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Disproportionate Reduction of Serotonin Transporter May Predict the Response and Adherence to Antidepressants in Patients with Major Depressive Disorder: A Positron Emission Tomography Study with 4-[18F]-ADAM

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Abstract

Background: Many lines of evidence suggest the role of serotonin transporter (SERT)-mediated reuptake of serotonin in the pathophysiology and treatment of major depressive disorder (MDD). This study aimed to examine whether the pretreatment of SERT binding potential or SERT binding ratio between terminal projection regions relative to the midbrain raphe nuclei was associated with treatment outcomes to SERT-targeted antidepressants.
Methods: We recruited 39 antidepressant-naïve patients with MDD and 39 heathy controls. Positron emission tomography with N,N-dimethyl-2-(2-amino-4-[18F]fluorophenylthio)benzylamine (4-[18F]-ADAM) was used to measure *in vivo* SERT availability prior to antidepressant treatment. The 21-item Hamilton Depression Rating Scale (HDRS) was use to assess the severity of depression from baseline to week 6. All the patients with MDD had HDRS scores of 18 or more.

Results: Pretreatment SERT binding in the thalamus and striatum positively correlated with an early reduction in HDRS scores at week 3. Nonresponders and dropout patients showed a proportionate reduction in SERT binding in the terminal projection regions and midbrain compared to healthy controls. In contrast, a disproportionate reduction in SERT binding in the terminal projection regions relative to midbrain was observed in responders.

Conclusions: The results of this study suggested that a disproportionate reduction in SERT binding between terminal projection regions and midbrain may predict better treatment outcomes in patients with MDD.

Keywords: adherence, antidepressant response, major depressive disorder, positron emission tomography, serotonin transporter

Introduction

Many lines of evidence suggest that serotonin transporter (SERT) plays an important role in the pathophysiology of major depressive disorder (MDD) and the therapeutic actions of SERT-targeted antidepressants (Owens and Nemeroff, 1994; Mann, 2013) that exert their function by blocking the SERT, thereby increasing serotonin (5-hydroxytryptamine [5-HT]) levels in the synapses of serotonergic projection terminals. The involvement of SERT gene (solute carrier family 6 (neurotransmitter transporter), member 4 [*SLC6A4*]) variants such as 5-hydroxytryptamine transporter-linked polymorphic region [5-*HTTLPR*] and *STin2* variable number tandem repeat in antidepressant efficacy has been widely investigated. However, the results are controversial and inconsistent across different ethnic populations (Ng et al., 2013). Furthermore, two meta-analysis studies reported a non-significant correlation between *SLC6A4* variants and antidepressant efficacy (Kato and Serretti, 2010; Niitsu et al., 2013). Epigenetic evidence in peripheral leukocytes has shown a higher methylation rate of the *SLC6A4* gene in drug-free patients with MDD, which may be associated with better therapeutic responses to antidepressants (Domschke et al., 2014; Okada et al., 2014). However, DNA methylation in peripheral blood cells may not be a reflection of that in the brain. Hence, *in vivo* imaging studies are necessary to examine the differences in SERT expression relative to the response to antidepressants.

The majority of SERT imaging studies have shown that patients with MDD have reduced SERT binding in a major depressive episode (MDE; Newberg et al., 2005, 2012; Parsey et al., 2006b; Reimold et al., 2008; Selvaraj et al., 2011; Ho et al., 2013; Gryglewski et al., 2014); however, there are some inconsistent reports of unaltered (Meyer et al., 2004a; Miller et al., 2013) or elevated SERT binding (Reivich et al., 2004; Cannon et al., 2007). Moreover, there are no differences in SERT binding in an euthymic state (Lehto et al., 2008; Hsieh et al., 2010) in patients with MDD compared to healthy controls. These findings suggest that a decrease in SERT binding occurs during the transition from a healthy state to a MDE, which then reverses during the transition from a MDE to the remitted (euthymic) state. Therefore, SERT binding could be a state marker for patients with MDD, and dynamic changes in SERT binding might be associated with pathophysiology of MDD and its treatment response.

Previous studies that examined the antidepressant occupancy of SERT and its relationship to treatment response have reliably shown an 80% striatal occupancy of the SERT after a 4-week treatment with selective serotonin reuptake inhibitors (SSRIs; Meyer et al., 2001, 2004b; Erlandsson et al., 2005; Klein et al., 2006; Parsey et al., 2006c). Nonetheless, these findings cannot explain the individual differences observed in antidepressant efficacy and treatment dropout rate. In addition, association studies of pretreatment brain SERT availability and treatment response have yielded diverse results (Kugaya et al., 2004; Miller et al., 2008; Lanzenberger et al., 2012), although this may be due to the difference in radioligands and treatment durations used. Moreover, these studies merely analyzed patients who had completed the treatment course; they did not compare SERT binding in patients with MDD who withdrew from treatment or in healthy controls.

Kugaya et al. (2004) first used single-photon emission computed tomography (SPECT) and the radioligand [((123)I]beta-CIT (2beta-carbomethoxy-3beta-(4-iodophenyl)tropane) ([^{123}I]-β-CIT) to investigate the association between SERT binding and therapeutic efficacy. They found that a higher pretreatment SERT density in the diencephalon, including the thalamus and hypothalamus, correlated with a better response after a 4-week SSRI treatment. This result suggested that higher pretreatment availability and greater SSRI occupancy of the SERT might predict better treatment response. A later study that used positron emission tomography (PET) with the radioligand *rel*-(6R,10bS)-6-[4-(Methylsulfanyl)phenyl]-1,2,3,5,6,10b-hexahydropyrrolo[2,1-α] isoquinoline ([^{11}C](+)McN5652; Miller et al., 2008) suggested that lower pretreatment SERT binding in depressed patients relative to healthy controls predict 1 year non-remitters. However, this finding was obtained in patients who were treated with a mixture of medications, including monoamine oxidase inhibitors, bupropion, lithium, and/or thyroid hormone supplementation, which may have consequently confounded the results (Miller et al., 2008).

The major drawback of these studies is the use of nonselective SERT radioligands, such as [^{123}I]-β-CIT and [^{11}C](+)McN5652. For example, [^{123}I]-β-CIT has near equal affinity for the SERT and dopamine transporters (Meyer, 2007) and the mixed binding of the SERT and dopamine transporters may mask the true levels of SERT binding. Although [^{11}C](+)McN5652 has higher selectivity for SERT compared with the other monoamine transporters, it has a low ratio of specific binding relative to free and nonspecific binding (Brust et al., 2006). A recent PET study that used the SERT-selective radioligand [(11C) 3-amino-4-(2-dimethylaminomethyl-phenylsulfanyl)benzonitrile ([^{11}C]DASB) reported no association between pretreatment SERT binding levels and the 3-week SSRI treatment response in patients with MDD (Lanzenberger et al., 2012). Hence, it remained to be elucidated whether the levels of SERT in drug-naïve patients are associated with treatment response and premature withdrawal from antidepressant treatment. The SERT binding in axon-projecting areas can be modulated by the tonic firing of serotonergic neurons through 5-hydroxytryptamine (serotonin) receptor 1A [5-HT$_{1A}$] autoreceptors. This has been suggested as a potential mechanism of antidepressant action (Lanzenberger et al., 2012). Therefore, a biomarker to predict SERT-targeted antidepressant efficacy should not be limited to regional SERT binding, but should also measure the interplay of SERT binding in terminal-projection regions and midbrain raphe nuclei.

In the present study, we used PET with the SERT-selective radioligand N,N-dimethyl-2-(2-amino-4-[^18F]fluorophenylthio) benzylamine (4-[^18F]-ADAM) to measure *in vivo* SERT binding in the human brain (Shiue et al., 2003; Huang et al., 2010; Huang et al., 2013). After completing the PET scan, patients with MDD were administered a SERT-targeted antidepressant and a 6-week naturalistic follow-up. The study aimed to examine whether pre-treatment regional SERT binding or terminal projection region/midbrain SERT binding ratios correlated with an early reduction in depressive symptoms. Secondly, it aimed to investigate whether the pretreatment levels of regional SERT binding or SERT binding ratios predicted responders, nonresponders, and dropout patients.

Method

Participants

We used the Chinese version of the modified Schedule of Affective Disorder and the Schizophrenia-Lifetime (SADS-L) to screen psychiatric conditions in all participants (Endicott and Spitzer, 1978; Huang et al., 2004). The inclusion criteria for patients with MDD were as follows: (1) age between 20 to 65 years; (2) meeting MDD according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM-IV-TR); (3) a score of $\geq$18 on the 21-item Hamilton Depression Rating Scale (HDRS) as is indicative of moderate to severe MDD (Hamilton, 1960). The exclusion criteria for patients with MDD were as follows: (1) patients diagnosed with other comorbid Axis I and/or Axis II disorders, with the exception of patients with nicotine dependence; (2) HDRS score of <18, as is indicative of a mild degree of depression; (3) significant physical illness; (4) women who were pregnant or lactating; (5) previous head trauma with loss of consciousness, epilepsy, and/or thyroid disease; (6) previous exposure to psychotropic medication, such as, antidepressants, mood stabilizers, or antipsychotics.

Thirty-nine healthy volunteers were recruited from the community. They were free of past or present major or minor mental illness, as determined by the SADS-L semi-structured interview, and none of the first-degree relatives of the control subjects had a history of psychiatric disorders, substance abuse/dependence, or attempted suicide. Thirty-nine patients with MDD were recruited from Tri-Service General Hospital. PET imaging was arranged after informed consent was obtained. After completing a PET scan, patients with MDD began 6 weeks of treatment with an open-label SERT-targeted antidepressant [either paroxetine (20–40mg), venlafaxine (75–150mg), or duloxetine (30–60mg)] with a prospectively naturalistic follow-up. Some participants were recruited from a previous study (Yeh et al., 2014) and agreed to receive a naturalistic follow-up. The 21-item HDRS was used to assess the severity of depression from baseline to endpoint (week 6); these time points are often considered clinically important for treatment response (Papakostas et al., 2006; Lin et al., 2011; Lanzenberger et al., 2012). A responder was defined as a patient with a reduction $\geq$50% in the HDRS score between baseline and week 6, and a nonresponder was defined as a patient with a reduction <50% in the HDRS score between baseline and week 6. Adherence was defined as the act of filling a new prescription or refilling prescriptions on time, whereas nonadherence was defined as the discontinuation of filling or refilling prescriptions before the 6 week endpoint of treatment. The protocol was approved by the Institutional Review Board for the Protection of Human Subjects at the Tri-Service General Hospital in Taipei, Taiwan (Figure 1).
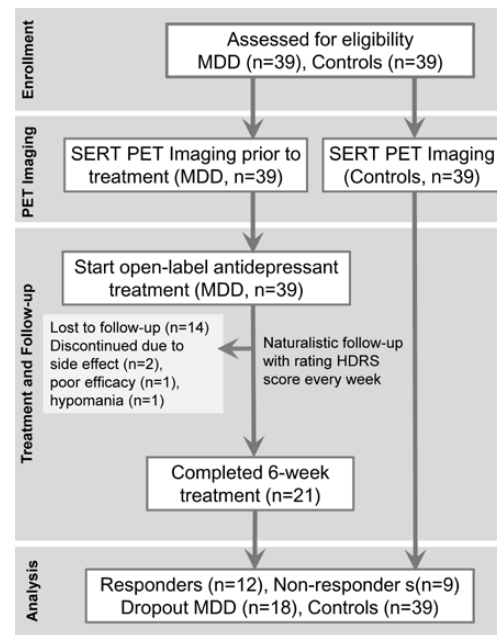

Figure 1. Protocol of positron emission tomography (PET) sudy in healthy controls andantidepressant-naïve subjects with major depressive disorder (MDD). HDRS, Hamilton Depression Rating Scale; SERT, serotonin transporter.

PET Procedure

The preparations for 4-[^18F]-ADAM synthesis were carried out in our PET Current Good Manufacturing Practice laboratory with an automated synthesizer as previously described (Peng et al., 2008); the synthesizer was inspected by the Council of Atomic Energy and the Department of Health, Taiwan. All participants underwent a low-dose computed tomography (CT) scan (130 kVp, 50 mAs, 0.8 s tube rotation, 4mm slice collimation, and pitch 3) and then a static PET scan in three-dimensional mode using a BIOGRAPH PET/CT scanner (Biograph Duo, Siemens). Total PET imaging began at 120 to 140min (total 20min) after intravenous bolus injection of 7.94±1.13 mCi 4-[^18F]-ADAM. This scanner had a transverse field-of-view of 58.5cm, an axial field-of-view of 15.5cm, and a spatial resolution of 4.8mm. PET images were reconstructed in a 512×512×64 matrix with a pixel size of 0.519×0.519×2.4mm using the ordered subset expectation maximization method (six iterations and 16 subsets) with a Gaussian filter of 3mm full-width half maximum.

PET Data Acquisition

PET images were analyzed with commercial PMOD software for Windows (version 3.0, PMOD Technologies Ltd). The PET image from each subject was automatically co-registered with the corresponding individual CT scan and was then manually adjusted by an experienced physician. The individual CT images provide an anatomical reference, and regions of interest (ROIs) were defined on reconstructed and summated PET images. The ROIs were drawn over the midbrain, striatum, thalamus, and prefrontal cortex (PFC), where loci of serotonergic cell bodies (e.g. midbrain) and the main projection regions (e.g. striatum, thalamus, PFC) could be found. We used the cerebellum as a background reference because of its low SERT concentration when compared to other brain regions. To reduce the confounding effects of SERT binding in the cerebellum, we only delineate the posterior half of the cerebellar cortex and exclude the white matter

and vermis. The non-displaceable binding potential (BP_{ND}) of 4-[^{18}F]-ADAM was quantified using a ratio method by comparing a specifically-bound radioligand to a nondisplaceable radioligand in brain tissue at equilibrium as previously described (Huang et al., 2013; Yeh et al., 2014). We applied the time period at 120–140 min for the transient equilibrium ratio model. The BP_{ND} of 4-[^{18}F]-ADAM in the target region was calculated by subtracting the tissue radioligand activity in the nondisplaceable region (C_{CB}) from the tissue radioligand activity in the target region (C_{ROI}), and then dividing the result by the tissue radioligand activity in the nondisplaceable region. The equation was defined as follows: BP_{ND} = binding potential' (BP') = (C_{ROI}- C_{CB})/C_{CB} (Ichise et al., 2001; Innis et al., 2007). The investigator using PMOD software to analyze images was blinded to all participant information.

Statistical Analysis

The continuous variables of the demographic data between groups were analyzed with either a Student's *t*-test or one-way analysis of variance. Nominal differences in the demographic data between groups were examined with the Pearson χ^2 test or Fisher's exact test. BP_{ND} data were analyzed by a linear mixed-effects model (McCulloch et al, 2008), with brain region and diagnostic group as fixed effects and subjects as the random effect (Parsey et al., 2006a, 2006b; Miller et al., 2008; Miller et al., 2013; Yeh et al., 2014). To assess the effects of demographic data, we successively set age, sex, smoking status, and body mass index (BMI) as other fixed effects in the linear mixed-effects models. A Bonferroni correction was used for multiple comparisons in the post hoc analysis. We used Spearman's rank correlation to analyze the relationship between pretreatment regional levels of SERT BP_{ND} and the reduction in the HDRS scores. A *p*-value < 0.05 was considered statistically significant (two-tailed).

A nonparametric analysis was applied in order to examine the differences in the pretreatment levels of regional SERT BP_{ND} and SERT BP_{ND} ratios in the terminal projection area/midbrain raphe nuclei among depressed subgroups and healthy controls. Due to the comparisons of SERT binding in the four ROIs and the three SERT binding ratios among the different groups (controls, responders, nonresponders, and dropouts), a conservative *p*-value of less than 0.05/7 = 0.007 was considered significant for multiple comparisons. We used Hedges' *g* to compute the effect size between two groups. Small, medium, and large effect sizes were defined, respectively, as 0.2 ≦ Hedges' *g* < 0.5, 0.5 ≦ Hedges' *g* < 0.8, and Hedges' *g* ≧ 0.8. Receiver operator characteristic (ROC) curves were obtained and the area under the curve (AUC) was calculated for each regression model. All data were analyzed with SPSS software for Windows (version 17, SPSS).

Results

Demographic and Clinical Characteristics

The characteristics of all of the participants are summarized in Table 1. The age of the controls ranged from 21–62 years [mean ± standard deviation (SD), 32.3 ± 8.3 years; 21 men and 18 women] and the age of the patients with MDD ranged from 20–60 years (mean ± SD, 34.3 ± 11.5 years; 20 men and 19 women). Twenty-one patients with MDD completed the 6-week course of antidepressant treatment, and 18 patients with MDD dropped out (Figure 1). Among the 21 patients with MDD who completed the 6-week treatment course, the response rate was 57.1%. There were no significant differences in age, onset age, sex, BMI,

smoking status, education years, or baseline HDRS scores among the responders, nonresponders, and dropout patients with MDD. A higher number of MDEs were seen in the dropout group than in the completed-treatment group. There were no significant differences in the mean injection dose of 4-[^{18}F]-ADAM among the groups. The types of antidepressants and their mean dosages used in the present study did not significantly differ among the responders, nonresponders, and dropout patients (Supplemental Table 1).

Possible Effects of Demographic Data and Current MDD on Regional SERT Binding

A scatter plot of the BP_{ND} values from the four ROIs in the healthy controls and depressed subgroups is presented in Figure 2. By using linear mixed-effects models with brain region and diagnostic group as the fixed effects and subjects as the random effect, we found a significant effect of brain region on SERT binding (F = 348.953, df = 3,231, p < 0.001). The values of BP_{ND}, in the order of highest to lowest, in the four ROIs was midbrain > thalamus > striatum > PFC. Across the four ROIs, BP_{ND} was significantly different according to the diagnosis of current MDD (F = 11.357, df = 1,76, p = 0.001). A post hoc analysis demonstrated significantly lower BP_{ND} values in the midbrain (t = -3.480, df = 1,152, uncorrected p < 0.001), thalamus (t = -4.204, df = 1,152, uncorrected p < 0.001), and striatum (t = -2.821, df = 1,152, uncorrected p = 0.005) in patients with MDD compared with healthy controls. Furthermore, the effect of current major depression on BP_{ND} remained significant in the midbrain, thalamus, and striatum after Bonferroni corrections for multiple comparisons (Bonferroni-adjusted p = 0.003, <0.001, and 0.022, respectively). To further assess the effects of the demographic data, we successively set age, sex, smoking status, and BMI as other fixed effects in the linear mixed-effects models. Across the four ROIs, there was no effect of age (F = 1.564, df = 1,75, p = 0.215) or sex (F = 1.732, df = 1,75, p = 0.192) on SERT binding in the combined depressed and control groups. BMI (F = 0.884, df = 1,75, p = 0.350) did not significantly influence SERT binding, and there was no effect of cigarette smoking on SERT binding (F = 0.129, df = 1,75, p = 0.720).

Because nicotine dependence might confound the results, we further applied a stratification analysis according to cigarette smoking status. A significant effect of brain region on SERT binding in smokers (F = 52.467 df = 3,60, p < 0.001) and nonsmokers (F = 336.854, df = 3,168, p < 0.001) remained. A significant effect of the diagnosis of current MDD on SERT binding in smokers (F = 7.960, df = 1,19, p = 0.011) and nonsmokers (F = 5.422, df = 1,55, p = 0.024) remained. There was no effect of age, sex, or BMI on SERT binding across the four ROIs in smokers or in nonsmokers (all *p*-values > 0.05).

The Association of Regional SERT BP_{ND} or Projection Area/Midbrain SERT BP_{ND} Ratios with Early Response to Antidepressants

Within the completed treatment group (n = 21), we observed that SERT binding in the thalamus (Spearman's ρ coefficient = 0.518, p = 0.016) and striatum (Spearman's ρ coefficient = 0.438, p = 0.047) positively correlated with a reduction in HDRS scores at week 3 (Figure 3, Supplemental Table 2) but not at the other weeks. We calculated the SERT binding ratio by dividing the SERT BP_{ND} in the terminal projection area by the SERT BP_{ND} in the midbrain. The projecting areas/midbrain SERT BP_{ND} ratios

Table 1. Clinical and demographic characteristics of the participants

	Healthy controls (n = 39)	MDD (n =39)	Healthy controls vs MDD		MDD (n = 39)			Responders vs nonresponders vs dropout MDD	
					Responders (n = 12)	Nonresponders (n = 9)	Dropout MDD (n = 18)		
	%, n	%, n	χ^2 (df = 1)	p	%, n	%, n	%, n	χ^2 (df = 2)	p [a]
Male, % (male/female)	38.9% (21/18)	51.3% (20/19)	0.05	0.82	66.7% (8/4)	55.6% (5/4)	38.9 % (7/11)	2.28	0.33
Smoker, % (smoker/nonsmoker)	12.8% (5/34)	41.0% (16/23)	7.89	**0.005****	50.0% (6/6)	44.4% (4/5)	33.3% (6/12)	0.88	0.70
First episode, % (first/recurrent)	-	56.4% (22/17)	-	-	66.7% (8/4)	77.8% (7/2)	38.9 % (7/11)	4.22	0.13
Antidepressant									
Paroxetine	-	22 (56.4)	-	-	9 (75.0)	6 (66.7)	7 (38.9)	4.56	0.30
Venlafaxine	-	15 (38.5)	-	-	3 (25.0)	3 (33.3)	9 (50.0)		
Duloxetine	-	2 (5.1)	-	-	0	0	2 (11.1)		
	Mean ± SD	Mean ± SD	t (df = 1)	p	Mean ± SD	Mean ± SD	Mean ± SD	F (df = 2)	p
Age (years)	32.3±8.3	34.3±11.5	-0.88	0.38	35.1±13.2	33.4±8.5	34.2±12.1	0.05	0.95
Onset age (years)	-	28.4±9.8	-	-	31.1±10.9	31.8±7.6	24.9±9.3	2.24	0.12
Education (years)	16.5±2.1	13.9±2.7	4.65	**<0.001****	14.2±1.7	14.6±1.8	13.2±3.5	0.85	0.44
Body mass index (kg/m²).	23.4±3.5	22.7±3.5	0.85	0.40	23.5±4.1	23.1±3.4	22.0±3.2	0.70	0.51
Number of MDE	-	1.7±0.9	-	-	1.4±0.7	1.2±0.4	2.1±1.1	3.72	**0.03***
Number of suicide attempts	-	0.9±1.3	-	-	0.4±0.9	1.0±1.5	1.1±1.5	1.05	0.36
Baseline HDRS score	0.4±1.1	27.8±5.4	-30.8	**<0.001****	26.5±5.1	26.8±4.1	29.2±6.2	1.07	0.35
Dose of 4-[18F]-ADAM (mCi)	8.1±1.2	7.8±1.0	1.28	0.20	7.8±0.8	8.1±0.9	7.6±1.2	0.60	0.55

MDD, major depressive disorder; MDE, major depressive episode; HDRS, 21-item Hamilton Depression Rating Scale; SD, standard deviation; df: degree of freedom. Bold numbers indicate significance.

[a] Fisher's exact test.

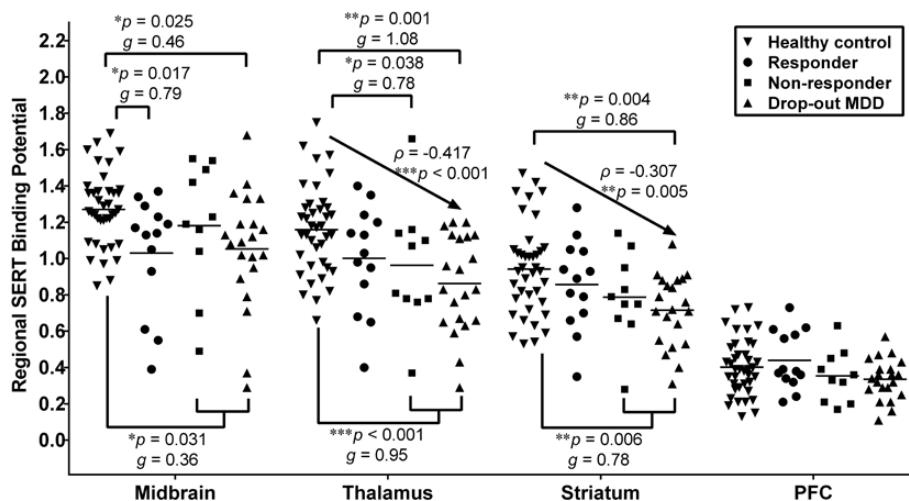*p<0.05, **p<0.01, ***p<0.001 is considered as significant.

Figure 2. Scatter plots of pretreatment levels of serotonin transporter (SERT) binding between healthy controls (n = 39) and subjects with major depressive disorder (MDD; n = 39) that were categorized by their outcomes to 6-week antidepressant treatment: responders (n = 12), nonresponders (n = 9), and dropout subjects (n = 18). The horizontal bar indicates the mean value of SERT binding ($*p < 0.05$, $**p < 0.01$, $***p < 0.001$ compared to healthy controls). Small, medium, and large effect sizes were defined respectively as $0.2 \leqq$ Hedges' $g < 0.5$, $0.5 \leqq$ Hedges' $g < 0.8$, and Hedges' $g \geqq 0.8$. We coded healthy controls, responders, nonresponders, and dropout subjects as an ordinal variables 0, 1, 2, and 3, respectively. The Spearman's rank correlations between the rank order and SERT binding were $\rho = -0.417$, $p < 0.001$ for thalamus; $\rho = -0.307$, $p = 0.005$ for striatum. PFC, prefrontal cortex.
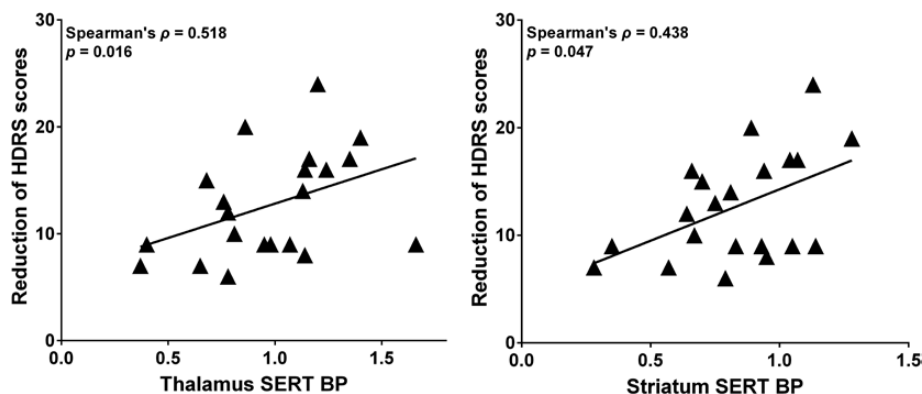

Figure 3. Correlation between the reduction of Hamilton Depression Rating Scale (HDRS) score at early stage (week 3) and pretreatment levels of serotonin transporter (SERT) binding potential (BP) in the thalamus and striatum (n = 21).

did not show any correlations with reductions in HDRS scores (Supplemental Table 2).

The Association of Regional SERT BP_{ND} or Projection Area/Midbrain SERT BP_{ND} Ratios in Association with Outcome to Antidepressants at Week 6

The SERT BP_{ND} did not differ among responders, nonresponders, and dropout patients across the four ROIs. However, SERT binding in the thalamus and striatum was significantly lower in dropout patients compared to healthy controls ($z = -3.35$, $p = 0.001$ for thalamus; $z = -2.87$, $p = 0.004$ for striatum). By coding the healthy controls, responders, nonresponders, and dropout patients as an ordinal variable in the order of 0, 1, 2, and 3, respectively, SERT binding negatively correlated with the ordinal variable in the thalamus ($\rho = -0.417$, $p < 0.001$) and striatum ($\rho = -0.307$, $p = 0.005$, Figure 2). This indicated a trend association in reduced SERT availability from healthy control to responder to nonresponders to dropout patients.

Furthermore, we compared the projecting areas/midbrain SERT BP_{ND} ratios among responders, nonresponders, and dropout patients, and found that there were significant differences

in thalamus/midbrain and striatum/midbrain SERT BP_{ND} ratios (Figure 4). A post hoc test revealed that responders had a higher striatum/midbrain SERT binding ratio compared with nonresponders ($z = -1.99$, $p = 0.047$). Because SERT BP_{ND} and SERT BP_{ND} ratios did not differ between nonresponders and dropout patients, we combined these two groups into the "other" group (n = 27). In comparison with the "other" group, responders had higher thalamus/midbrain, striatum/midbrain, and PFC/midbrain binding ratios (all p-values < 0.05). After Bonferroni corrections for multiple testing, the significance remained in the thalamus/midbrain SERT binding ratio between the responder group and the "other" group. Moreover, the PFC/midbrain binding ratio was higher in the responders than in the healthy controls ($z = -2.74$, $p = 0.006$). There were no differences in the SERT BP_{ND} ratios among the healthy controls, nonresponders, and dropout patients.

The Prediction of Responders Based on Projection Area/Midbrain SERT BP_{ND} Ratios

The ROC curve and the AUC are presented in Figure 5A and B. The AUC for the logistic regression models for each projection
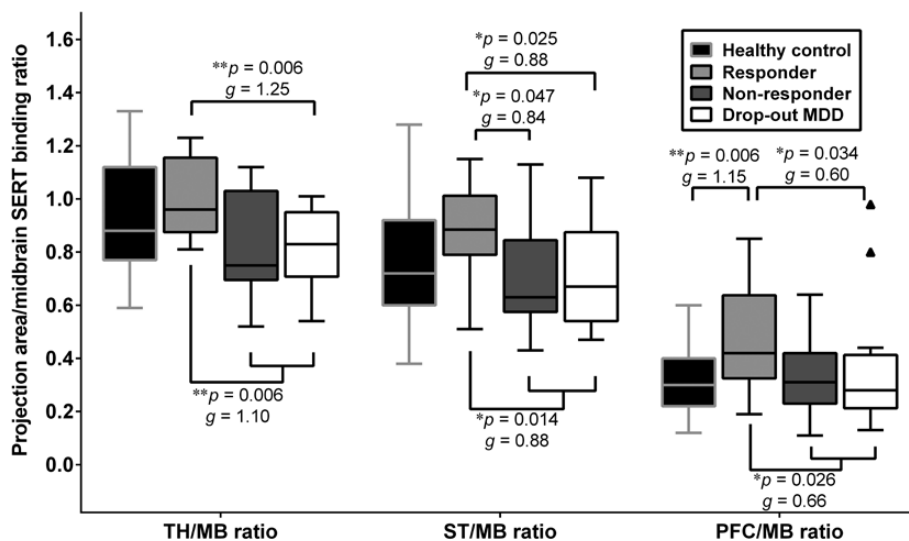
Figure 4. The box-and-whisker plot of pretreatment serotonin transporter (SERT) binding ratios in the terminal projection regions relative to the midbrain raphe nuclei among healthy controls (n = 39), responders (n = 12), nonresponders (n = 9), and dropout subjects (n = 18) with major depressive disorder (MDD). The short horizontal bar within the box indicates the median value of SERT binding ratios (*$p < 0.05$, **$p < 0.01$ compared to responders). The interquartile range (IQR), which is the length of the box, can be used as a measure of how spread-out the values are. There were two outliers in the PFC/MB ratio in dropout subjects. Small, medium, and large effect sizes were defined respectively as $0.2 \leqq$ Hedges' $g < 0.5$, $0.5 \leqq$ Hedges' $g < 0.8$, and Hedges' $g \geqq 0.8$. MB, midbrain; PFC, prefrontal cortex; ST, striatum; TH, thalamus.
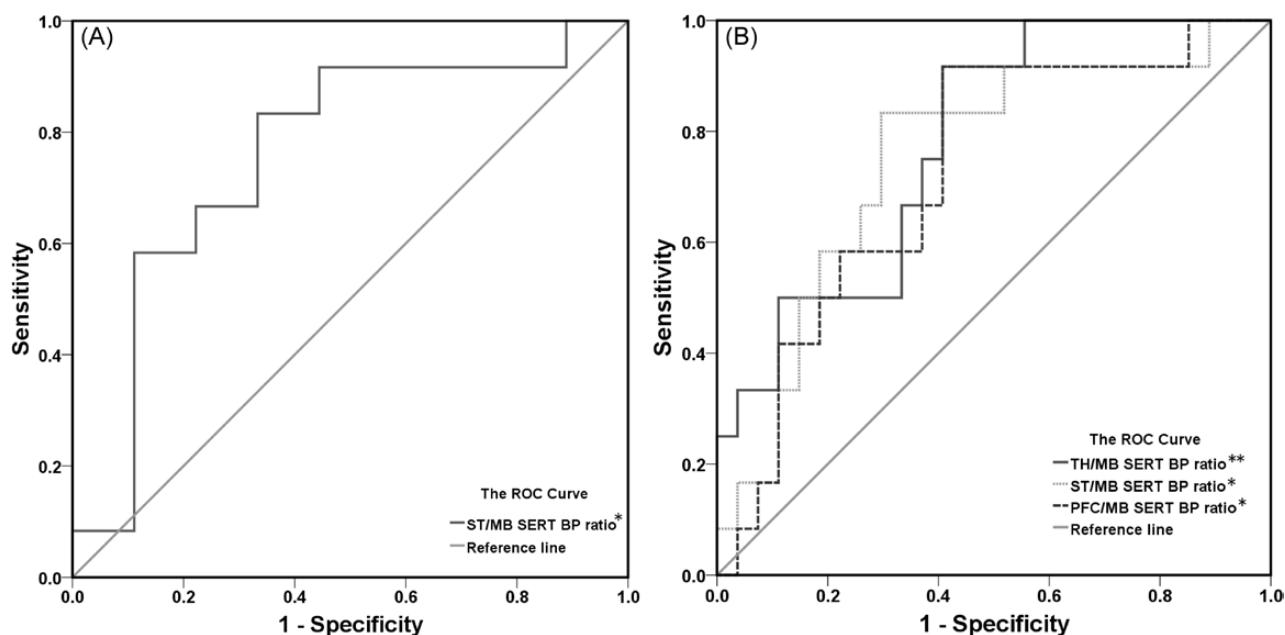

Figure 5. Prediction of (A) responders versus nonresponders; (B) responders versus "other" group (combined nonresponders and dropout subjects) using receiver operator characteristic (ROC) curves and the area under the curve (AUC) based on serotonin transporter (SERT) binding ratios (*$p < 0.05$, **$p < 0.01$). BP, binding potential; MB, midbrain; PFC, prefrontal cortex; ST, striatum; TH, thalamus.

area/midbrain SERT BP_{ND} ratios is presented in Table 2. The striatum/midbrain SERT BP_{ND} ratio predicted responders versus nonresponders (AUC = 0.76, $p = 0.047$, Table 2, Figure 5A). We found that the thalamus/midbrain SERT BP_{ND} ratio can distinguish the responder group from the "other" group (AUC = 0.78., $p = 0.006$, Table 2, Figure 5B). Both the striatum/midbrain (AUC = 0.75, $p = 0.014$) and PFC/midbrain (AUC = 0.73, $p = 0.026$) SERT BP_{ND} ratios predicted the responders versus the "other" group. The predictive power of SERT BP_{ND} ratios was fair because all the AUC values were greater than 0.7.

Discussion

Our naturalistic cohort study of 4-[^{18}F]-ADAM PET imaging in antidepressant-naïve patients with MDD demonstrated that greater pretreatment levels of SERT binding in the thalamus and striatum are associated with a greater reduction in HDRS scores at the 3-week stage of treatment. Our findings regarding the prediction of early improvement in depressive symptoms was in accordance with those of a previous study that used SPECT with the nonspecific SERT radioligand [^{123}I]-β-CIT (Kugaya et al., 2004).

Table 2. Predictive Ability of Serotonin Transporter (SERT) Non-displaceable Binding Potential (BP_{ND}) Ratios to Distinguish Responders from Nonresponders or "Other" Group.

	Responders vs nonresponders (total n = 21)					Responders vs other group (total n = 39)				
	Cutoff point	Sensitivity (%)	Specificity (%)	Predictive power (%)	AUC (%)	*p value*	Cutoff point	Sensitivity (%)	Specificity (%)	Predictive power (%)	AUC (%)	*p value*
TH/MB ratio	0.78	100	56	78	73	0.076	0.86	92	59	75	78	0.006**
ST/MB ratio	0.76	83	67	75	76	0.047*	0.77	83	70	77	75	0.014*
PFC/MB ratio	0.31	92	56	74	71	0.102	0.31	92	59	75	73	0.026*

The cutoff point of SERT BP ratios as the predictor by plotting the proportion of true positive result (sensitivity) versus the proportion of false-positive results (1-specificity). The area under the curve (AUC) indicates area under the receiver operator characteristic (ROC) curves (*$p < 0.05$, **$p < 0.01$). Other group indicates combined nonresponders and dropout subjects. MB, midbrain; PFC, prefrontal cortex; ST, striatum; TH, thalamus.

They showed that higher levels of SERT binding in the diencephalon, including the thalamus and hypothalamus, predict a better 4-week treatment response to SSRIs. However, our findings were inconsistent with those of a study that used PET with the SERT-selective radioligand [^{11}C]DASB (Lanzenberger et al., 2012) and that revealed no association between any regional SERT binding and reduction in HDRS scores at week 3. Although we found that the SERT BP_{ND} was lower in the midbrain, thalamus, and striatum in patients with MDD compared with healthy controls, similar to the findings of previous reports (Newberg et al., 2005, 2012; Parsey et al., 2006b; Reimold et al., 2008; Selvaraj et al., 2011; Ho et al., 2013; Nye et al., 2013; Gryglewski et al., 2014), we did not find any differences in SERT BP_{ND} across the four ROIs between responders and nonresponders. This suggested that pretreatment SERT availability might contribute to the initial change in HDRS scores in the first 3 weeks; nevertheless, the final therapeutic efficacy may be regulated by another mechanism.

As previous studies did not recruit healthy controls (Kugaya et al., 2004; Lanzenberger et al., 2012), the differences among responders, nonresponders, and healthy controls could not be investigated. To the best of our knowledge, this is the first study to explore whether regional SERT binding and SERT binding ratios differ between healthy controls and depressed subgroups that were categorized by their outcomes to antidepressant treatment, including responders, nonresponders, and dropout patients. In the present study, we observed a trend towards reduced SERT availability, in order, from healthy controls to responders to nonresponders to dropout patients. Furthermore, the SERT BP_{ND} ratios between the terminal projection regions and the midbrain in healthy controls were nearly equal to those in nonresponders and dropout patients (Figure 4). This finding indicated that both nonresponders and dropout patients demonstrated a proportionate decrease in SERT BP_{ND} in the terminal projection regions and midbrain compared with controls. In contrast, responders showed a pronounced decline in SERT BP_{ND} in the midbrain raphe nuclei and a disproportionally smaller decline in SERT BP_{ND} in serotonergic terminal projection regions compared to controls. Therefore, greater SERT BP_{ND} ratios in the projection regions (e.g. thalamus and striatum) relative to the midbrain might predict better treatment outcomes to SERT-targeted antidepressants at week 6.

This concept was partially in accordance with the findings of an earlier PET imaging study by Lanzenberger et al. (2012) that showed that higher SERT binding in the projection regions (amygdala and habenula) relative to the median raphe nuclei was beneficial for SSRI therapeutic efficacy and that these SERT binding ratios positively correlated with a reduction in HDRS scores at week 3. We found that higher SERT binding ratios (thalamus/midbrain and striatum/midbrain) might predict

SERT-targeted antidepressant efficacy at week 6. The inconsistent results at the various response times (week 3 versus week 6) might be due to differences in the antidepressants used, because antidepressants have different efficacies and response times (Cipriani et al., 2009). Previous studies that defined 3 or 4 weeks as the treatment endpoints might have underestimated the number of responders (Kugaya et al., 2004; Lanzenberger et al., 2012). Therefore, our study analyzed antidepressant-naïve depressed patients and followed them for 6 weeks, which might be a more complete treatment course.

It was unclear why a disproportionate reduction of SERT BP_{ND} between the projection regions and the midbrain raphe nuclei was associated with better clinical response after 6 weeks of antidepressant therapy in drug-naïve patients with MDD. We propose three possible mechanisms to explain this finding. First, the down-regulation of SERT in the serotonergic cell bodies and axon terminals takes longer than 4–6 weeks after administration of an antidepressant (Mirza et al., 2007; Descarries and Riad, 2012). Higher pretreatment levels of SERT binding in projection regions (e.g. thalamus and striatum) may lead to greater occupancy by the antidepressant (Kugaya et al., 2004; Baldinger et al., 2014), thereby rapidly enhancing 5-HT levels in the thalamus and striatum, which could ameliorate depressive symptoms at an early stage. Lower pretreatment levels of SERT BP_{ND} in the midbrain might be a compensatory response to the fewer numbers of serotonergic axons and neurons in major depression in order to overcome the lower levels of 5-HT (Arango et al., 2002; Austin et al., 2002). The responders showed the lowest SERT binding in the midbrain and a smaller decline in SERT binding in projection regions, which suggested that responders had a superior compensatory response and neuronal plasticity in response to lower serotonin neurotransmission among patients with major depression (Celada et al., 2001; Artigas, 2013). These patients might therefore have a better response to antidepressants. Second, higher pretreatment levels of SERT BP_{ND} in the thalamus or striatum could be moderated by inhibitory gamma-aminobutyric acid or excitatory glutaminergic inputs from the cortex and suppressed by 5-HT$_{1A}$ autoreceptors in order to regulate the serotonergic neuron firing rate. After initiating SERT-targeted antidepressants (Artigas, 2013), the serotonergic tonic firing rate would be suppressed through 5-HT$_{1A}$ autoreceptors in the midbrain raphe nuclei (Celada et al., 2001), which would consequently activate serotonergic neurons and promote 5-HT neurotransmission in terminal areas (Descarries and Riad, 2012). Therefore, the unequal reduction in the SERT density in terminal projection regions relative to the midbrain might be involved in therapeutic efficacy in MDD, and could aid in predicting the treatment outcomes of patients with MDD. Third, microRNA expression are down-regulated in the terminal projection

regions in patients with depression and suicide (Smalheiser et al., 2012; Serafini et al., 2014) and up-regulated after the administration of antidepressants (Baudry et al., 2010; Issler et al., 2014). These findings suggest that microRNA might modulate the level of SERT and 5-HT$_{1A}$ receptor in response to antidepressant treatment.

Our naturalistic follow-up study raised the issue of nonadherence to antidepressant treatment. The 53.8% complete-treatment rate in our study was in line with previous studies showing that approximately 55% of the patients with depression maintained their complete antidepressant therapy during the first month, whereas the other patients prematurely discontinued medication (Brown et al., 2005; Wu et al., 2013). Adherence to the antidepressant is essential for therapeutic efficacy in patients with MDD (Demyttenaere, 1998; Pampallona et al., 2002). However, earlier studies investigating adherence to antidepressants have focused on personal insight, education level, and concerns about the antidepressant side effects without assessing the biological factors in dropout patients (Pampallona et al., 2002; Vergouwen et al., 2003). Our results showed that SERT BP$_{ND}$ ratios did not differ between dropout patients and nonresponders, but a trend of greater decline in regional SERT binding was noted in dropout patients compared to nonresponders. This implied that the withdrawal from antidepressant in patients with major depression might not only be due to poor insight into the illness and medication but also because of a poor response to antidepressants, similar to nonresponders. Furthermore, a significant difference in the SERT BP$_{ND}$ ratios was observed between dropout patients and responders. Therefore, the SERT BP$_{ND}$ ratios between the terminal projection regions and the midbrain raphe nuclei might be a potential biomarker for prediction of responders versus nonresponders and dropouts. Because the predictive power of the SERT BP$_{ND}$ ratios was fair (75%–78%, Table 2), this biological evidence may help clinicians to more closely monitor those patients who have low thalamus/midbrain and striatum/midbrain SERT BP$_{ND}$ ratios while they are on antidepressant therapy.

Finally, the basal ganglia, including the striatum, are strongly interconnected with the cortex, thalamus, brain stem, and other brain areas. Among these, the corticobasal ganglia pathway plays a crucial role in the neuropathology of mood disorders (Marchand et al., 2012) and reward prediction (Tanaka et al., 2004), and could contribute to the response and decision of antidepressant adherence. Serotonin levels have been suggested to modulate cerebral glucose metabolism in patients with major depression (Smith et al., 2002, 2009; Geday et al., 2005). Regional SERT density can affect 5-HT levels in order to modulate glucose uptake in the subcortical and cortical regions, thus influencing the response and adherent behaviors of patients to antidepressants (Kennedy et al., 2007; Milak et al., 2009). As we previously mentioned, the higher projection region/midbrain BP$_{ND}$ ratios distinguished responders from nonresponders and dropout patients, and the disproportional reduction in SERT binding between terminal projection regions and the midbrain might be involved in the treatment outcome through the regulation of cerebral glucose metabolism. Although nicotine can inhibit 5-HT reuptake and stimulate 5-HT release in platelets (Rausch et al., 1989), which potentially increases SERT levels, previous imaging studies did not show a significant association between cigarette smoking and SERT availability (Staley et al., 2001; Ruhe et al., 2009; Erritzoe et al., 2010; Ho et al., 2013). Consistent with these studies, we did not find any associations between cigarette smoking and SERT binding.

A few limitations in our study should be addressed. First, we did not measure the plasma levels of the antidepressant or SERT occupancy by the antidepressant during the 6-week treatment course. The association between SERT occupancy by the antidepressant and antidepressant concentration could not be further analyzed. However, previous studies had shown that pretreatment SERT binding levels positively correlated with antidepressant occupancy (Baldinger et al., 2014). Hence, our result based on pretreatment SERT availability was able to predict the response to SERT-targeted antidepressants. Second, we recruited patients with MDD and treated them with common SERT-targeted antidepressants, including SSRIs, such as paroxetine, and serotonin-norepinephrine reuptake inhibitors (SNRIs), such as venlafaxine and duloxetine. The different antidepressants that we used in the present study may have had different affinities to norepinephrine transporter (NET). An antidepressant with a higher NET/SERT affinity ratio, such as duloxetine, might have a higher blockade of NET compared with other SNRIs and SSRIs. Thus, our prediction based on SERT binding might merely explain the SERT effect and not the NET effect. We could not rule out the effect of NET in the present study (Owens et al., 2008; Nogami et al., 2013), because pretreatment levels of NET binding and NET occupancy by the antidepressants might also contribute to therapeutic efficacy. Third, the dosage may also confound the outcome of antidepressant treatment. However, both SSRIs and SNRIs at their minimal standard therapeutic dosages show around 80% occupancy of the SERT (Meyer et al., 2001, 2004b), and higher dosages would result in a plateau effect of SERT occupancy. Even though the doses of the SERT-targeted antidepressants varied, the SERT occupancy by the antidepressants should be consistent with those in previous studies. In contrast, the occupancy of NET by SNRIs had a dose-dependent effect (Owens et al., 2008), and dosage of SNRIs may influence the therapeutic efficacy. Nevertheless, we did not observe any dosage differences among different types of antidepressants between responders and nonresponders. Fourth, our small sample size (n = 78) was insufficient to explore the association of *SLC6A4* variant effects with each group's SERT BP$_{ND}$. However, previous postmortem (Mann et al., 2000) and *in vivo* imaging studies (Parsey et al., 2006a; Ho et al., 2013) have shown no significant differences in 5-*HTTLPR* and *STin2* variable number tandem repeat polymorphisms on SERT binding. Therefore, *in vivo* SERT availability might account for treatment response more than *SLC6A4* variants in patients with MDD. Nevertheless, the effects of other genes or epigenetic modulations on SERT binding in the brain should be considered. Fourth, psychosocial problems and personality traits may also contribute to treatment response (Kaneda et al., 2011); however, we did not discuss this in the present study.

Conclusion

Taken together, our study provides new insight into pretreatment SERT availability and its association with treatment outcomes in patients with major depression. The disproportionate reduction in SERT binding in the projection area and in the midbrain raphe nuclei may be attributed to a response to antidepressants, whereas a proportionate reduction in SERT density may be associated with nonresponse and nonadherence to antidepressants. These findings may help clinicians to predict which patients may be nonresponsive or reluctant to adhere to SERT-targeted antidepressants treatment. For such patients, the use of other treatment strategies, including augmentation, use of antidepressants with other mechanisms of action, or

psychotherapy, is recommended.Supplemental Table 1. Dosages of antidepressants used in present study.Supplemental Table 2. Correlation between the reduction of HDRS score at each week and pretreatment regional SERT binding and SERT binding ratios.

Acknowledgements

Statement of Interest

None.

Supplementary Material

For supplementary material accompanying this paper, visit http://www.ijnp.oxfordjournals.org/

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