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Temperament and character profiles of Japanese university students with depressive episodes and ideas of suicide or self-harm: A PHQ-9 screening study

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Abstract

Objective: The aim of our study was to reveal the personality traits of individuals with major and other depressive episodes among the young adult population. Furthermore, character traits of individuals with ideas of suicide or self-harm were also investigated in this study.
Methods: The subjects of this study were 1421 university students who completed the Patient Health Questionnaire (PHQ-9) and the Temperament and Character Inventory (TCI). The subjects were divided into three separate groups: the major depressive episode group (N = 41), the other depressive episode group (N = 97), and the non-depressive controls (N = 1283). This separation was achieved using the PHQ-9 algorithm diagnosis. We compared the TCI scores using an analysis of variance. Moreover, the Cochran-Armitage trend test was used to determine the diagnosis, ideas of suicide or self-harm, and analysis of *character profiles*.
Results: The major depressive episode group had significantly higher HA ($P < 0.001$), lower RD ($P < 0.001$), lower SD ($P < 0.001$), and lower C ($P < 0.001$) scores than non-depressive controls. The other depressive episode group had significantly higher HA scores ($P < 0.001$) and lower SD scores ($P < 0.001$) than non-depressive controls. The Cochran-Armitage trend test revealed that the prevalence of depressive episodes decreased as the character profiles matured ($\chi^2_{trend} = 57.2$, $P < 0.0001$). The same tendency was observed in individuals who had ideas of suicide or self-harm ($\chi^2_{trend} = 49.3$, $P < 0.0001$).
Conclusion: High HA and low SD scores were common personality traits among young adults with major depressive episodes. Furthermore, the immaturity of *character profiles* was clearly associated with depressive episodes and ideas of suicide or self-harm.

1. Introduction

Kessler et al. reported that the highest risk of initial suicide ideation, planning, and attempts took place during an individual's late teens to early 20s [1]. Accordingly, it is very important to study depression and ideas of suicide or self-harm in young adults to prevent suicide. Several studies have examined the pathogenic and predictive role of personality in depressive symptoms among the young adult population using the Temperament and Character Inventory (TCI) [2–5]. The TCI is a widely used self-rating scale for assessing personality among adult samples. The TCI consists of four dimensions of temperament [i.e., novelty seeking (NS), harm avoidance (HA), reward dependence (RD), and persistence (P)] and three dimensions of character [i.e., self-directedness (SD), cooperativeness (C), and self-transcendence (ST)].

Among the four temperament dimensions, high HA scores were consistently associated with depressive symptoms in both clinical samples and general populations [6]. Recently, Kampman et al. reviewed 12 studies that focused on the relationship between TCI temperament dimensions and depressive symptoms. He concluded that high HA scores were associated with both current depressive symptoms and a depressive trait [6]. Four recent studies, comprised of young adult participants, also demonstrated the correlation

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between depressive symptoms and HA scores [2–5]. In these studies, the Beck Depression Inventory (BDI), Self-Rating Depression Scale (SDS), and the Hospital Anxiety and Depression Scale (HADS) were used to evaluate depressive symptoms. These instruments can be used as continuous measures of depression severity, but they cannot make a criteria-based diagnosis of depression. Therefore, it is difficult to differentiate threshold and sub-threshold depressive episodes, i.e., major and minor depressive episodes, using the BDI, SDS, and HADS. Hence, these previous studies did not properly judge the temperaments of individuals with major depressive episodes among the young adult population. Moreover, the relationship between minor depressive disorder and personality has yet to be reported. Although minor depressive disorder is thought to be a risk factor for developing major depressive disorder [7–9], the personality traits of minor depressive disorder have not been made clear.

Concerning *character profiles*, several previous studies conducted in clinical settings [10,11] have reported that depressive symptoms are associated with character immaturity. This character immaturity was indicated by the presence of low SD and low C scores [12]. In a general adult sample, depressive symptoms are often observed in individuals who have an immature *character profile* [13]. Moreover, a low SD score was suggested as one of the predictors of vulnerability to a future major depressive disorder [14]. Focusing on the young adult population, previous studies reported that SD scores are negatively correlated with depressive symptoms [2–5]. The other character dimensions, C [2–4] and ST [2], are also negatively correlated with depressive symptoms. Cloninger et al. proposed eight *character profiles* based on eight possible configurations of high or low scores of SD, C, and ST [15]. For example, the *character profile* that includes low scores in three different character dimensions will most likely embody a depressive personality. The melancholic *character profile* is the most common in depression [15]. Although the link between character immaturity and depression is anticipated among the young adult population, the prevalence of depressive episodes among young adults who fit one of Cloninger's eight *character profiles* has not yet been reported. Another reason for major depressive episode screening among the general population is the early detection of individuals with a high suicide risk. Few studies, at least among the young adult population, have analyzed the association between personality and ideas of suicide or self-harm. According to clinical studies, individuals who had previously attempted suicide [16,17] and had suicidal thoughts, [16] along with depression, showed high HA scores and low SD scores. Our recent study demonstrated that young adults who completed suicide consistently had high HA scores [18]. The association between ideas of suicide or self-harm and *character profiles* has not yet been studied among the young adult population.

This study aims to verify a number of hypotheses regarding young adults. First, young adults with major depressive episodes have higher HA scores and lower SD scores than did non-depressive controls. Second, young adults with other depressive episodes defined by the PHQ-9 also have higher HA scores and lower SD scores than non-depressive controls. Third, major or other depressive episodes are more often observed in individuals with low SD scores and low C *character profiles* than those with high SD scores and high C *character profiles*. Fourth, ideas of suicide or self-harm are more often observed in individuals with low SD scores and low C *character profiles* than those with high SD scores and high C *character profiles*. To screen for major and other depressive episodes and ideas of suicide or self-harm and to study the relationship between these disorders and personality, we administered the PHQ-9, a self-report questionnaire, and TCI to university students. Though the PHQ-9 requires less than 1 minute for patients to complete, it is as good a screener for major depression as longer instruments in various settings, countries, and populations, and has a validity for measuring its severity [19–21].

2. Methods

2.1. Subjects

The PHQ-9 and TCI were administered to 2117 university students who enrolled in Hokkaido University in April 2010. Both self-rating scales were completed by 1421 students (67.1%). We defined these 1421 students as the "subjects" of our study. According to the PHQ-9 algorithm diagnosis, 41 (2.9%) were classified as having a major depressive episode, 97 (6.8%) were classified as having other depressive episodes, and 1283 (90.3%) were classified as non-depressive controls (NC).

Written informed consent was obtained from all subjects prior to completion of the TCI and PHQ-9. This study was approved by the Ethical Committee of Hokkaido University Graduate School of Medicine and was conducted in accordance with the ethical standards established in the 1964 Declaration of Helsinki (amended in Seoul, October 2008).

2.2. Measures

2.2.1. PHQ-9

The PHQ was developed as a self-report version of the Primary Care Evaluation of Mental Disorders (PRIME-MD). It was designed for the criteria-based diagnosis of several mental disorders commonly observed in primary care [19]. The validity of the depression module (PHQ-9) of the PHQ for screening major depressive episodes was confirmed in primary care, medical outpatient services and specialist medical services [20,22]. Two recent meta-analyses have reliable sensitivity numbers (0.80 and 0.77, respectively) and specificity data (0.92 and 0.94, respectively) for the PHQ-9. These numbers correspond with the DSM-IV diagnosis of major depressive disorder or major depressive episodes in primary care clinics and non-psychiatric clinics [20,22]. In this study, we used the Japanese version of the PHQ-9. The

Japanese version of the PHQ-9 also has excellent validity in primary care and in psychiatric settings [23,24]. A major depressive episode is diagnosed in two ways using the PHQ-9: it is diagnosed by a diagnostic algorithm and a summary score [19]. In this study, we adopted the diagnostic algorithm of the PHQ-9 for screening major and other types of depressive episodes because this algorithm is based on DSM-IV criteria. It is thus suitable for identifying other types of depressive episodes with similar criteria (2 to 4 characteristics) [19]. The diagnostic algorithmic threshold for diagnosing a major depressive episode was considered fulfilled if the answer to question #1a or question #1b and five or more of questions #1a–#1i was at least "more than half the days" (question #1i was counted if present at all). The diagnostic algorithmic threshold for diagnosing other depressive episode was regarded as fulfilled if the answer to question #1a or question #1b and two, three or four of questions #1a–#1i was at least "more than half the days" (question #1i was counted if present at all).

After reviewing the data, when an individual responded that he or she had, "Thoughts that you would be better off dead or of hurting yourself in some way" at least "several days" out of the week, subjects were considered to have "ideas of suicide or self-harm".

2.2.2. TCI

The TCI is a self-report method of personality testing based on a theory proposed by Cloninger [13]. In this study, we used the 125-item Japanese version of the TCI with a 4-point scale. Kijima et al. showed that a 4-point scale was superior to a dichotomous scale in terms of internal consistency, as expressed by Cronbach's α coefficient [25]. This Japanese version of the TCI is a valid and reliable measure of temperament and character for the young adult population [26]. *Character profiles* were created according to Cloninger's methods [27]. We then compared the prevalence of depressive episodes (major and other depressive episodes) and the prevalence of ideas of suicide or self-harm for at least "several days," as defined by the PHQ-9. To form the *character profiles*, the sample was divided into subjects above and below the median for each of the three character traits (i.e., SD, C, and ST) after excluding the 33 participants who were in the middle third of the distribution for all three traits. Then, the participants were grouped according to their character scores to define the eight possible character configurations shown in Table 1. The character profiles are listed in the order that was previously observed to be associated with less happiness and character integration [27]. The variables "s", "c", and "t" denote low SD scores, low C scores, and low ST scores, respectively. The variables "S", "C", and "T" denote high SD scores, high C scores, and high ST scores. Individuals who have sct *character profiles* are described as "melancholic" because they are selfish, immature, and emotionally reactive [15]. Individuals who have scT *character profiles* are designated as "disorganized" because they tend to be illogical, suspicious, and immature [15]. The countertype to the melancholic personality is the creative character (SCT). They are inventive, thoughtful, mature, and frequently feel the positive emotions of joy, love, and hope [15]. The organized character SCt is described as logical, trusting, and mature [15].

2.3. Statistical analyses

Descriptive statistics were given for demographic data and were subjected to chi-square tests and an analysis of variance (ANOVA). For an analysis of TCI scores, a two-way ANOVA was used. Diagnoses and gender were used as two categorical factors for the analyses because gender differences have been reported for the TCI [11,13]. Tukey's HSD test was applied as post-hoc analysis. We then analyzed the association between *character profiles* and depressive episodes or, ideas of suicide or self-harm. To confirm the order of character dimension, a logistic regression analysis was performed. Thereafter, the Cochran-Armitage trend test was performed to analyze the association between character maturity and the prevalence of depressive episodes or ideas of suicide or self-harm. Bonferroni corrections were made, and the differences were

Table 1
Demographic data.

	NC	Other	Major	Statistics	*P*
	N = 1283	N = 97	N = 41		
Gender[a]					
Female	377	36	15	$\chi^2 = 3.398$	0.183
Male	906	61	26		
Age, mean (S.D.)[b]					
Female	18.5 (1.2)	18.4 (0.7)	18.7 (0.9)	$F = 1.008$	0.366
Male	18.6 (1.6)	18.4 (0.6)	19.0 (1.9)	$F = 0.792$	0.453
PHQ-9 total score, mean (S.D.)[b]					
Female	3.2 (2.5)	11.4 (1.4)	17.7 (2.6)	$F = 388.3$	<0.000*
Male	3.1 (2.6)	11.4 (1.4)	18.6 (3.2)	$F = 673.3$	<0.000*

[a] Chi-square test.
[b] ANOVA; NC, non-depressive controls; Other, Other depressive episode; Major, Major depressive episode.
* Statistically significant.

considered significant at $P < 0.001$. SPSS software, version17.0 (SPSS Inc., Japan) and JMP pro10.0 (SAS Institute Inc., Japan) were used for our analysis.

3. Results

3.1. Demographic data

Demographic data were shown in Table 1. The ratio of male to female individuals was not significantly different between groups ($\chi^2 = 3.398$, $P = 0.138$). The ANOVA showed no significant difference in mean age between female groups ($F = 1.008$, $P = 0.366$) and male groups ($F = 0.792$, $P = 0.453$). Meanwhile, as expected, the ANOVA showed significant differences in PHQ-9 total scores between female groups ($F = 388.3$, $P < 0.001$) and male groups ($F = 673.3$, $P < 0.001$).

3.2. TCI scores

Mean TCI scores are shown in Table 2. Concerning interaction effects between gender and diagnosis, we performed a two-way ANOVA test (diagnosis × gender) (Table 3). The results of ANOVA revealed significant effects of diagnosis on HA scores (F[2,1415] = 20.389, $P < 0.001$), RD scores (F[2,1415] = 10.051, $P < 0.001$), SD scores (F[2,1415] = 55.108, $P < 0.001$), and C scores (F[2,1415] = 16.292, $P < 0.001$). Gender was also shown to have an effect on RD scores (F[1,1415] = 14.089, $P < 0.001$). No interaction effect, however, was found between gender and diagnosis when analyzing TCI scores (Table 3). We then performed a post-hoc analysis using the HSD test. No significant difference in TCI scores was found between major depressive episodes and other depressive episode groups. The major depressive episode group had significantly higher HA scores ($P < 0.001$), lower RD scores ($P < 0.001$), lower SD scores ($P < 0.001$), and lower C scores ($P < 0.001$) than did non-depressive controls. The other depressive episode group had significantly higher HA scores ($P < 0.001$) and lower SD scores ($P < 0.001$) than did the non-depressive controls.

3.3. Character profiles of major and other depressive episodes and ideas of suicide or self-harm

A logistic regression analysis revealed that SD had the greatest contribution to major and other depressive episodes, followed by C (Table 4). ST did not contribute to major or other depressive episodes significantly. We compared the prevalence of major and other depressive episodes among four categories of possible combinations, which were sc (sct, scT), sC (sCt, sCT), Sc (Sct, ScT), and SC (SCt, SCT). The major or other depressive episodes were observed frequently (16.1%) in individuals who were depressive (sct) or disorganized (scT). Alternately, these episodes were rarely observed (2.2%) in individuals who were creative (SCT) or organized (SCt) (Table 5). The Cochran-Armitage trend test revealed that the prevalence of depressive episodes increased as SD and C scores lowered, i.e., *character profiles* became immature ($\chi^2_{trend} = 57.2$, $P < 0.0001$). The same tendency was also observed for ideas of suicide or self-harm ($\chi^2_{trend} = 49.3$, $P < 0.0001$) (Table 6).

4. Discussion

In this study, we confirmed that young adults with major and other depressive episodes had higher HA scores and lower SD scores than did non-depressive controls. A significant difference in personality traits between individuals with major and other depressive episodes was not observed. As low SD and low C scores increased in number, the prevalence of major and other depressive episodes, as well as ideas of suicide or self-harm, tended to increase. These results suggest that *character profiles* with high SD and high C scores are protective factors against depressive episodes and ideas of suicide or self-harm among young adults.

Table 2
Comparison of TCI scores among non-depressive controls (NC), other depressive episode group (Other) and major depressive episode group (Major).

	NC (N = 1283)		Other (N = 97)		Major (N = 41)	
	Female (N = 377)	Male (N = 906)	Female (N = 36)	Male (N = 61)	Female (N = 15)	Male (N = 26)
	Mean (S.D.)	Mean (S.D.)	Mean (S.D.)	Mean (S.D.)	Mean (S.D.)	Mean (S.D.)
Temperament						
NS	49.5 (6.7)	49.8 (6.4)	52.3 (8.5)	49.9 (7.0)	50.1 (5.7)	51.7 (6.9)
HA	55.3 (9.4)	55.4 (9.0)	57.7 (11.1)	61.1 (9.9)	63.7 (5.2)	62.5 (7.7)
RD	45.6 (5.6)	43.2 (5.5)	45.5 (5.9)	41.5 (6.2)	41.3 (6.8)	39.4 (8.6)
P	10.8 (2.7)	10.3 (2.8)	9.9 (3.2)	10.2 (2.9)	11.1 (3.9)	9.7 (4.0)
Character						
SD	89.2 (9.0)	86.7 (9.7)	79.5 (11.2)	78.0 (8.8)	77.8 (10.0)	78.3 (12.3)
C	75.4 (7.7)	72.9 (7.8)	74.1 (7.6)	69.4 (8.5)	68.1 (10.0)	66.9 (11.8)
ST	15.1 (6.8)	14.2 (6.9)	17.9 (6.9)	13.2 (8.1)	16.2 (6.8)	13.1 (8.1)

NS; Novelty Seeking, HA; Harm Avoidance, RD; Reward Dependence, P; Persistence, SD; Self-Directedness, C; Cooperativeness, ST; Self-Transcendence.

Table 3
Comparison of TCI scores using a two-way ANOVA.

	Source of variations	F statistic	P	Tukey HSD test		
				Major vs. Other	Major vs. NC	Other vs. NC
NS	Gender	0.033	0.855			
	Diagnosis	2.532	0.080	-	-	-
	Gender × diagnosis	1.869	0.155			
HA	Gender	0.400	0.527			
	Diagnosis	20.389	<0.001*	0.168	<0.001↑*	<0.001↑*
	Gender × diagnosis	1.566	0.209			
RD	Gender	14.089	<0.001*	(Female > Male)		
	Diagnosis	10.051	<0.001*	0.015	<0.001↓*	0.307
	Gender × diagnosis	0.906	0.404			
P	Gender	2.044	0.153			
	Diagnosis	1.105	0.332	-	-	-
	Gender × diagnosis	1.236	0.291			
SD	Gender	0.859	0.354			
	Diagnosis	55.108	<0.001*	0.966	<0.001↓*	<0.001↓*
	Gender × diagnosis	0.517	0.596			
C	Gender	7.349	0.007			
	Diagnosis	16.292	<0.001*	0.029	<0.001↓*	0.006
	Gender × diagnosis	0.940	0.391			
ST	Gender	10.039	0.002			
	Diagnosis	0.644	0.526	-	-	-
	Gender × diagnosis	3.429	0.033			

Major; Major depressive episode group, Other; Other depressive episode group, NC; Non-depressive controls, NS; Novelty Seeking, HA; Harm Avoidance, RD; Reward Dependence, P; Persistence, SD; Self-Directedness, C; Cooperativeness, ST; Self-Transcendence.
↑, higher in major or other depressive groups than in NC.
↓, lower in major or other depressive groups than in NC.
* Statistically significant.

In this study, high HA scores were observed in both the major depressive episode group and other depressive episode group. Harm avoidance is the temperament dimension that corresponds to the inhibition of behavior [28]. Thus, individuals who had high harm avoidance scores tended to have anticipatory worry, fear of uncertainty, shyness, and rapid fatigability [13]. These features of HA may predispose the subjects to depression [28]. High HA scores in major depressive disorder were reported repeatedly in clinical settings [6,29,30]. That being said, temperaments of individuals with minor depressive episodes, which correspond to other depressive episodes defined by the PHQ-9, have not been studied. In our study, HA scores in the other depressive episode group were as high as those in the major depressive episode group. Previous findings showed that high HA scores reflect a depressive trait [14,31–33] and that minor depression is a predictor of major depressive disorder [8]. Consistent with these results, the results of this study indicate that high HA scores are a common temperament trait of major and other depressive episodes.

Table 4
Logistic regression analysis to confirm the effect size of character dimensions.

	SE	Wald	OR	P	95% C.I.
SD	0.011	53.681	1.086	0.000	1.062–1.110
C	0.013	9.264	1.041	0.002	1.014–1.069
ST	0.014	2.978	0.976	0.084	0.949–1.003

SE, Standard error, OR, Odds ratio, C.I., Confidence interval.

Low RD scores were observed only in the major depressive episode group. Previous studies involving young adults reported a correlation between depressive symptoms and low RD scores [2–4]. Kampman and Poutanen suggested that low RD scores in depression may be state-dependent because low RD scores were raised by treatment during clinical trials [6]. Therefore, low RD scores may be a sign of severe and untreated depressive symptoms. In the future, we will conduct a longitudinal study to follow-up the same group of university students and to evaluate whether a low RD score is a result of state effects of depression or a trait, which might lead to depression.

Table 5
Major or other depressive episode and character profiles.

Character profiles			Non-depressive controls	Major or other depressive episode	Total	%
sc	sct	Depressive	396	76	472	16.1%
	scT	Disorganized				
sC	sCt	Dependent	210	37	247	15.0%
	sCT	Moody				
Sc	Sct	Autocratic	252	14	266	5.3%
	ScT	Fanatical				
SC	SCt	Organized	394	9	403	2.2%
	SCT	Creative				

Cochran-Armitage trend test; $\chi^2_{trend} = 57.2$, $P < 0.0001$, $\chi^2_{linearity} = 3.83$, $P = 0.28$.

Table 6
Ideas of suicide or self-harm and character profiles.

Character profiles			No. of subjects without ideas of suicide or self-harm	No. of subjects with ideas of suicide or self-harm	Total	% of ideas of suicide or self-harm
sc	sct	Depressive	406	66	472	14.0%
	scT	Disorganized				
sC	sCt	Dependent	221	26	247	10.5%
	sCT	Moody				
Sc	Sct	Autocratic	253	13	266	4.9%
	ScT	Fanatical				
SC	SCt	Organized	396	7	403	1.7%
	SCT	Creative				

Cochran-Armitage trend test; $\chi^2_{trend} = 49.3$, $P < 0.0001$, $\chi^2_{linearity} = 0.41$, $P = 0.94$.

In this study, Japanese young adults with major or other depressive episodes showed lower SD and C scores. When the *character profiles* of participants had low SD and low C, the prevalence of depressive episodes and ideas of suicide or self-harm was markedly increased. This result suggests that *character profiles* with low SD and/or low C would have a strong impact on the prevalence of depressive episodes and ideas of suicide or self-harm among young adult populations. In previous studies, both low SD scores and low C scores were shown during major depression in clinical settings [14,15]. Conversely, previous studies have also demonstrated that only SD scores in the character dimension were negatively associated with depressive symptoms [5,14,34]. The character dimension of SD measures self-determination and the ability of an individual to control a situation in accordance with their individually chosen goals and values [13]. The character dimension of C measures an individual's social tolerance, empathy, helpfulness, and compassion [13]. In our study, the combination of high SD and C seems to lower the risk of depressive episodes and ideas of suicide or self-harm. Moreover, a recent study on well-being, which is a broader concept of mental health, reported that character has a strong impact on all aspects of health, including social, emotional, and physical well-being [27]. The evaluation of *character profiles* using the TCI is useful for a broad assessment of mental health among young adult populations. This study suggests that a relationship exists between depression and *character profiles*.

Cross-sectional design proved to be a limitation of this study. In the future, longitudinal studies will be needed to analyze the risks and protective factors for major depressive disorders and ideas of suicide or self-harm. The association between *character profiles* and oncoming depression or suicide needs to be studied further. Another limitation of this study was that "other depressive episode" derived from the PHQ-9 includes not only minor depressive episode but also dysthymia and other depressive disorders not otherwise specified in DSM-IV. Although time-consuming, structured clinical interviews are necessary to overcome this limitation; therefore, it is difficult to apply these interviews to a large-scale screening test, such as in this study. Finally, in this study, we evaluated ideas of suicide or self-harm by the item of PHQ-9. However, the relationship between suicide and self-injurious behaviors is reportedly complex in a college population. More than half of the students who had engaged in self-injurious behaviors reported never having considered or attempted suicide [35]. Accordingly, ideas of self-harm are not always related to suicidal ideas. That we could not distinguish between ideas of suicide and those of self-harm is also one limitation in this study.

In conclusion, high HA scores, low RD scores, low SD scores and low C scores were prevalent in young adults who had major depressive episodes. High HA scores and low SD scores were also common in our major and other depressive episode groups. *Character profiles* have a strong impact on the prevalence of major depressive episodes, depressive episodes and ideas of suicide or self-harm.

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Neuropsychiatric Disease and Treatment

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CASE REPORT

Selegiline remarkably improved stage 5 treatment-resistant major depressive disorder: a case report

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Abstract: We report a case in which selegiline, an irreversible monoamine oxidase B (MAO-B) inhibitor, greatly improved depressive symptoms in an adult with stage 5 treatment-resistant major depressive disorder. Four antidepressants and four augmentation therapies had previously been ineffective or intolerable, and electroconvulsive therapy had only a temporary effect. After 20 weeks of treatment with selegiline (10 mg/day), the patient's score on the 17-item Hamilton Depression Rating Scale (HDRS) had decreased from 19 to 4 points. [^{18}F]-Fluorodeoxyglucose positron emission tomography (FDG-PET) showed increased glucose metabolism in the bilateral basal ganglia after initiating selegiline treatment; blood dopamine levels were also increased after selegiline treatment. These results raise the possibility that selegiline enhances dopaminergic neural transmission in treatment-resistant depression, thus leading to an improvement in depressive symptoms.

Keywords: treatment-resistant depression, FDG-PET, glucose metabolism, basal ganglia

Introduction

Transdermal selegiline, an irreversible selective monoamine oxidase B (MAO-B) inhibitor, is approved as an antidepressant in the USA.[1] Some reports have shown that oral selegiline treatment can also be effective in treating depression. For example, oral selegiline was effective for treatment-resistant elderly depressive patients at high doses[2] and exhibited antidepressant effects on severe refractory depression at more typical dosing regimens.[3] On the other hand, one report indicates that low-dose transdermal selegiline treatment, but not oral selegiline treatment, was effective for treatment-resistant depression.[4]

We experienced a patient with severe stage 5 treatment-resistant major depression, which is defined by the persistence of significant or moderate depressive symptoms despite at least two treatment trials with antidepressants from different pharmacological classes and two augmentation therapies, as well as electroconvulsive therapy (ECT), as classified by Thase and Rush.[5] Because selegiline may enhance dopaminergic neural transmission,[6] [^{18}F]-fluorodeoxyglucose positron emission tomography (FDG-PET) of his brain was performed, and blood levels of dopamine, noradrenalin, and homovanillic acid (HVA) were measured. In addition, ß-phenylethylamine (PEA) levels in the patient's urine were measured because selegiline increases PEA levels through MAO-B inhibition. PEA stimulates trace amine-associated receptors (TAAR), which respond to trace amines, p-tyramine, and PEA, but not classic biogenic amines.[7]

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Case description

A 51-year-old man had been diagnosed with major depression 6 years prior to beginning selegiline treatment. During that time, he was treated with imipramine, a tricyclic antidepressant (TCA) (150 mg/day), and his symptoms remitted. However, although he continued medication for 4 years, his depression relapsed. Imipramine was thus replaced with milnacipran, a serotonin and noradrenalin reuptake inhibitor (150 mg/day) and then paroxetine, a selective serotonin reuptake inhibitor (40 mg/day), but those were ineffective, and he was later admitted to our hospital. Concurrent administration of amoxapine, a tetracyclic antidepressant (TeCA) (150 mg/day), lithium carbonate (800 mg/day), and cabergoline (1 mg/kg) improved his symptoms, and he was discharged. His plasma lithium concentration was 0.61 mEq/L. However, he again relapsed into depression 8 months later, despite receiving treatment. His major symptoms included fatigue, loss of motivation, and decline in work efficacy. Subsequently, he was readmitted to our hospital.

During this second hospitalization, amoxapine (200 mg/day), amitriptyline, a TCA (200 mg/day), imipramine (250 mg/day), and clomipramine, a TCA (150 mg/day) were consecutively used for at least 4 weeks, but none were effective. Additional lithium augmentation had no effect, and the addition of olanzapine (5 mg/day) was ineffective. Likewise, pramipexole (2.625 mg/day) induced the side effects of oral dyskinesia and auditory hyperesthesia, while the addition of triiodothyronine was stopped because of palpitations. ECT was administered ten times, and depressive symptoms disappeared transiently. However, the patient's depression relapsed again 1.5 months later, despite the continuation of clomipramine after ECT. Selegiline (2.5 mg/day) treatment was started after he signed the informed consent form. The daily dose of selegiline was increased by 2.5 mg every week up to a maximum of 10 mg/day. Mianserin, a TeCA, (60 mg/day), and valproate (600 mg/day) were also concurrently used, but the dosages of those drugs remained constant during selegiline treatment. Before initiating selegiline treatment, the patient received a score of 19 on the 17-item Hamilton Depression Rating Scale (HDRS). During selegiline treatment, his score on the HDRS decreased to 9 points at week 8 and 4 points at week 20. He returned to work after 49 weeks of selegiline treatment. No side effects from selegiline treatment were either observed or reported. We followed up with the patient approximately 2 years and 5 months after the trial with selegiline. During this period, no relapse occurred.

FDG-PET scans were measured twice, before selegiline treatment and after 20 weeks of selegiline treatment. Two experienced nuclear medicine physicians visually interpreted the images. Twenty weeks after selegiline treatment, selegiline-induced enhancement of glucose metabolism was observed in the bilateral basal ganglia by FDG-PET, while decreases were shown in those in the occipital lobe (Figure 1). The methods used to conduct and analyze the PET scan were as follows: a 10-minute regional static scan was performed with a Siemens ECAT HR+ scanner using the three-dimensional (3D) mode, and the image was reconstructed in the brain mode, using Siemens' ecat software, version 7.2.2 (Siemens AG, Munich, Germany). The images were acquired via 3 minutes of transmission scanning and 10 minutes of emission scanning. The energy window was 350–650 keV. In the brain mode, the acquired 3D sonograms were converted into two-dimensional (2D) sonograms, using Fourier rebinning. The images were reconstructed using the direct inversion Fourier transformation method. We used a Hanning reconstruction filter with a 4 mm full width at half-maximum (FWHM). The reconstruction matrix was 256 × 256, and the field of view was 33 cm in diameter. The FWHM was 6.4 mm after reconstruction.

Moreover, the patient's plasma dopamine levels (<5 to 9 pg/mL) and urine PEA levels (<0.5 to 179.4 ng/mg CRE [creatinine]) were greater after 12 weeks of selegiline treatment compared with baseline, although the plasma HVA and noradrenalin levels were not changed. The MAO-B activity

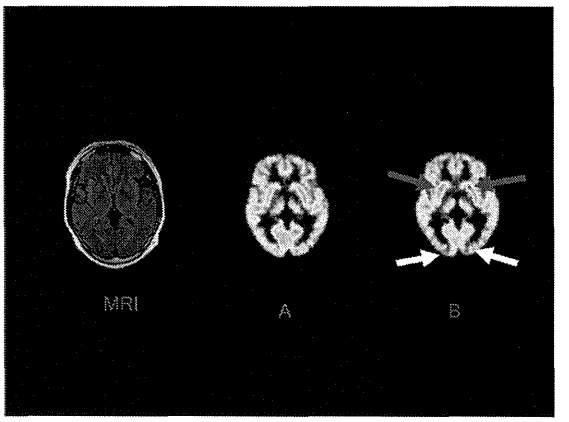

Figure 1 The changes in glucose metabolism using FDG-PET before and after selegiline treatment.
Notes: (**A**) Before selegiline treatment, the patient's 17-item HDRS score was 19 points; (**B**) after 20 weeks of selegiline treatment, a 17-item HDRS score was 4 points. Selegiline treatment increased glucose metabolism in the bilateral basal ganglia (red arrows) and decreased glucose metabolism in the occipital lobe (white arrows).
Abbreviations: FDG-PET, [^{18}F]-fluorodeoxyglucose positron emission tomography; HDRS, Hamilton Depression Rating Scale; MRI, magnetic resonance imaging.

of blood platelets was nearly completely inhibited by administering selegiline 10 mg/day.

Discussion

In the history of this case, various antidepressants were tried, ultimately without success. Lithium and olanzapine augmentation therapies, for example, were ineffective. The addition of a dopamine agonist or triiodothyronine had been intolerable, and ECT showed only transient effects. In short, this case was considered to be a stage 5 treatment-resistant major depressive disorder, as classified by Thase and Rush.[5] There have been few studies of stage 5. This is the first report of successful selegiline treatment for a stage 5 treatment-resistant major depressive patient and of the FDG-PET time course before and after selegiline.

Increases in the glucose metabolism of the bilateral basal ganglia and decreases in those of the occipital lobe were observed using FDG-PET, after selegiline treatment.

Recently, two FDG-PET studies[8,9] reported different results from ours, showing that repetitive transcranial magnetic stimulation decreased abnormally elevated glucose metabolism in the left temporal cortex and fusiform gyrus, in persons with stage 2 major depressive disorder, which is defined as insufficient improvement despite at least two antidepressant trials[8] and the presence of increased glucose metabolism in subdivisions of the anterior cingulate cortex (Brodmann areas 24 and 32).[9] Differences in the staging of treatment resistance and in the studied treatments between our study and previous studies may account for the inconsistencies in the FDG-PET data. Nevertheless, the changes in glucose metabolism after selegiline treatment found in our study suggest that such changes are involved in the treatment response for persons with stage 5 major depression.

The main pharmacological functions of selegiline are (1) the reinforcement of dopamine transmission by MAO-B inhibition and (2) neuroprotective action.[10] Because dopamine is abundant in the basal ganglia, the increases in glucose metabolism in the basal ganglia may occur via increases in dopamine by MAO-B inhibition after selegiline treatment. Moreover, a previous study has reported that volumetric decreases and regional blood flow decreases in the basal ganglia occur in major depressive disorders.[11] However, there have been no studies about the effect of selegiline on FDG-PET in Parkinson's disease or other diseases. An earlier study has suggested that the antidepressant effects in poststroke depression, in which biogenic amine-containing axons may be interrupted by ischemic lesions, may extend beyond mood symptoms, eg, to motor recovery.[12] In addition, depressive symptoms are common in patients with Parkinson's disease, and improvement in mood, motor symptoms, and cognitive symptoms are found after the treatment of depression.[13] These findings suggest that selegiline may not only improve mood, but also affect motor function. On the other hand, research using an animal model reported that selectively bred helpless rats treated with selegiline were no longer helpless when the tests were repeated.[14] This suggests that selegiline also participates in the learning process.[14] Although the glucose metabolism of the occipital lobe was decreased after selegiline treatment in this case, other antidepressant treatments, cognitive behavior therapy, and venlafaxine reportedly have increased the glucose metabolism of the occipital lobe.[15] Thus, there seems to be a difference between selegiline and the conventional treatment for major depression. It is uncertain whether the decreased glucose metabolism in the occipital lobe in this case is related to the clinical efficacy of selegiline.

In addition to the FDG-PET data, increased plasma dopamine levels were also observed after selegiline treatment. Previously, we reported that a dopamine agonist (pramipexole) was effective for stage 2 treatment-resistant major depression;[16] those results suggested that dopamine neurotransmission might be involved in treatment-resistant depression. Although it is not clear whether blood dopamine levels reflect dopaminergic neural transmission in the central nervous system, this dopaminergic effect may reflect MAO-B inhibition in the whole body, including the brain. Moreover, PEA levels in the urine of our patient were increased due to MAO-B inhibition by selegiline. PEA increases extracellular dopamine levels in the brain[6] and acts at TAAR as an agonist.[7] Together, these results raise the possibility that selegiline enhances dopaminergic neural transmission through one or more mechanisms, contributing to an overall antidepressive effect.

One limitation of this case report must be noted. Because selegiline had been added to mianserin and valproate, we cannot eliminate the possibility that the combined treatment was completely effective.

In conclusion, we reported on the progress of a stage 5 treatment-resistant major depressive patient, for whom selegiline greatly improved depressive symptoms. These results raise the possibility that selegiline produces antidepressant effects by enhancing dopaminergic neural transmission in the basal ganglia.

Disclosure

The authors report no conflicts of interest in this work.

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Research report

The influence of childhood abuse, adult stressful life events and temperaments on depressive symptoms in the nonclinical general adult population

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ABSTRACT

Background: Previous studies have shown the interaction between heredity and childhood stress or life events on the pathogenesis of major depression. We hypothesized that childhood abuse, affective temperaments, and adult stressful life events interact and influence depressive symptoms in the general adult population and tested this hypothesis in this study.
Methods: The 294 participants from the nonclinical general adult population were studied using the following self-administered questionnaire surveys: the Patient Health Questionnaire-9 (PHQ-9), Life Experiences Survey (LES), Temperament Evaluation of the Memphis, Pisa, Paris, and San Diego auto-questionnaire (TEMPS-A), and Child Abuse and Trauma Scale (CATS). The data were analyzed with single and multiple regressions and structural equation modeling (Amos 20.0).
Results: Childhood abuse indirectly predicted the severity of the depressive symptoms through affective temperaments measured by TEMPS-A in the structural equation modeling. Four temperaments – depressive, cyclothymic, irritable, and anxious – directly predicted the severity of depressive symptoms and the negative appraisal of life events during the past year. The negative appraisal of life events during the past year mildly, but significantly, predicted the severity of depressive symptoms.
Limitations: The subjects of this study were nonclinical. The findings might not be generalized to patients with mood disorders.
Conclusions: This study suggests that childhood abuse, especially neglect, indirectly increased depressive symptoms through increased affective temperaments, which, in turn, increase the negative appraisal of stressful life events. An important role of affective temperaments in the effect of childhood abuse and stressful life events on depressive symptoms was suggested.

1. Introduction

Various factors, such as genetic factors, environmental factors, and personality traits, predispose individuals to depressive symptoms or the development of a major depressive disorder (Caspi et al., 2010; Mitsui et al., 2013). The heritability of major depressive disorders is low (37%) compared with schizophrenia (81%) and bipolar disorder (85%) (Bienvenu et al., 2011). Other environmental factors and personality traits likely contribute to the development of major depressive disorder. Adult stressful life events and child abuse are major environmental factors for major depressive disorder (Kendler et al., 1999; Kessler and Magee, 1993; Weich et al., 2009; Wise et al., 2001), and these two factors interact with genetic factors in Gene-by-Environment (G × E) interactions (Caspi et al., 2010, 2003). An epidemiological study showed that *s*-carriers in a repeat length polymorphism in the promoter region of the human serotonin transporter gene (5-*HTTLPR*) exhibited elevated depressive symptoms, diagnosable depression, and suicidality after experiencing adult stressful life events and childhood mistreatment (Caspi et al., 2003). To the best of our knowledge, no

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study has reported an interaction of adult stressful life events and childhood mistreatment with depressive symptoms or a major depressive disorder.

Personality traits, another predisposition factor for depressive symptoms or major depressive disorders, are known to be a risk factor for major depression (Kendler et al., 2004, 1993). Adult stressful life events interact with neuroticism and sex in the etiology of major depression, and the risk of neuroticism affecting illness is greater at high than at low levels of adult stressful life events (Kendler et al., 2004). Because neuroticism is related to the 5-*HTTLPR* polymorphism and *s*-carriers have high levels of neuroticism (Greenberg et al., 2000; Lesch et al., 1996), the interaction between neuroticism and adult stressful life events might be partly explained by the 5-*HTTLPR* G × E interaction (Caspi et al., 2003). Other personality traits shown on tests such as the Temperament and Character Inventory (TCI) and the Temperament Evaluation of Memphis, Pisa, Paris, and San Diego auto-questionnaire version (TEMPS-A) are related to neuroticism and the 5-*HTTLPR* polymorphism (Gonda et al., 2009). The items on the TEMPS-A, formulated on the basis of the diagnostic criteria for affective temperaments, are divided into five groups as follows: the cyclothymic, depressive, irritable and hyperthymic groups based on the subscales representing the Kraepelinian temperaments, and the anxious group, based on the subscale developed secondarily by Akiskal and coworkers (Akiskal et al., 2005; Akiyama et al., 2003). The subscales of the TEMPS-A provided unique profiles of major depressive disorder and bipolar disorder (Matsumoto et al., 2005; Mendlowicz et al., 2005a) and are very useful for clinical practice, especially for bipolar spectrum diagnosis. An interaction of personality traits (temperaments) measured by the TEMPS-A with childhood abuse and adult stressful life events in influencing depressive symptoms has not been reported, however, identifying such an interaction is important for psychological and psychiatric evaluation of the general and clinical population.

We hypothesized that childhood abuse, adult stressful life events (stressful events within the last year) and affective temperaments interact with one another and influence depressive symptoms or the development of major depressive disorder (Fig. 1). Temperaments identified on the TEMPS-A are the putative 'fundamental states' that Kraepelin considered to be enduring subclinical states without or before the florid symptoms of manic-depressive illness (Kraepelin, 1913). For this reason, the factor 'temperaments' was located between two factors, childhood abuse and adult stressful life events, in this schema shown in Fig. 1. In this study, the effect and interaction of these three factors on depressive symptoms in the general adult population was examined, and we plan a further study that will examine these interactions in clinical subjects with mood disorder. The covariance structure analysis was used to analyze this sophisticated interaction model.

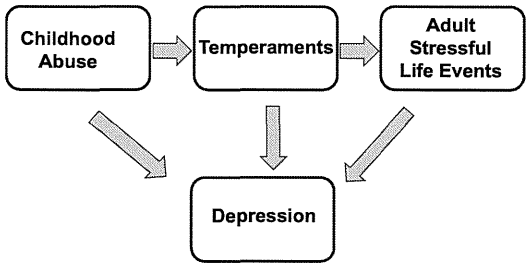

Fig. 1. Structural equation model of the hypothesis of this study. In this model, depressive symptoms are predicted by childhood abuse, temperament and stressful life events in adulthood.

2. Subjects and methods

2.1. Subjects

This research was conducted during July 2011 and December 2011 on 500 Japanese volunteers from the general adult population, who had no history of psychiatric disease. Of 500 volunteers, 294 subjects (58.8%) provided a complete response to the questionnaires. Five questionnaires, which are shown below (Section 2.2), and a questionnaire on demographic data (gender, age, education, marital status, family members, employment status, past history of physical and psychiatric diseases, and family history) were distributed. The completed questionnaires were returned anonymously to the research group by mail for complete confidentiality. Of the 294 subjects, 170 subjects (103 male, 67 female), who did not fulfill the criteria of the Mini-International Neuropsychiatric Interview screen (M.I.N.I screen) or did not fulfill the criteria of major or other depressive episode as screened by the Patient Health Questionnaire-9 (PHQ-9), were classified as 'healthy controls'. Written informed consent was obtained from all of the subjects. This study was performed in accordance with the Declaration of Helsinki and was approved by the institutional review board of Hokkaido University Hospital.

2.2. Questionnaires

2.2.1. Patient health questionnaire-9 (PHQ-9)

The Japanese version of the PHQ-9 was self-completed by the patient in written form (Muramatsu et al., 2007). Major depressive episodes were diagnosed in two ways using the PHQ-9: diagnostic algorithm and a summary score. This study employed the diagnostic algorithmic threshold for diagnosing a major depressive episode that was regarded as fulfilled if the answer to question #1a or question #1b and five or more questions from #1a to #1i was at least "more than half the days" (question #1i was counted if present at all) (Spitzer et al., 1999). The diagnostic algorithmic threshold for diagnosing other depressive episodes was regarded as fulfilled if the answer to question #1a or question #1b and two, three or four of the questions from #1a–#1i was at least "more than half the days" (question #1i was counted if present at all). This study employed a summary score for assessing the severity of depressive symptoms.

2.2.2. Life experiences survey (LES)

The LES is a 57-item self-report measure that allows respondents to indicate events that they have experienced during the past year (Sarason et al., 1978). The format of the LES calls for subjects to rate separately the desirability and effect of the events that they have experienced. They are asked to indicate those events experienced during the past year (0–6 months or 7 months–1 year) as well as (a) whether they viewed the event as being positive or negative and (b) the perceived impact of the particular event on their life at the time of occurrence. Ratings are on a 7-point scale ranging from extremely negative (−3) to extremely positive (+3). Summing the impact ratings of those events designated as positive by the subject provides a *positive change score*. A *negative change score* is derived by summing the impact ratings of those events experienced as negative by the subject.

In this study, the LES was translated from English to Japanese, and Dr. J. H. Johnson, one of the developers of the LES, confirmed the accuracy of this Japanese translation of the LES through back translation. Our previous study confirmed the validity and reliability of the Japanese version of the LES (Nakai et al., 2012) as follows: the negative change score was significantly and positively correlated with depressive symptoms measured by the PHQ-9

scores (Pearson $r=0.21$), state anxiety measured by the State-Trait Anxiety Inventory X (STAI-X) (Pearson $r=0.22$) and trait anxiety measured by the STAI-X (Pearson $r=0.28$). The positive change score was not correlated with depressive symptoms, state anxiety or trait anxiety. These results were consistent with the results of the English version of the LES (Sarason et al., 1978). The test–retest reliability of the LES was confirmed with a moderate intraclass correlation coefficient (ICC) of 0.47 for the positive change score and 0.45 for the negative change score when administered twice within an 8-week period.

2.2.3. Temperament evaluation of the Memphis, Pisa, Paris, and San Diego auto-questionnaire (TEMPS-A)

The TEMPS-A is a self-rating questionnaire consisting of 109 items for men and 110 for women (Akiskal et al., 2005). The subjects completed the Japanese standardized version of the TEMPS-A, which is a true (=2) - false (=1) questionnaire measuring the following temperament dimensions: depressive, cyclothymic, hyperthymic, irritable and anxious (Matsumoto et al., 2005).

2.2.4. Child abuse and trauma scale (CATS)

The CATS is a 38-item scale. Initial findings have demonstrated that this measure has strong internal consistency (Cronbach's *alpha*=0.63–0.90) and test–retest reliability ($r=0.71$–0.91) (Sanders and Becker-Lausen, 1995). The CATS has been shown to correlate significantly with outcome measures such as dissociation, depression, stressful life events and interpersonal difficulties. On each item, participants rate how frequently a particular abusive experience occurred to them during their childhood and adolescence, using a scale of 0–4 (0=never; 4=always). The score for each subscale is the mean score on the items that make up that subscale. There are three subscales, measuring subjective reports of three aspects of adverse childhood experience—neglect/negative home atmosphere, punishment, and sexual abuse.

H. Tanabe, one of the authors, developed and validated the Japanese version of the CATS by the classic translation–back translation technique with the permission and confirmation of Dr. Sanders, the developer of the CATS (Tanabe et al., 2010).

2.2.5. Mini-international neuropsychiatric interview (MINI) screen

The MINI screen version was self-completed by the subjects in written form to screen for the 13 putative major psychiatric disorders (Sheehan et al., 1998).

2.3. Data analysis

According to the hypothesis presented in Fig. 1, we designed a structural equation model, in which depressive symptoms were predicted by childhood abuse, temperaments and adult stressful life events. Two latent variables, childhood abuse and temperaments were composed of three and four observed variables, respectively that were evinced from the original questionnaire subscales. We used AMOS 20.0 (SPSS, Chicago, IL) to perform this path analysis to obtain the direct and indirect effects among all of the variables, and we used maximum likelihood covariance estimation to analyze the model. For the inferential statistical evaluation of structural equation modeling (SEM), we calculated the indices of goodness of fit, such as the Goodness of Fit Index (GFI), Adjusted GFI (AGFI), Comparative Fit Index (CFI), and Root Mean Square Error of Approximation (RMSEA). According to the conventional criteria, GFI greater than 0.90, AGFI greater than 0.85, CFI greater than 0.95, and RMSEA less than 0.08 indicate an acceptable fit; GFI greater than 0.95, AGFI greater than 0.90, CFI greater than 0.97, and RMSEA less than 0.05 indicate a good fit (Schermelleh-Engel et al., 2003). We standardized and indicated

Table 1
Characteristics, PHQ-9, CATS, TEMPS-A, LES and correlation with PHQ-9 or effects on PHQ-9 in 294 general adult subjects.

Characteristics or measures		Value (number or mean ± SD)	Correlation with PHQ-9 (ρ) or effect on PHQ-9 (mean ± SD of PHQ-9 scores, *U*-test)
Age		42.4 ± 11.2	$\rho=-0.10$, n.s.
Gender (male:female)		171:123	male 2.7 ± 3.3 vs female 4.6 ± 4.5** *U*-test)
Education, years		14.9 ± 2.2	$\rho=-0.12$, n.s.
Employment status (employed:unemployed)		241 : 49	employed 3.4 ± 3.9 vs unemployed 3.8 ± 3.9
Homemakers of unemployed persons		43	n.s. (*U*-test)
Marital status	Never Married	45	Married 3.0 ± 3.6 vs Unmarried 5.3 ± 4.5** (*U*-test)
	Married	228	
	Divorce	16	
	Widowed	3	
Living-alone	(Yes:No)	222:62	Yes 4.0 ± 3.4 vs No 3.3 ± 4.1*(U-test)
Number of offspring		1.4 ± 1.0	$\rho=-0.11$, n.s.
Presence of offspring (Yes : No)		209:85	Yes 3.3 ± 3.9 vs No 4.1 ± 3.9, n.s.(*U*-test)
Comorbidity of physical disease (Yes:No)		63:229	Yes 3.5 ± 4.0 vs No 3.5 ± 3.9, n.s.(*U*-test)
1st-degree relative with psychiatric diseases (Yes:No)		40:254	Yes 3.8 ± 4.0 vs No 3.5 ± 3.9, n.s.(*U*-test)
PHQ-9 score		3.5 ± 3.9	
CATS (average score)	Sexual Abuse	0.03 ± 0.12	$\rho=0.03$, n.s.
	Neglect	0.58 ± 0.66	$\rho=0.32$**
	Punishment	1.50 ± 0.64	$\rho=0.06$, n.s.
	Total	0.66 ± 0.47	$\rho=0.25$**
TEMPS-A (average score)	Depressive	1.33 ± 0.17	$\rho=0.40$**
	Cyclothymic	1.17 ± 0.20	$\rho=0.54$**
	Hyperthymic	1.25 ± 0.21	$\rho=-0.04$, n.s.
	Anxious	1.18 ± 0.17	$\rho=0.47$**
	Irritable	1.13 ± 0.16	$\rho=0.43$**
LES (change score)	Negative	2.25 ± 4.28	$\rho=0.29$**
	Positive	2.01 ± 3.39	$\rho=0.08$, n.s.

Data presented as means ± SD or numbers.
ρ=Spearman's rank correlation coefficient.
n.s. not significant.
* $P<0.05$.
** $P<0.01$.

all of the coefficients (with a maximum of 1 and a minimum of −1) for the covariance structure analysis.

We conducted the Mann-Whitney U-test for comparison of the demographic characteristics and the questionnaire data between the two groups. Spearman's rank correlation coefficient and multiple regression analysis were used for correlation between the parameters and the predictive factors.

The statistical analyses were conducted using IBM SPSS AMOS 20.0 (SPSS, Chicago, IL) for the covariance structure analysis, Excel Statistics for Macintosh (Esumi Co, Ltd., Tokyo, Japan) for the multiple regression analysis, Spearman's rank correlation coefficient, and GraphPad Prism 4 (GraphPad Software, La Jolla, CA, USA) for the Mann-Whitney *U*-test.

The differences were considered to be statistically significant at $p < 0.05$.

3. Results

3.1. Demographic characteristics, PHQ-9, CATS, TEMPS and LES of the subjects

The demographic characteristics, PHQ-9, CATS, TEMPS and LES of 294 subjects are presented in Table 1. Gender (female), marital status (unmarried), and living-alone were associated with high PHQ-9 summary scores as determined by the Mann-Whitney *U*-test. Neglect and the total scores of the CATS, the depressive, cyclothymic, anxious, and irritable temperament scores of TEMPS-A, and the negative change scores of LES were significantly correlated with the PHQ-9 summary scores as determined by Spearman's rank correlation coefficients.

3.2. Stepwise multiple regression analysis of the putative explanatory variables on the PHQ-9 suggested from Table 1

The putative explanatory variables that showed significant correlations with the PHQ-9 as determined by Spearman's rank correlation coefficients or had significant effects on the PHQ-9 summary scores as determined by the Mann-Whitney *U*-test in Table 1 were further analyzed by a stepwise multiple-regression analysis.

Table 2 shows the results of a stepwise multiple regression analysis where a PHQ-9 summary score was the dependent factor, and gender (female=2, male=1), marital status (married=2, unmarried=1), living alone (yes=2, no=1), a neglect score on the CATS, depressive, cyclothymic, anxious, and irritable temperament scores on the TEMPS-A, and a negative change score on the LES were independent factors. A total score on the CATS that had a significant correlation with PHQ-9 (Table 1) was excluded from the stepwise multiple regression analysis because it had a high correlation with a neglect score on the CATS ($\rho=0.84$). When entering these independent factors in a stepwise multiple regression analysis, a neglect score on the CATS, cyclothymic and anxious scores on the TEMPS-A, and a negative change score on the LES were significant predictors of PHQ-9 ($F=43.7$, $p < 0.0001$, adjusted $R^2=0.38$), whereas other factors were excluded from the model. Multicollinearity was denied in this multiple regression analysis.

Table 2
The results of a stepwise multiple regression analysis of PHQ-9.

Positive variables selected	Beta	*p*
Cyclothymic score of TEMPS-A	0.29	< 0.0001
Anxious score of TEMPS-A	0.18	0.0069
Neglect score of CATS	0.22	< 0.0001
Negative change score of LES	0.16	0.0016
Adjusted R^2	0.38	< 0.0001

Beta=standardized partial regression coefficient.
Dependent factor, PHQ-9 summary score.
Nine independent factors: gender (female=2, male=1), marital status (married=2, unmarried=1), living-alone (yes=2, no=1), neglect and total scores of CATS, depressive, cyclothymic, anxious, and irritable temperament scores of TEMPS-A, and a negative change score of LES. A total score of CATS, that had a significant correlation with PHQ-9 (Table 1), was excluded from the stepwise multiple regression analysis because it had a high correlation with a neglect score of CATS ($\rho=0.84$).

3.3. Correlation between the CATS subscale scores and the temperament scores on the TEMPS-A

As shown in Table 3, four temperament scores (excluding the hyperthymic temperament score) were significantly and positively correlated with the neglect and punishment subscale scores. The sexual abuse subscale score was significantly and positively correlated with the cyclothymic and anxious temperament scores.

Multiple regression analysis was performed to identify the independent predictors of the CATS subscales for each affective temperament on the TEMPS-A. Table 4 shows the results of the multiple regression analysis where each temperament score was the dependent factor, and the neglect, punishment, and sexual abuse subscale scores on the CATS were the independent factors. The hyperthymic temperament score was not analyzed because Spearman's rank correlation coefficients showed no correlation between the hyperthymic temperament score and the three subscale scores of the CATS. Only the neglect subscale score was a significant predictor of four temperament scores (Table 4).

Table 3
Correlation (ρ) between CATS subscales and temperament scores of TEMPS-A.

	neg	pun	sex	dep	cyc	hyp	anx	irr
neg	1.00	0.34**	0.23**	0.30**	0.32**	−0.01	0.38**	0.39**
pun		1.00	0.14*	0.17**	0.17**	−0.09	0.16**	0.20**
sex			1.00	0.08	0.12*	−0.09	0.15*	0.09
dep				1.00	0.47**	−0.22**	0.56**	0.37**
cyc					1.00	0.19**	0.61**	0.60**
hyp						1.00	0.01	0.26**
anx							1.00	0.53**
irr								1.00

ρ=Spearman's rank correlation coefficient,
neg, neglect subscale; pun, punishment subscale; sex, sexual abuse subscale; dep, depressive temperament; cyc, cyclothymic temperament; hyp, hyperthymic temperament; anx, anxious temperament; irr, irritable temperament.
* $P < 0.05$.
** $P < 0.01$.

Table 4
Multiple regression analysis of each temperament score of TEMPS-A.

Independent factor	Dependent factor			
	Depressive	Cyclothymic	Anxious	Irritable
Neglect subscale	0.31**	0.33**	0.43**	0.37**
Punishment subscale	0.06	0.03	−0.003	0.03
Sexual abuse subscale	0.03	0.003	0.06	−0.05
ANOVA	$F=14.2$**	$F=13.0$**	$F=24.3$**	$F=15.3$**
adjusted R^2	0.12	0.11	0.19	0.13

Figures except for *F* values and adjusted R^2 present beta values (standardized partial regression coefficients).
Hyperthymic temperament was not analyzed because it was not correlated with any CATS subscale score (Table 3).
** $p < 0.01$.

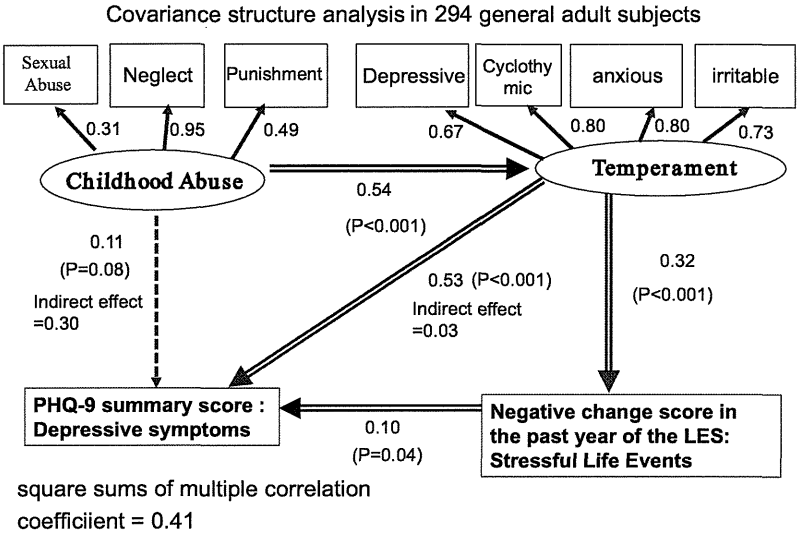

Fig. 2. The results of the covariance structure analysis in the structural equation model with childhood abuse (CATS), affective temperaments (TEMPS-A), adult stressful life events (LES), and depressive symptoms (PHQ-9): Results of 294 subjects from the nonclinical general adult population. Rectangles indicate the observed variables associated with the latent variables, which are shown as ovals. The arrows with double lines represent the statistically significant paths, and the broken lines show the non-significant paths. The numbers beside the arrows show the standardized path coefficients (minimum −1, maximum 1). The *P* values reveal the levels of statistical significance. Indirect effects indicate the effect mediated by the other variables.

3.4. Analysis of the structural equation modeling

To examine the causality of all of the variables, we built a structure equation model based on the results of the above correlation analysis and multiple regression analysis (Fig. 2). The results of the path coefficients calculated by AMOS are shown in Fig. 2.

A good fit of the model was obtained as follows: RMSEA= 0.078, GFI=0.951, AGFI=0.904, CFI=0.950. Only the path coefficient (0.11) of childhood abuse to the PHQ-9 summary score (depressive symptoms) was not significant ($p=0.08$). The other path coefficients were substantially significant ($p < 0.001$) except for that relating the LES to the PHQ-9 summary score (depressive symptoms) ($p < 0.05$). According to the structural equation modeling and consistent with the results of the multiple regression analysis (Table 4), four temperament scores on the TEMPS-A were significantly predicted by the subscales of the CATS. Hyperthymic temperament was excluded from the observed variables of the latent variable "temperament" because hyperthymic temperament was not correlated with the PHQ-9 summary score (Table 1) nor correlated with any subscales of the CATS (Table 3). A neglect subscale score showed a very high-standardized coefficient with the latent variable "childhood abuse". The PHQ-9 summary score was significantly predicted by four temperament scores on the TEMPS-A and a negative change score on the LES. The effect of the CATS subscales on the PHQ-9 summary score was indirect and mediated by the effect of the CATS subscales on four temperament scores on the TEMPS-A (indirect path coefficient=0.30). Four temperament scores on the TEMPS-A significantly predicted a negative change score on the LES, which, in turn, predicted the PHQ-9 summary score.

3.5. Comparison between the subjects with a major depressive episode and healthy subjects

The 294 subjects in this study did not have a history of psychiatric diseases, as indicated in the Subjects and methods section. The results of this study are associated with depressive symptoms in the general adult population, not with depressive symptoms of a major depressive episode. The PHQ-9, which could screen a major depressive episode, as mentioned in the Subjects and methods section (Furukawa, 2010), enables comparison between the subjects with a major depressive episode and the healthy subjects in terms of childhood abuse, negative stressful life events and temperaments. The diagnostic algorithmic threshold of the PHQ-9 for diagnosing a major depressive episode found 7 subjects (1 male, 6 female) with a major depressive episode, although these diagnoses were tentative because these subjects did not have psychiatric interviews. Because of the large gender imbalance, six female subjects with a major depressive episode were compared with 67 healthy female subjects (Table 5).

As shown in Table 5, the female subjects with a major depressive episode showed significantly higher scores on the PHQ-9, the neglect and total scores on the CATS, depressive, cyclothymic, anxious, and irritable temperaments, and a negative change score on the LES than the healthy female subjects, consistent with the model shown in Fig. 2.

4. Discussion

This study is the first report showing that childhood abuse indirectly predicted the severity of depressive symptoms through the affective temperaments measured by the TEMPS-A in the structural equation modeling of nonclinical general adult population. Four temperaments – depressive, cyclothymic, irritable, and anxious – directly predicted the severity of depressive symptoms and the negative change score on the LES during the past year. The negative change score of the LES during the past year mildly, but significantly, predicted the severity of depressive symptoms. Compared with the effect of the negative change score on the LES, the direct effect of temperament and the indirect effect of childhood abuse were more marked. The validity of this result of the structural equation modeling was supported by the results of the multiple regression analysis based on the clinical demographic characteristics and the questionnaire data that were correlated with the severity of depressive symptoms as follows: a stepwise multiple regression analysis showed that four factors - neglect,

Table 5
Comparison of PHQ-9, CATS, and LES of 67 healthy female subjects and 6 female subjects with a major depressive episode.

Characteristics or measures		Healthy female subjects $n=67$	Female subjects with a major depressive episode ($n=6$)
Age		44.6 ± 11.4	50.0 ± 16.5
PHQ-9		2.2 ± 2.5	16.5 ± 3.2**
CATS (average score)	Sexual abuse	0.03 ± 0.12	0.06 ± 0.12
	Neglect	0.50 ± 0.52	1.64 ± 0.80*
	Punishment	1.57 ± 0.56	2.03 ± 0.42
	Total	0.61 ± 0.35	1.40 ± 0.60**
TEMPS-A (average score)	Depressive	1.34 ± 0.14	1.50 ± 0.16*
	Cyclothymic	1.10 ± 0.10	1.27 ± 0.11**
	Hyperthymic	1.19 ± 0.15	1.17 ± 0.09
	Anxious	1.13 ± 0.13	1.46 ± 0.18**
	Irritable	1.07 ± 0.10	1.29 ± 0.14**
LES (change score)	Negative	1.6 ± 4.0	8.6 ± 7.7**
	Positive	1.5 ± 2.9	1.3 ± 1.3

Data presented as means ± SD.
Two groups were compared by Mann-Whitney *U*-test.
* $P<0.05$.
** $P<0.01$.

cyclothymic and anxious temperaments, and a negative change score on the LES – predicted the severity of depressive symptoms. These factors were statistically significant predictors or factors with high path coefficients in the structural equation modeling.

There has been no study that examined the effect of childhood abuse on the TEMPS-A. Pompili et al. (2009) reported that psychiatric inpatients with a history of childhood abuse showed a higher incidence of the irritable temperament trait than did the non-abused patients. In the multiple regression analysis of this study, only neglect among the childhood abuse subscales significantly predicted high scores of depressive, cyclothymic, irritable, and anxious temperaments in the nonclinical general adult population. The affective temperaments measured by the TEMPS-A are considered antecedents or subsyndromal manifestations of mood disorders. The depressive, cyclothymic, and anxious temperaments scores of mood disorder patients are reportedly higher than those of healthy controls (Matsumoto et al., 2005). In particular, cyclothymic temperament is more evident in bipolar disorders (Mendlowicz et al., 2005a, 2005b) and is the major and important factor in the soft bipolar spectrum (Akiskal and Pinto, 1999; Goto et al., 2011; Takeshima and Oka, 2013). This study suggests the possibility that childhood abuse might increase soft bipolarity in the general adult population, which, in turn, might affect the pathogenesis or clinical outcomes of mood disorders. Several studies have reported that the history of childhood abuse is closely related to the onset, course, and treatment response of mood disorders (Alloy et al., 2006; Caspi et al., 2003; Daruy-Filho et al., 2011; Levitan et al., 1998; Nanni et al., 2012). The results of this study link childhood abuse to affective temperaments in aspects of depressive symptoms. Because this study examined the nonclinical general adult population, which constitutes a limitation of this study, the linkage between childhood abuse and affective temperaments should be studied further in a large sample size of mood disorder patients. Although this study shows the relative importance of neglect compared with sexual abuse and punishment, the results with a different population such as psychiatric patients might show a different contribution from each abuse.

Among the five temperaments of the TEMPS-A, depressive, cyclothymic, irritable, and anxious, but not hyperthymic, temperaments, were significantly and positively correlated with depressive symptoms in single regression analyses. Similar findings have been reported in nonclinical subjects by earlier studies (Iliceto et al., 2011; Rozsa et al., 2008). The multiple regression analyses of this study clarified that only cyclothymic and anxious temperaments significantly predicted depressive symptoms. This finding was supported by higher path coefficients from the latent variable "temperament" to cyclothymic and anxious temperaments in the structural equation modeling. The earlier studies reported only correlations between temperaments and depressive symptoms, and they did not investigate the other factors that influenced temperaments (Iliceto et al., 2011; Rozsa et al., 2008). Our study revealed the direct effects of childhood abuse on temperaments, resulting in increased depressive symptoms.

In the LES, each subject rates separately the desirability and effect of events that they have experienced on a 7-point scale ranging from extremely negative (−3) to extremely positive (+3) (Sarason et al., 1978). A negative change score indicates the subjective severity of stressful life events rather than objective severity; i.e., it indicates a negative appraisal of life events. In the structural equation modeling of this study, four temperaments predicted negative scores on the LES, suggesting that these temperaments (depressive, cyclothymic, irritable, and anxious) might increase vulnerability to stressful life events. There has been only one report that studied the relationship between affective temperaments and stress. Sakai et al. (2005) showed that affective temperament measured by the TEMPS-A influenced interpersonal relationship stressors, i.e., conflicts, more than workload-related stressors and that irritable temperament was associated with the most prominent vulnerability, followed by cyclothymic and anxious temperaments. Their results are consistent with ours.

A positive correlation ($r=0.24$) between depressive symptoms and negative change scores on the LES in nonclinical university students was reported in the original manuscript of the LES (Sarason et al., 1978). In our study, this correlation coefficient between depressive symptoms and negative change scores on the LES showed a similar value ($\rho=0.29$); however, it was unexpectedly much lower than those between depressive symptoms and temperaments in the single regression analyses (Table 1) and obviously lower than those between depressive symptoms and childhood abuse (indirect) or temperament (direct) in the structural equation modeling (Fig. 2). This finding indicates that baseline depressive symptoms in nonclinical adults are influenced more strongly by childhood abuse (indirect effect) and temperament (direct effect) than by negative life events. Because there has been no study that examined the complex interaction between childhood abuse, temperament, and negative life events, our results could not be compared with earlier studies.

This study, using the structural equation model, showed that childhood abuse, especially neglect, increases affective temperament, which, in turn, increases the negative appraisal of stressful life events and increases depressive symptoms. The important role of affective temperament in the effect of childhood abuse and stressful life events on depressive symptoms was suggested. Further studies to investigate the effect of childhood abuse, affective temperaments, and adult stressful life events in mood disorder patients are necessary for further understanding.

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Conflict of interest

The authors report no financial or other relationship that is relevant to the subject of this article.

TI has received honoraria from GlaxoSmithKline, Pfeizer, Astellas, Eli Lilly, Mitsubishi Tanabe Pharma, Mochida Pharmaceutical, Otsuka Pharmaceutical, Meiji Seika Pharma, Asahi Kasei Pharma, Shionogi, Janssen Pharmaceutical, Takeda Pharmaceutical and Yoshitomi Pharmaceutical, has received research/grant support from Otsuka Pharmaceutical, and is a member of the advisory boards of GlaxoSmithKline, Eli Lilly, Mochida Pharmaceutical and Mitsubishi Tanabe Pharma.

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The potential of SLC6A4 gene methylation analysis for the diagnosis and treatment of major depression

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ABSTRACT

We examined the utility of DNA methylation profiles at the CpG island of SLC6A4 (DMS) as a diagnostic biomarker for major depression (MD). In addition, the relationship between DMS and the serotonin transporter gene-linked polymorphic region (5-HTTLPR) allele, the severity of symptoms, number of early adversities, and therapeutic responses to antidepressants were examined. Genomic DNA was extracted from peripheral blood of Japanese healthy controls and patients with MD before and after treatment. DMS was analyzed using a MassARRAY Compact System. The severity of depression was evaluated using the Hamilton Rating Scale for Depression, and early adversity was evaluated using the Early Trauma Inventory. We were unable to distinguish between and healthy controls, or between unmedicated patients and medicated patients using DMS. The 5-HTTLPR allele had no significant effect on DMS. The methylation rates for several CpGs differed significantly after treatment. Notably, the methylation rate of CpG 3 in patients with better therapeutic responses was significantly higher than that in patients with poorer responses. Although further studies examining the function of specific CpG units of SLC6A4 are required, these results suggest that the pre-treatment methylation rate of SLC6A4 is associated with therapeutic responses to antidepressants in unmedicated patients with MD.

1. Introduction

Major depression (MD) is a common illness worldwide and was the second-leading contributor to global disease burden in 2010 (Vos et al., 2012). Since long-lasting emotional and psychomotor disturbances due to MD can induce functional difficulties at work, school, or home, MD is predicted to become the second-leading cause of disability-adjusted life years in 2020 based on the systematic analyses of population health data (Murray and Lopez, 1997). In addition, MD is a leading cause of suicide and is responsible for 1 million suicide-related deaths every year (WHO, 2012). In this context, early diagnosis and intervention are necessary to prevent worsening of MD and MD-related suicide attempts. However, the diagnostic system for MD, which relies on subjective assessment of patient symptoms using, for example, the Diagnostic and Statistical Manual of Mental Disorders Fourth Edition (DSM-IV) or the International Classification of Diseases Tenth Edition (ICD-10) rather than an objective laboratory test, may be associated with misdiagnoses, poor outcomes in the treatment of MD, and development of refractory depression.

To establish objective diagnostic biomarkers for MD, numerous approaches have been undertaken. For example, Carroll et al. (1981) and Carroll (1982) proposed dexamethasone suppression

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test (DST) results as a biomarker for the diagnosis of melancholia based on dysregulation of the hypothalamo–pituitary–adrenal (HPA) axis in MD, but a major drawback of DST was its modest sensitivity. Subsequently, a refined laboratory test, the combined dexamethasone/corticotrophin-releasing hormone (DEX/CRH) test, was developed (Heuser et al., 1994), although the sensitivity for detection of MD was only 80% at best (Mossner et al., 2007).

We recently proposed that the methylation profile of the promoter region of exon I of the brain-derived neurotrophic factor (BDNF) gene could serve as a valuable diagnostic biomarker for MD (Fuchikami et al., 2011). It is well known that the concordance rate for MD in monozygotic twins is almost 40% (Fu et al., 2002; McGuffin et al., 1996; Sullivan et al., 2000). The heritability of MD is lower than that of bipolar disorder or schizophrenia (Goodwin and Jamison, 2007; Moldin and Gottesman, 1997). These epidemiological studies suggest that gene–environment interactions play a pivotal role in the etiology of MD. With regard to this interaction, growing evidence indicates that the regulation of DNA methylation in response to environmental stimuli plays an important role in the development of stress vulnerability, predisposing to MD under stressful situations (McGowan et al., 2009; Weaver et al., 2004; Zhang et al., 2013). Thus, it is plausible that epigenetic factors could be used for the development of a more sophisticated diagnosis system for MD.

The serotonin transporter (5-HTT) is a major target of antidepressants and the activity of 5-HTT is inhibited by different types of antidepressants. In vivo neuroimaging and postmortem histochemical studies have shown decreased 5-HTT binding density mainly in the prefrontal cortex of patients with MD (Stockmeier, 2003). Whereas a recent in vivo neuroimaging study using positron emission tomography (PET) demonstrated increased 5-HTT binding in the thalamus, insula, and striatum of patients with MD (Cannon et al., 2007), another PET study reported lower 5-HTT binding in the midbrain of patients with MD, particularly those who were unmedicated (Parsey et al., 2006). Based on these findings, it is conceivable that altered 5-HTT function in the brain plays an important role in the pathophysiology of depression.

Furthermore, it is well known that the short (*s*) allele of the 5-HTT gene-linked polymorphic region (5-HTTLPR: 43-bp deletion or insertion in the promoter of exon I) is associated with anxiety-related personality traits (Lesch et al., 1996), and that this polymorphism is associated with decreased expression of the 5-HTT gene in the 5-HTTLPR *s* allele (Bradley et al., 2005). The 5-HTTLPR *s* allele has been reported to be associated with an increased risk of developing depression under stress (Karg et al., 2011).

A recent meta-analysis revealed a significant association between 5-HTTLPR and the clinical response to selective serotonin reuptake inhibitor (SSRI) treatment in terms of both the remission rate and response rate in depressed patients. This suggests that 5-HTTLPR could be a predictor of the response to SSRIs (Serretti et al., 2007). Together, these observations indicate that individual differences in the transcriptional activity of 5-HTT might be involved in the pathophysiology of MD and the response to antidepressant treatment.

With regard to the regulation of 5-HTT expression, the methylation rate of the CpG island at the 5′ region of the SLC6A4 gene have been reported to be associated with the levels of 5-HTT mRNA in human lymphoblast cells (Philibert et al., 2007). In addition, Wang and associates (Wang et al., 2012) reported that in vitro methylation in the promoter of exon I of the SLC6A4 gene in a luciferase-reporter construct suppressed its transcriptional activity. Furthermore, the possibility of a joint effect of 5-HTT methylation and 5-HTTLPR *s* allele carriage on the risk for depression was reported recently (Olsson et al., 2010). These findings suggest that the methylation status of the promoter of exon I of the SLC6A4 gene alters transcription of the SLC6A4 gene, and subsequently leads to the occurrence of MD.

It has also been reported that early adversity is associated with increased prevalence, earlier onset (Widom et al., 2007) or severity of symptoms, treatment resistance of MD (Tunnard et al., 2013; Widom et al., 2007), and elevated DNA methylation across the SLC6A4 gene promoter in subjects without MD as well as in those without psychiatric disorders (Beach et al., 2010, 2011). Furthermore, increased DNA methylation of the SLC6A4 gene has been reported to be associated with bullying victimization in childhood (Ouellet-Morin et al., 2013).

In the present study, we assessed the degree of symptoms and early adversity of patients with MD, analyzed DNA methylation rates of the CpG island in the promoter of exon I of the SLC6A4 gene (DMR), and conducted genotyping of 5-HTTLPR using genomic DNA from the peripheral blood of patients with MD or healthy controls.

First, we examined whether the profile of the SLC6A4 gene was an appropriate diagnostic biomarker for MD. Next, we analyzed the effect of genotype on DMR, the relationship between DMR and the severity of symptoms, and the association between DMR and the number of early adversities. Finally, the effect of antidepressant treatment on DMR and the relationship between DMR and response rates was analyzed by comparing data sets from patients with MD before and after antidepressant treatment to evaluate the potential of DMR as a predictor of the treatment response.

2. Materials and methods

2.1. Subjects

Fifty patients with MD and 50 healthy controls participated in this study. Demographic characteristics of the participants are shown in Table 1. All participants were Japanese. All patients were diagnosed by trained psychiatrists according to DSM-IV criteria, on the basis of unstructured interviews, information from medical records, and the use of a structured clinical interview (the Japanese version of the Mini-International Neuropsychiatric Interview) by a research psychiatrist. The criteria for the selection of samples in this study was as follows: (1) median age (21–62 years old), (2) distribution of sex was almost equal among groups, (3) severity of symptoms were moderate so as to be able to give written informed consent, (4) all patients did not have treatment history or previous depressed episodes. The severity of depression was evaluated using the Hamilton Rating Scale for Depression (HAM-D; Hamilton, 1967; Cusin et al., 2009) and early adversity was evaluated using the Early Trauma Inventory Self Report-Short Form (ETISR-SF; Bremner et al., 2000, 2007).

None of the patients had current or past diagnoses of substance-related disorders or physical diagnoses. Healthy controls were recruited by advertisement. They had no current or past psychiatric or physical diagnoses, and they had no first-degree relatives with MD. Blood samples were collected at Hiroshima University Hospital, Hokkaido University Hospital, Oita University Hospital and Showa University Hospital. Medical treatment was initiated in 50 patients; 40 patients were available for a follow-up interview 6 weeks later, at which time additional blood samples were collected.

This study was approved by the respective ethics committees of Hiroshima University School of Medicine, Hokkaido University School of Medicine, Oita University School of Medicine, and Showa University School of Medicine. All subjects received a description of the study and gave written informed consent.