared to Val66 BDNF homozygotes by the Kaplan-Meier analyses (log rank statistic: 7.51, $p = 0.023$), and that the BDNF polymorphism significantly affects clinical symptoms (Numata et al., 2006). The multiple regression analyses of age at onset were performed as dependent variables. Plausible predictors (sex, education, family history, marriage status and BDNF or COMT polymorphisms) were included in the original models. The final linear regression model included the number of BDNF 66Met alleles ($p = 0.022$) and marriage status ($p < 0.001$) as significant variables influencing age at onset in schizophrenia. COMT polymorphism was eliminated as a significant variable ($p < 0.2$). The dose of Met66 BDNF was weakly but significantly correlated with the onset age (Spearman: $r = 0.162$, $p = 0.042$). On the other hand, the COMT polymorphism was not significantly correlated with the onset age ($p = 0.824$).

We performed two way ANOVA analyses in clinical symptoms because we could not find linear correlation between the BDNF polymorphism and BPRS scores (Numata et al., 2006). By two-way ANOVA followed by multiple comparison testing using the Tamhane correction, BDNF Val66Met polymorphism significantly affects the BPRS in our schizophrenic in-patients samples ($p = 0.040$), however, there was no significant effect seen with COMT Val108/158Met polymorphism to the BPRS ($p = 0.845$). There were no significant effects of DIEPSS or the medication dose in genotypes of either gene.

Discussion

We determined whether any association exists between COMT Val108/158Met polymorphism and clinical variables of schizophrenia and investigated the interaction between COMT Val108/158Met polymorphism and BDNF Val66Met polymorphism. The genotypic frequencies of two polymorphisms of those genes in our sample were almost the same ratio as those of the precedent reports of Japanese samples (Inada et al., 2003; Kunugi et al., 2004).

COMT Val108/158Met polymorphism was not related to either the onset age or the levels of clinical symptoms that remained after long-term antipsychotic treatment in our sample. It has been reported that an association between COMT Met/Met genotype and schizophrenia patients with aggressive behavior as well as suicidal behavior (Nolan et al., 2000; Strous et al., 2003). A possible interaction between low activity COMT and poor response to conventional neuroleptics has been suggested (Illi et al., 2003). However, the lack of association between this COMT polymorphism and clinical variables of schizophre-

nia in this study is consistent with previous reports (Herken et al., 2003; Strous et al., 2006). No significant effects of age at onset or duration of illness were observed in the effect of the COMT polymorphism on the BPRS.

Since COMT knockout mice are known to have increased brain dopamine, especially in the frontal cortex and to show aberrant behavior (Gogos et al., 1998), there may be a distinct effect of the functional single nucleotide polymorphism, COMT Val108/158Met, in human behaviors and diseases. Several studies have revealed that subjects with COMT Met/Met homozygotes performed better than COMT Val/Val homozygotes on executing the Wisconsin Card Sorting Test (WCST), a test associated with prefrontal cortical function (Egan et al., 2001; Malhotra et al., 2002). Ohnishi et al found that this COMT polymorphism is associated with morphological changes in schizophrenia, particularly in the limbic and paralimbic systems (Ohnishi et al., 2006). More extensive studies on the association between the COMT polymorphism and clinical variables are necessary.

Schizophrenia is a complex psychiatric disorder with multiple factors including genetic inheritances. We hypothesized that gene-by-gene interaction might contribute to the different effects of COMT Val108/158Met polymorphism on clinical variables of schizophrenia. We have previously found that the BDNF gene Val66Met polymorphism is related to the onset age of schizophrenia and also influences to the levels of clinical symptoms that are refractory to long-term ordinary antipsychotic treatment in the same sample (Numata et al., 2006). Gourion et al. reported that interaction between BDNF Val66Met and dopamine D3 receptor Ser9Gly polymorphisms was significantly associated with an earlier emergence of psychosis by three years (Gourion et al., 2005). So we investigated the gene by gene interaction between COMT Val108/158Met polymorphism and BDNF Val66Met polymorphism, but the COMT Val108/158Met × BDNF Val66Met genotype interactions were not detected in this study. However, The BDNF Val66Met polymorphism still indicates a weak but significant effect to onset age and the BPRS even after adjusting for the COMT genetic effect. Kaufman et al. reported that children with one or two of Met66 BDNF alleles are vulnerable to environmental stress in depression (Kaufman et al., 2006). We suggest that the schizophrenic patients with Met66 BDNF might also show vulnerability to environmental stress and suffer the disease earlier.

Our study has several limitations. First, the BPRS is a cross sectional rating scale but not a life time scale, although our patients showed little fluctuation in their symptoms at the time of the interview under long-term antipsychotic treatment. Second, all the patients were long-term inpatients and might not represent schizophrenic patients in general. Third, the sample size is relatively small. Larger studies will be needed to confirm these results.

In summary, our finding suggests that, unlike BDNF Val66Met polymorphism, COMT Val108/158Met polymorphism is not related to the onset age of schizophrenia and does not influence to the levels of clinical symptoms that are refractory to long-term ordinary antipsychotic treatment at least in the Japanese population.

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Altered HDAC5 and CREB mRNA expressions in the peripheral leukocytes of major depression

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Abstract

Background: Gene expressions of the peripheral leukocytes in depressive patients might reflect the systemic dysfunction of major depression. We determined mRNA expression levels of Histone deacetylase 5 (HDAC5) gene and cyclic AMP response element-binding protein 1 (CREB) gene in the leukocyte of depressive patients. HDAC5 and CREB are reported to be important targets of antidepressants, the latter being located in the downstream of the former in lymphocyte calcium signaling.
Methods: 25 patients with major depression and 25 age- and sex-matched healthy controls were included in this study. Twenty patients were able to be followed up until the 8 week-treatment. The mRNA levels were determined by a quantitative RT-PCR method.
Result: Levels of *HDAC5* and *CREB* mRNA were significantly higher in drug-free depressive patients than those of controls and the higher mRNA levels decreased to control levels after 8-week paroxetine treatment. There were positive correlation between levels of *HDAC5* and *CREB*.
Conclusion: Our results suggest the alteration of HDAC5 and CREB gene expression in the systemic pathophysiology of major depression.

Keywords: Calcium signaling; CREB; HDAC5; Leukocyte; Major depression; mRNA expression

1. Introduction

Several studies including our own have shown the altered mRNA expressions in the peripheral leukocytes of patients with major depression (Rocca et al., 2002; Iga et al., 2005, 2006). For example, we reported that the level of serotonin transporter mRNA in leukocytes was higher in depressive patients than that of control subjects, and normalized after antidepressant treatment (Iga et al., 2005). Not only neurochemical transmitter systems, such as serotonergic and noradrenergic, but also hormones, cytokines and even structural molecules in the whole body may take part in the occurrence of depressive states (Raison and Miller, 2003; Henn and Vollmayr, 2005). Thus, gene expressions of the peripheral leukocytes in depressive patients may reflect the systemic dysfunction and the response to antidepressants.

Recent progress in molecular pharmacology of antidepressants highlighted the importance of chromatin remodeling in controlling gene expression by regulating the histone acetylation and methylation (Berton and Nestler, 2006; Newton and Duman, 2006). Histone deacetylase 5 (HDAC5) is one of the interesting targets of antidepressants, because it was reported that the down-regulation of HDAC5 by chronic antidepressant treatment was critical for its therapeutic efficacy in the animal model of depression (Tsankova et al., 2006). HDAC5 is also known to be involved in calcium/calmodulin-dependent protein kinase signaling and control of cellular differentiation (McKinsey et al., 2000).

Cyclic AMP response element-binding protein 1 (CREB), which is one of transcriptional targets of the calcium signaling, has been reported to be a molecular marker for the response to antidepressants in neurons (Blendy, 2006). Interestingly, alteration of calcium signaling in the peripheral leukocytes has been reported in major depression (Bohus et al., 1996; Iga et al., 2006; Vollmayr et al., 1995). For example, we reported that the

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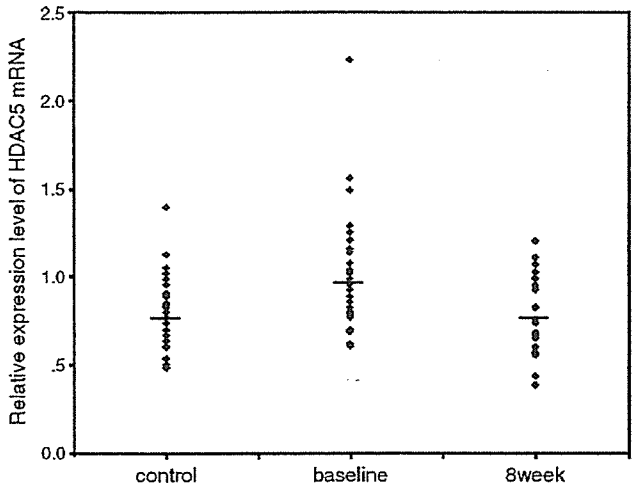

Fig. 1. *HDAC5* mRNA expression levels in the peripheral leukocytes. Bars indicate the mean of the values of each subject (control, age- and sex-matched controls, $N=25$; baseline, patients before treatment, $N=25$; 8-week, Patients after 8-week paroxetine treatment, $N=20$). The *HDAC5* mRNA levels were significantly higher in patients (control; 0.92±0.21, baseline; 0.77±0.16: Student t test $P=0.008$) and were significantly decreased at 8-week compared with those at baseline ($N=20$, baseline; 0.92±0.20, 8-week; 0.76±0.23: paired t test $P=0.031$).

PDLIM5 gene, which is involved in calcium signaling in neurons, showed lower expression in the leukocytes of depressive patients and the expression was increased up to healthy control levels after paroxetine treatment. We suggest that the calcium signaling in the leukocytes may be a useful biological marker of major depression (Iga et al., 2006). Since CREB is downstream of HDAC5 in lymphocyte calcium signaling and both are targets of antidepressants in neurons (Gallo et al., 2006; West et al., 2002), we hypothesize that the levels of *HDAC5* and *CREB* mRNA may be altered in the leukocytes of major depression.

2. Materials and methods

The subjects were 25 patients diagnosed as Major Depressive Disorder according to DSM-IV (APA, 1994) (8 males, 17 females and mean age 41.1±13.1) and 25 age- and sex-matched controls (mean age 41.7±13.3). Before study participation, all subjects signed an informed consent form approved by the Ethical Committee of University of Tokushima School of Medicine. All patients underwent extensive medical, neurological, psychological and laboratory evaluations before participating in the study. The persons who had axis II disorders were removed from the study. The diagnosis and the eligibility of the patients were reconfirmed during follow-up periods. Eighteen patients were in the first and other seven were in the recurrent depressive episode. All patients did not receive any antidepressants for the current episode before blood sampling. We were able to follow up twenty patients treated with paroxetine for 8 weeks. The dose of paroxetine was started with 10 or 20 mg for the first 2 weeks and gradually increased up to 40 mg based on judgment of the trained clinician. At baseline and 8-week, patients were rated with Structured Interview Guide for the 17-item Hamilton Depression Rating Scale (SIGH-D 17, Williams, 1988) before blood collection. Peripheral blood was also collected from 25 sex- and age-matched volunteers who were in good physical health with a history of neither psychiatric nor serious somatic disease and were not taking any medication. Probands who had first-degree relatives with psychiatric disorders were excluded from the control subjects.

2.1. Quantification of blood paroxetine concentration

The paroxetine quantification was performed using high performance liquid chromatography with 4-fluoro-7-nitrobenzo-2-oxa-1, 3-diazole (NBDF)-derivatization, according to the method of Irie et al. (2000) with slight modification in which the separation was performed on a Phenomenex C18 column (4.6×250 mm).

2.2. Quantitative real-time PCR

Total RNA was extracted from the peripheral leukocytes of whole blood samples using the PAXgene Blood RNA kit (Qiagen, Tokyo, Japan) according to the manufacturer's recommendations. Residual genomic DNA was digested with RNase-free DNase I (Qiagen). 2 μg of total RNA was used for cDNA synthesis by random (N6) primers and Quantiscript Reverse Transcriptase (Qiagen, Tokyo, Japan) after assessing RNA quality and quantity with NanoDrop (NanoDrop Technologies, Delaware, USA). For quantitative PCR method, we used commercially available TaqMan probe according to the manufacturer's recommendations (Assay ID: Hs00608366_m1 for HDAC5, Hs01081733_m1 for CREB, Applied Biosystems, CA, USA). We used two control genes (Glyceraldehyde-

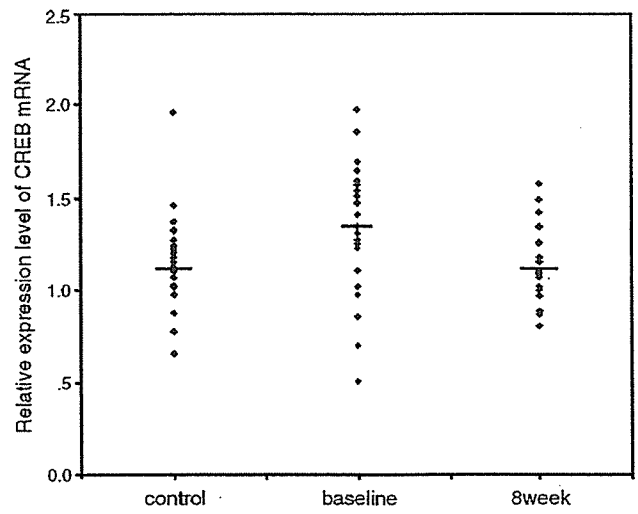

Fig. 2. *CREB* mRNA expression levels in the peripheral leukocytes. Bars indicate the mean of the values of each subject (control, age- and sex-matched controls, $N=25$; baseline, patients before treatment, $N=25$; 8-week, Patients after 8-week paroxetine treatment, $N=20$). The *CREB* mRNA levels were significantly higher in patients. (control; 1.17±0.25, baseline; 1.35±0.35: Student t test, $P=0.034$) and were significantly decreased at 8-week compared with those at baseline ($N=20$, baseline; 1.38±0.35, 8-week; 1.15±0.21, paired t test $P=0.021$).

Table 1
Characteristics of patients

Age/sex[a]	Episode	Age of onset	Hereditary load
25/M (25/M)	Single	25	–
26/F (27/F)	Single	25	–
26/F (29/F)	Single	26	–
26/F (29/F)	Single	25	+
30/F (33/F)	Single	30	–
30/M (30/M)	Single	30	–
32/M (32/M)	Single	32	–
33/M (31/M)	Single	32	–
33/F (36/F)	Single	32	–
37/M (37/M)	Single	37	–
41/F (44/F)	Single	41	–
46/F (48/F)	Single	46	–
49/M (48/M)	Single	49	–
52/F (53/F)	Single	52	–
56/M (61/M)	Single	56	–
60/F (58/F)	Single	60	–
63/M (64/M)	Single	63	–
66/F (68/F)	Single	66	+
28/F (28/F)	Recurrent	16	+
30/F (29/F)	Recurrent	28	–
38/F (33/F)	Recurrent	37	+
43/F (40/F)	Recurrent	42	–
45/F (45/F)	Recurrent	43	–
56/F (58/F)	Recurrent	54	–
57/F (57/F)	Recurrent	53	–

[a]Data of the respective matched control subject presented in parentheses.

3-phosphate dehydrogenase: GAPDH and Hypoxanthine guanine phosphoribosyltransferase 1: HPRT) for normalization of possible fluctuations in quantitative values of the target transcripts (Applied Biosystems). Measurements of each gene expression with Delta CT method were conducted in triplicate.

2.3. Statistical analysis

Statistical calculations were carried out using the SPSS Statistical Software Package 11.5 (SPSS, Tokyo, Japan). Expressional differences between patients and control subjects were calculated using the Mann–Whitney *U* test. The changes before and after treatment were calculated with the Wilcoxon rank sum test. Spearman correlation coefficients were used to evaluate the correlations. All significance levels were two-sided. The criterion for significance was set at $P<0.05$ for all tests. Data are presented as mean±standard deviation.

3. Result

The relative amount of *HDAC5* mRNA in the peripheral leukocytes was standardized with *GAPDH* mRNA. We also used HPRT mRNA as a standard but obtained almost the same results (data not shown).

The *HDAC5* mRNA levels are shown in Fig. 1. The *HDAC5* mRNA levels were significantly higher in patients (Patients; 0.92±0.21, Controls; 0.77±0.16: Mann–Whitney *U* test, $P=0.020$) and were significantly decreased at 8-week compared with those at baseline ($N=20$, baseline; 0.92±0.20, 8-week; 0.76±0.23: Wilcoxon rank sum test, $P=0.033$).

The *CREB* mRNA levels are shown in Fig. 2. The *CREB* mRNA levels were significantly higher in patients (Controls; 1.17±0.25, Patients; 1.35±0.35: Mann–Whitney *U* test, $P=0.009$) and were significantly decreased at 8-week compared with those at baseline ($N=20$, baseline; 1.38±0.35, 8-week; 1.15±0.21, Wilcoxon rank sum test, $P=0.028$).

The characteristics of patients are shown in Table 1. No clinical variables (ages, sexes, number of episodes, age of onset, hereditary load or HAM-D scores (21.2±7.3)) showed significant correlations with the *HDAC5* or *CREB* levels before treatment. Interestingly, positive correlation of the mRNA expression was observed between *HDAC5* and *CREB* before treatment ($P=0.007$ $r=0.529$; Spearman correlation test, Fig. 3).

Mean paroxetine doses and concentration at 8 week-treatment were 30.5±8.9 mg/day and 77.3±58.9 ng/ml, respectively. HAM-D scores were significantly improved after

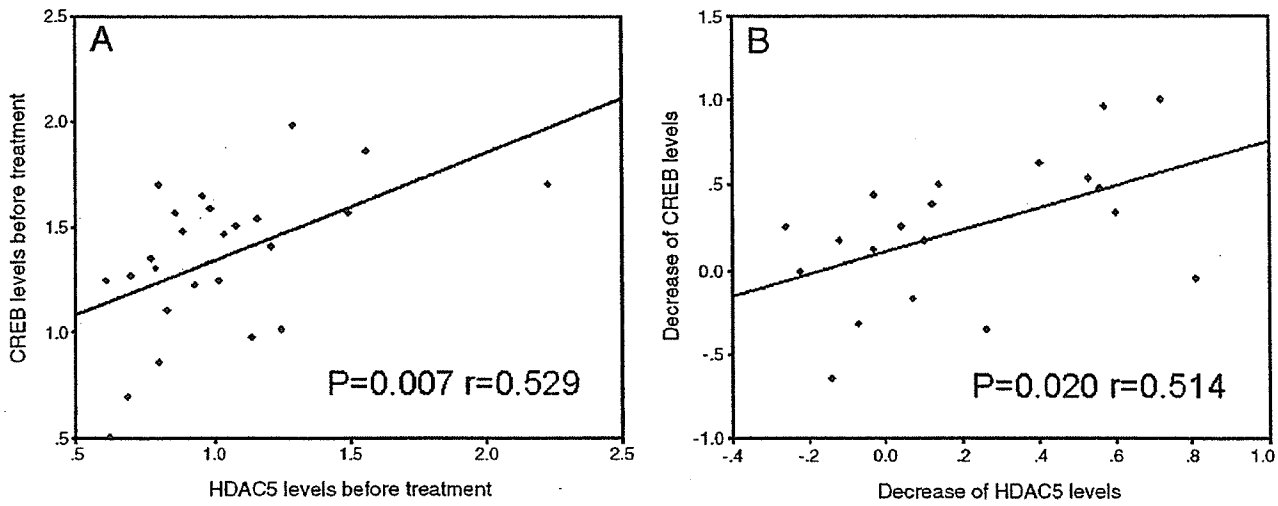

Fig. 3. Correlations between the levels of *HDAC5* mRNA and *CREB* mRNA before treatment (A: $N=25$) and decrease of *HDAC5* and *CREB* during the treatment (B: $N=20$) are shown. Each point represents an individual and the solid lines represent the regression of the correlations. Both axes are expressed as relative expression levels of target genes.

8-week paroxetine treatment (8.4±6.6, $P<0.001$). Neither paroxetine concentrations nor the changes of HAM-D scores showed significant correlations with the changes of the *HDAC5* or *CREB* levels. Positive correlation was also observed between the changes of *HDAC5* and the changes of *CREB* levels during 8 week-treatment $P=0.020$ $r=0.514$; Spearman correlation test, Fig. 3).

4. Discussion

The importance of specific chromatin remodeling is supported by recent articles demonstrating that modification of histone acetylation, by administration of inhibitors or viral-mediated over expression of specific HDACs, alters behavior in models of depression (Cassel et al., 2006; Tsankova et al., 2006). One particularly interesting target is HDAC5 because *HDAC5* mRNA is decreased by antidepressant treatment and when over-expressed was found to block the behavioral effects of antidepressants (Tsankova et al., 2006). It is generally believed that hyperacetylation of histones catalyzed by histone acetyl-transferases (HATs) facilitates gene transcription and the action of HATs is opposed by HDACs. HDACs and HATs are controlled mainly at the level of their recruitment to target promoters, but some evidences suggest that at least *CREB*-binding protein, a type of HAT, may be regulated directly through calcium signaling (West et al., 2002). Because both HDAC5 and CREB are also involved in the lymphocyte calcium signaling and may be associated with histone modification, our finding that their mRNA levels are up-regulated in the leukocytes of depressive patients before treatment may reflect impairments of calcium signaling which lead to the abnormal chromatin remodeling. The higher expression of *CREB* mRNA in antidepressant-free depressive patients is not consistent with a previous study showing no significant difference of CREB mRNA levels in the peripheral lymphocytes of depressive patients (Lai et al., 2003). Some differences in the experimental procedures such as extraction kit (TriZOL vs. Pax gene) or housekeeping gene (beta actin vs. GAPDH and HPRT) may explain this discrepancy. The changes of CREB levels in postmortem brain tissue of major depression are also controversial. For example, Odagaki et al. (2001) reported that the increases in CREB were specifically observed in prefrontal cortex of antidepressant drug-free subjects but not in the antidepressant-treated subjects, while Yamada et al. (2003) reported that CREB was significantly decreased in orbitofrontal cortex of antidepressant-free depressive patients compared to controls.

Down-regulation of *HDAC5* mRNA after paroxetine treatment is consistent with a study showing down-regulation of *Hdac5* mRNA in the hippocampal neurons after chronic antidepressant administration (Tsankova et al., 2006). Our result suggests that the chromatin remodeling induced by the down-regulation of *HDAC5* mRNA is an important mechanism controlling long-term adaptive changes of the antidepressant in not only mice but human tissues. Down-regulation of *CREB* mRNA after paroxetine treatment is also consistent with the Lai's study showing down-regulation of *CREB* mRNA in the lymphocytes of depressive patients after chronic antidepressant administration (Lai et al., 2003). Although many animal studies demonstrate that chronic antidepressants or electroconvulsive seizures increase CREB in hippocampus (Nibuya et al., 1996; Jeon et al., 1997; Thome et al., 2000), it has been reported that inhibition of CREB by viral-mediated over expression of a dominant-negative mutant CREB that blocks CREB function in the nucleus accumbens results in antidepressant-like responses (Newton et al., 2002; Pliakas et al., 2001).

There were positive correlations between mRNA levels of *HDAC5* and *CREB* before treatment as well as between their changes during 8 week-treatment. Because CREB is down-stream of HDAC5 in lymphocyte calcium signaling (Gallo et al., 2006), it is not surprising that the mRNA expression of CREB and HDAC5 showed the same changes to the same treatment. Because there was no correlation between mRNA levels of HDAC5 and CREB in controls ($P=0.679$ $r=0.087$; Spearman correlation test), the positive correlations between mRNA levels of *HDAC5* and *CREB* may be associated with the pathophysiology and treatment of major depression.

Both HDAC5 and CREB may be important factors in the pathophysiology of bipolar disorder (BPD), because valproate, a therapeutic agent for BPD, is known to regulate gene expression by acting as a histone deacetylase inhibitor and CREB has been reported to be involved in the neurotrophic hypothesis of bipolar disorder (reviewed in Zarate et al., 2006). Although it is difficult to point out the similarities and differences between the findings in MDD and BPD, we can make some discussions. Valproate is known to inhibit non-selectively all subtypes of HDACs (Gottlicher, 2004), while antidepressant imipramine selectively inhibit HDAC5 and promote BDNF expression in hippocampus (Tsankova et al., 2006). The different selectivity may be associated with the different therapeutic properties between antidepressant and valproate. Antidepressant treatment may increase CREB levels in temporal cortex of subjects with major depressive disorder, but anticonvulsant mood stabilizing drugs may have opposite effects on cortical CREB levels in patients with bipolar disorder (Stewart et al., 2001).

In conclusion, increased levels of *HDAC5* and *CREB* mRNA in the leukocytes may be a useful biological marker of major depression. Altered expression of these genes may be associated with the systemic pathophysiology of depression and normalization to control levels after treatment may be related to the antidepressant effect. Further clinical and experimental studies are necessary to confirm and extend the present results.

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