Dose-escalation in the picture: pharmacological and imaging studies in depression
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Citation for published version (APA):

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SEROTONIN TRANSPORTER GENE PROMOTER POLYMORPHISMS MODIFY THE ASSOCIATION BETWEEN PAROXETINE SEROTONIN TRANSPORTER OCCUPANCY AND CLINICAL RESPONSE IN MAJOR DEPRESSIVE DISORDER

Pharmacogenetics and Genomics 2008; in press

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Abstract

Background
In major depressive disorder (MDD), selective serotonin reuptake inhibitors (SSRIs) target the serotonin transporter (SERT). Their 30-50% response-rates are modified by SERT promoter polymorphisms (5-HTTLPR).

Aim
To quantify the relation between SERT occupancy and response, and whether 5-HTTLPR is a modifier.

Methods
Drug-free depressed outpatients (n= 49; both sexes; 25-55 years), received paroxetine 20 mg/day. We quantified SERT occupancy with $[^{123}\text{I}]\beta$-CIT SPECT imaging at baseline and after 6 weeks; we genotyped 5-HTTLPR (S, L, L). Primary outcomes: percentage decrease in Hamilton Depression Rating Scale (HDRS$_{17}$) and response ($\geq$50% decrease of HDRS$_{17}$).

Results
A significant positive relation between SERT occupancy and clinical response existed only in the L$_{A}$/L$_{A}$ genotype ($p<0.002$). Relative to paroxetine serum concentrations midbrain SERT occupancy was numerically higher for L$_{A}$/L$_{A}$ compared with other genotypes, but this difference was not significant ($p=0.188$).

Conclusions
Higher SERT occupancy is only associated with more clinical improvement in the L$_{A}$/L$_{A}$ genotype. We hypothesize that the L$_{A}$/L$_{A}$ carriers have a more dynamic serotonergic system, which appears more responsive to SSRIs.

ISRCTN register ISRCTN44111488
Introduction

Major depressive disorder (MDD) is often treated with antidepressants, most commonly selective serotonin reuptake inhibitors (SSRIs). SSRIs occupy the serotonin transporter (SERT) with high selectivity and affinity to block presynaptic serotonin reuptake. Unfortunately, clinical response rates for SSRIs are modest (30-50%) and difficult to predict. Unraveling the factors that modify clinical response may contribute to more effective SSRI treatment.

SERT occupancy can be estimated in-vivo using radioligands and SPECT or PET-imaging. SERT occupancy reaches a plateau at relatively low SSRI serum concentrations in healthy subjects, social phobia patients, and MDD patients, explaining why SSRI-serum levels do not predict clinical response. Previous studies postulated that 80% SERT occupancy by SSRIs would be required for clinical response. However, when SERT occupancy-rates and clinical response were correlated, the same studies found no clear relationship between striatal SERT occupancy and clinical response after 4 weeks of SSRI treatment in MDD patients. Contrary, Kugaya et al. found that higher pretreatment SERT availability and greater SERT occupancy in diencephalon predicted better treatment response after 6 weeks of SSRIs. Recently, Zitterl et al. found a correlation between clomipramine diencephalon SERT occupancy and severity-scores in obsessive compulsive disorder. However, in these two studies SERT occupancies ranged between 23-61%.

Polymorphisms of the SERT gene promoter region (5-HTTLPR) are associated with its transcriptional activity and influences the rate of serotonin uptake. In human lymphoblasts, cells homozygous for the long (L) allele produce higher concentrations of SERT mRNA than cells containing one or two copies of the short (S) allele. Furthermore, serotonin uptake by the transporter is >2-fold higher in cells homozygous for the L-allele. In-vivo imaging inconsistently showed lower midbrain SERT availability for S-allele carrying healthy subjects compared with L-allele carriers. Individuals with an S-allele have increased anxiety related traits, elevated risk of depression after stress, increased amygdala reactivity, and increased adverse events after SSRI treatment, compared with subjects with an L-allele. An elegant, large MRI-study in healthy volunteers showed associations of the 5-HTTLPR S/S polymorphism with unfavorable alterations in anatomy and function of the amygdala-cingulate feedback circuit. This study points to an important role of the 5-HTTLPR-polyorphism in the development and functioning of emotional networks involved in MDD. A meta-analysis showed associations between the 5-HTTLPR-polyorphism and clinical SSRI efficacy within 4 weeks of treatment. Depressed patients without S-alleles had higher response rates to SSRIs. Thus, apart from developmental, functional, and stress reactivity effects on emotional networks, the 5-HTTLPR polymorphism also appears to influence the response to SSRIs.

Nowadays, the 5-HTTLPR-polyorphism is considered tri-allelic. The L-allele is subdivided as L_G and L_A by a common single nucleotide polymorphism (SNP; rs25531). The L_G SNP creates a functional AP2 transcription factor binding site, behaving like an S-allele. The tri-allelic S:L_A:L_G ratio is approximately 4:5:1, and can be reclassified into a modified bi-allelic genotype. In-vivo studies predominantly found higher SERT availability for L_A/L_A carriers in healthy and MDD subjects. Recently, a significant association between the L_A/L_A allele and citalopram adverse effects but not with response was found in MDD patients.

In summary, the relation between SERT occupancy and clinical response to SSRIs is unclear, a SERT polymorphism likely influences the development and function of the serotonergic system (e.g. SERT availability) and affects clinical response to SSRIs. Since it was not investigated whether the relation between SERT occupancy and clinical response is modified by the 5-HTTLPR-polymorphism, we aimed to quantify (1) the relation between SERT occupancy and clinical response, (2) pre-treatment SERT availability for different tri-allelic 5-HTTLPR genotypes and (3) the modification by 5-HTTLPR with respect to the relation between SSRI-occupancy and clinical response.
We hypothesized that (1) the relation between occupancy and clinical response is nonlinear with increased response-rates above 80% occupancy after 6 weeks of treatment, (2) we would find lower pretreatment SERT availability for the tri-allelic S'/S' 5-HTTLPR genotype and (3) because of the higher response-rates in patients with a L-allele, the association between occupancy and clinical response would be distinct for tri-allelic 5-HTTLPR genotypes. We studied these questions in a 6 week open trial of paroxetine 20 mg/day, in subjects who participated in the first phase of a randomized dose-escalation trial with \([^{123}\text{I}]\beta\text{-CIT SPECT assessment of SERT occupancy}\).34

Methods

Participants

Following approval by the institutional medical ethical committee and written informed consent, we recruited drug-free outpatients (25-55 years)35 from primary care, our outpatient department, and public psychiatric settings (October 2003 - August 2006) and included them in the study before antidepressants were started. Inclusion criteria were: MDD determined by the Structured Clinical Interview for DSM-IV (SCID),36 and a Hamilton Depression Rating Scale (HDRS 17)37 score above 18. Patients were drug-naïve or drug-free (>4 weeks and ≥ 5 half-lives of a previous antidepressant, when treated previously) and had used no more than one antidepressant treatment (other than paroxetine) at an effective dose for ≥ 6 weeks for the present MDD-episode. Exclusion criteria were pregnancy (or wish), bipolar disorder, psychotic features, neurological cognitive impairments (i.e. dementia), primary anxiety and/or substance abuse disorders and acute, severe suicidal ideation. We allowed secondary co-morbid anxiety and/or substance abuse to increase applicability of the findings of our main study.34

SSRI treatment

After baseline assessment, patients were treated open-label with paroxetine 20 mg/day for six weeks. When severe adverse effects occurred, dosages were reduced to 10 mg/day and again increased to 20 mg/day after one week. We supplied paroxetine in pill-boxes to improve treatment adherence. We checked adherence by pill-counts and medical history.38 Benzodiazepines (temazepam 10-20 mg/day or oxazepam 10-30 mg/day) were allowed if necessary.

Questionnaires and measurements

Primary clinical outcomes were the percentage decrease in HDRS_17-score, and the proportion of patients achieving clinical response (≥50% decrease in HDRS_17). We administered questionnaires at study-entry and after 6 weeks of treatment. In addition, depressive symptoms were monitored at week 2 and 4 using the Maier and Bech subscales of the HDRS_17,39,40 and the self-rated Inventory for Depressive Symptomatology (IDS-SR_30) scores.41 Three trained investigators who administered the clinician-rated questionnaires (HDRS_17 and subscales) had good inter-rater agreement (intra-class correlation coefficient = 0.98).

SPECT imaging and analysis

We performed single photon emission computed tomography (SPECT) imaging for in-vivo assessment of SERT availability at study-entry and after 6 weeks between 2 to 10 pm as described previously.42 The acquisition started 230 ±18 (SD) minutes after intravenous injection of 100 MBq iodine-123-labeled 2β-carbomethoxy-3β-(4-iodophenyl)-tropane ([^{123}\text{I}]\beta\text{-CIT}), when the radioligand is at equilibrium for SERT binding in brain areas expressing high densities of SERTs (i.e. midbrain and diencephalon).43 To prevent thyroid uptake of \([^{123}\text{I}]\), all subjects received oral potassium-
iodide solution. We performed SPECT imaging using a 12-detector single slice brain-dedicated scanner (Neurofocus 810, Strichmann Medical Equipment; Cleveland, OH) with a full-width at half-maximum resolution of 6.5 mm, throughout the 20 cm field-of-view (www.neurophysics.com).

After attenuation correction and reconstruction in 3D mode (www.neurophysics.com), we defined regions of interest (RoIs) for midbrain, diencephalon and cerebellum by using validated templates (see Figure 2.3). One investigator, blinded for scan session (study-entry/6 weeks), positioned all RoIs in two series. Intra-class correlation coefficients between series were >0.97 for all RoIs. If the two series differed by >5%, scans were re-evaluated by a second investigator. In the analyses the counts were averaged for the two series.

Using activity in the cerebellum (CER) as indicator of non-displaceable activity (non-specific binding and free radioactivity), we estimated the non-displaceable binding potential (BP_{ND}) of the radioligand to SERT by calculating the ratio of specific to non-specific binding per scan as $BP_{ND} = \frac{activity_{scn} - activity_{neg}}{activity_{neg}}$. BP_{ND} is proportional to SERT availability under equilibrium conditions. In a different study, we found high reproducibility of SERT imaging with $\left[^{123}\text{I}\right]\beta$-CIT SPECT after repeated scanning of subjects, using the same camera and scanning-protocol (de Win et al., submitted). We calculated SERT occupancy at 6 weeks relative to the untreated SERT BP_{ND} (study-entry): $OCC_{6\text{ weeks}} = \frac{activity_{6\text{ weeks}} - activity_{neg}}{BP_{neg}}$.

**Paroxetine serum concentrations**

We collected blood for paroxetine serum concentrations (PSC; therapeutic range 10-75 µg/L) after 6 weeks, immediately before SPECT scanning. Serum was stored at -20° C until analysis. PSCs were determined using a validated High Pressure Liquid Chromatography-MS/MS method (available on request). The lower limit of quantification was 5µg/L, the lower limit of detection was 0.3 µg/L.

**Genotyping Procedures and Analysis**

Genomic deoxyribonucleic acid (DNA) was isolated out of blood using a filter-based method (QIAamp DNA Mini Kit, Qiagen Ltd, United Kingdom). The length of 5-HTTLPR was determined by gel electrophoresis. The region around the polymorphism was amplified by PCR using forward primer $\text{tgtaaaacgacggccagtgcagcagcatcaactaat}$ and reverse primer $\text{cagggagatcctgggaga}$ (M13 primer sequence in italics). The PCR reaction was performed in 10µl 1.5mM MgCl$_2$, 0.2µM forward and reverse primer, 0.1mM dNTP’s, 0.5 Units Hotfire Polymerase (Solis Biodyne, Estonia), Buffer B (Solis Biodyne, Estonia) and 20ng genomic DNA. The lengths of the different alleles were short ≈ 250 bp and long ≥ 298 bp. Genotyping of the rs25531 SNP was done by sequencing (Sanger) using Big Dye Terminators (Applied Biosystems). The M13 forward primer $\text{tgtaaaacgacggccagt}$ was used for sequencing. Reactions were performed containing 5ng of a forward primer, 5µl PCR product, BDT mix (Applied Biosystems) and 2.5xBDT buffer (Applied Biosystems). The length of the 5-HTTLPR polymorphism was confirmed by looking at the length of the sequenced PCR product. We reclassified the genotypes as S'/S' (S/S, LG/S, LG/LG), S'/LA (S/LA, LG/LA) and LA/LA. In post-hoc analyses we grouped S'/S' and S'/LA genotypes to contrast these with the ‘high-expression’ LA/LA genotype.

**Statistical Analysis**

For cross tabulations and differences between groups we used $\chi^2$ tests, Fisher’s exact test and ANOVA in SPSS for Windows v15.0.1 (www.spss.com). To investigate the relation between SERT occupancy and PSC, we modeled SERT occupancy (OCC) after 6 weeks in an $E_{\text{max}}$ model as $OCC_{\text{max}} = \frac{PSC}{b + PSC}$, in which $a$ represents maximal SERT occupancy in the model ($OCC_{\text{max}}$) and $b$ the PSC with 50% SERT occupancy ($EC_{50}$). We calculated $a$ and $b$ by fitting a nonlinear regression model that minimizes the sum of squares of the residuals (GraphPad Prism v5.00, www.graphpad.com). To assess whether PSC-occupancy or occupancy-response curves were improved by sub-grouping (e.g. genetic subgroups or responders versus non-responders), we fitted either one curve, or separate curves and determined whether separate curves decreased...
the Akaike Information Criterion (AIC; lower is better), which expresses the -2 log-likelihood of the (nested) model penalized for the number of independent variables in the model. We furthermore verified these analyses by regression models including interaction terms for genotype.

To predict clinical response by SERT occupancy, we performed $\chi^2$ and ANOVA tests to estimate group differences in clinical response-rate and proportional decrease in HDR$S_{17}$, respectively, with SERT occupancy stratified as poor <50%, intermediate 51-79%, and high ≥80%, and linear and logistic regression analyses for continuous outcomes (proportional decrease in HDR$S_{17}$) and dichotomous outcomes (response). In these models occupancy was entered as a continuous and dummy-coded group variable (stratified SERT occupancy), while the models were corrected for age, baseline midbrain/diencephalon SERT availability and sex.

One responder and one non-responder were potentially non-adherent after 6 weeks (PSC <5µg/L), but were included in the analyses for three reasons. First, this estimation of medication adherence was only based on a single measurement of PSC over a 6-week treatment period, second, patients had empty pill-boxes and had claimed adherence, and third, potential non-adherence in other patients before the measurement of PSC could not be excluded either.

### Table 8.1. Characteristics of patients, stratified by clinical response after 6 weeks of paroxetine 20 mg/day.

<table>
<thead>
<tr>
<th></th>
<th>Responders* (n= 10)</th>
<th>Non-responders* (n= 32)</th>
<th>$p$†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at baseline (years)</td>
<td>43.5 ±10.8</td>
<td>41.5 ±7.6</td>
<td>0.51</td>
</tr>
<tr>
<td>Female sex - n (%)</td>
<td>6 (60.0)</td>
<td>21 (65.6)</td>
<td>1.00</td>
</tr>
<tr>
<td>Current smoking - n (%)</td>
<td>5 (50.0)</td>
<td>17 (53.1)</td>
<td>1.00</td>
</tr>
<tr>
<td>Alcohol use: n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 7 Units/week</td>
<td>7 (70.0)</td>
<td>30 (93.8)</td>
<td></td>
</tr>
<tr>
<td>&gt;7 Units/ week</td>
<td>3 (30.0)</td>
<td>2 (6.2)</td>
<td></td>
</tr>
<tr>
<td>HDR$S_{17}$ at baseline</td>
<td>23.8 ±3.7</td>
<td>25.0 ±4.7</td>
<td>0.47</td>
</tr>
<tr>
<td>IDS-SR$S_0$ baseline</td>
<td>41.0 ±7.2</td>
<td>44.7 ±9.1</td>
<td>0.28</td>
</tr>
<tr>
<td>First episode - n (%)</td>
<td>4 (40.0)</td>
<td>18 (58.1)</td>
<td>0.48</td>
</tr>
<tr>
<td>No of episodes</td>
<td>2.0 ±1.1</td>
<td>1.9 ±1.8</td>
<td>0.92</td>
</tr>
<tr>
<td>Drug-naïve - n (%)</td>
<td>7 (70.0)</td>
<td>22 (68.8)</td>
<td>1.00</td>
</tr>
<tr>
<td>Used AD in current episode - n (%)</td>
<td>0 (0.0)</td>
<td>3 (9.4)</td>
<td>1.00</td>
</tr>
<tr>
<td>Melancholic - n (%)</td>
<td>7 (87.5)</td>
<td>24 (96.0)</td>
<td>0.43</td>
</tr>
<tr>
<td>Duration of episode: n (%)</td>
<td></td>
<td></td>
<td>0.97</td>
</tr>
<tr>
<td>≤5 months duration</td>
<td>3 (30.0)</td>
<td>10 (31.3)</td>
<td></td>
</tr>
<tr>
<td>5 months – 2 years duration</td>
<td>6 (60.0)</td>
<td>18 (56.3)</td>
<td></td>
</tr>
<tr>
<td>≥2 years</td>
<td>1 (10.0)</td>
<td>4 (12.5)</td>
<td></td>
</tr>
<tr>
<td>Age of first episode (years)</td>
<td>33.3 ±11.2</td>
<td>36.4 ±9.6</td>
<td>0.40</td>
</tr>
<tr>
<td>Co-morbidity - n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anxiety disorder</td>
<td>3 (30.0)</td>
<td>3 (9.4)</td>
<td>0.14</td>
</tr>
<tr>
<td>Dysthymia</td>
<td>0 (0.0)</td>
<td>1 (3.1)</td>
<td>1.00</td>
</tr>
<tr>
<td>Drug abuse / dependence‡</td>
<td>3 (30.0)</td>
<td>1 (3.1)</td>
<td>0.04</td>
</tr>
<tr>
<td>Alcohol abuse / dependence</td>
<td>2 (20.0)</td>
<td>1 (3.1)</td>
<td>0.14</td>
</tr>
<tr>
<td>SERT genotype</td>
<td></td>
<td></td>
<td>0.56</td>
</tr>
<tr>
<td>S'/S'/ (S/LC, L/C/LC)</td>
<td>4 (40.0)</td>
<td>8 (25.0)</td>
<td></td>
</tr>
<tr>
<td>S'/L' (S/LA, L/SA)</td>
<td>5 (50.0)</td>
<td>17 (53.1)</td>
<td></td>
</tr>
<tr>
<td>L'/L' (L/NA, L/NA)</td>
<td>1 (10.0)</td>
<td>7 (21.9)</td>
<td></td>
</tr>
<tr>
<td>SERT availability Bsl-scan (BPND)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Midbrain</td>
<td>0.60 (0.24)</td>
<td>0.61 (0.18)</td>
<td>0.93</td>
</tr>
<tr>
<td>Diencephalon</td>
<td>1.16 (0.29)</td>
<td>1.15 (0.23)</td>
<td>0.89</td>
</tr>
</tbody>
</table>

* Responders defined as patients with ≥50% decrease in baseline HDR$S_{17}$-score
† p values for $\chi^2$ for categorical, Fisher’s exact test for dichotomous, and independent T-tests for continuous data
‡ cannabis, benzodiazepines
Results

Patients

From 177 patients assessed, 53 patients were excluded and 73 refused participation. Of the 51 patients included in the study, 7 patients dropped out due to paroxetine adverse effects (n=5) or refusal of a second scan (n=2), leaving 44 patients who were scanned at baseline and after six weeks of treatment. Of these 44 patients, 12 patients were treatment-responder after 6 weeks. Of responders, two SPECT scans could not be used for analysis due to technical reasons, leaving 42 patients for the final analysis. Benzodiazepines were prescribed in 11/42 (26%) patients.

At study-entry, no significant differences were found between responders and non-responders except for drug (cannabis/benzodiazepine) abuse or dependence with higher prevalence in responders compared with non-responders (Fisher’s Exact; p=0.036; Table 8.1). None of the patients reported lifetime use of 3,4-methylenedioxymethamphetamine (MDMA).

SERT occupancy and clinical response

When SERT occupancy was stratified as poor (<50%), intermediate (51-79%) and high (≥80%), we found no significant relation between SERT occupancy and response-rate or the percentage decrease in HDRS17 after 6 weeks of paroxetine treatment, neither in midbrain nor in diencephalon (p>0.05) (Table 8.2). Neither in linear nor in logistic regression models occupancy significantly predicted clinical response (p>0.05). The minimum occupancy rate at which response occurred in at least one patient was 29% in midbrain and 49% in diencephalon. We found no increase in response-rate for SERT occupancy >80%. Only midbrain SERT-occupancy was associated with PSC (p=0.02). PSC significantly predicted response (OR=1.04 (95% CI=1.00-1.09)). After exclusion of 2 potential non-adherent patients, the association of PSC with response became non-significant, other results did not change.

Table 8.2. SERT occupancy by paroxetine and clinical response after 6 weeks of paroxetine 20 mg/day.

<table>
<thead>
<tr>
<th>Occupancy after 6 weeks paroxetine</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Diencephalon</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n=</td>
<td>9</td>
<td>25</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Mean occupancy (%)</td>
<td>28.9 ±7.43</td>
<td>65.4 ±7.22</td>
<td>85.0 ±1.29</td>
<td></td>
</tr>
<tr>
<td>Mean PSC (µg/L)</td>
<td>22.2 ±10.82</td>
<td>46.4 ±7.25</td>
<td>46.0 ±8.83</td>
<td>0.176</td>
</tr>
<tr>
<td>Responders n (%)</td>
<td>(33.3)</td>
<td>(41.6)</td>
<td>(37.5)</td>
<td>0.347</td>
</tr>
<tr>
<td>% decrease HDRS17</td>
<td>23.9 ±4.72</td>
<td>27.5 ±4.96</td>
<td>37.7 ±8.38</td>
<td>0.530</td>
</tr>
<tr>
<td>Midbrain</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n=</td>
<td>9</td>
<td>12</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Mean occupancy (%)</td>
<td>30.4 ±5.40</td>
<td>68.6 ±2.46</td>
<td>93.2 ±2.18</td>
<td></td>
</tr>
<tr>
<td>Mean PSC (µg/L)</td>
<td>18.2 ±6.27</td>
<td>38.5 ±7.90</td>
<td>56.3 ±19.18</td>
<td>0.020</td>
</tr>
<tr>
<td>Responders n (%)</td>
<td>(44.4)</td>
<td>(16.7)</td>
<td>(16.7)</td>
<td>0.149</td>
</tr>
<tr>
<td>% decrease HDRS17</td>
<td>30.2 ±10.89</td>
<td>26.1 ±6.88</td>
<td>30.6 ±4.29</td>
<td>0.896</td>
</tr>
</tbody>
</table>

Values represent means (±SEM) unless indicated different. N=42, including 2 non-adherent patients. PSC = Paroxetine serum concentration* χ2 for linear trend for response rates; ANOVA for % decrease† after 6 weeks of treatment

Modification of the relation between PSC, occupancy and response by genotype

PSC-occupancy curves in both the midbrain and diencephalon were curvilinear, with significantly different (non-stratified) curves for midbrain and diencephalon (determined by a decrease in AIC; figure available on request). The curves for responders versus non-responders were not significantly different in both brain regions. Stratification of the PSC-occupancy curves by SERT genotype indicated numerically higher OCCmax and lower EC50 in the LA/LA genotype (n=8/42; 19%) compared with the S’/S’ (n=12/42; 29%) and S’/LA genotypes (n=22/42; 52%), especially in midbrain (OCCmax for LA/LA=99.6; p=0.188) but not in diencephalon (OCCmax for LA/LA=79.3; p=1.0;
Figures 8.1A and B. The difference in AIC between one or separate curves indicated no significant improvement by including genotype in the models, both in midbrain (AIC increase 2.359) and diencephalon (AIC increase 4.037).

SERT availability and SERT occupancy by genotype
Mean pretreatment SERT availability and mean SERT occupancies after 6 weeks in midbrain or diencephalon did not significantly differ between genotypes (Table 8.3). Six of 8 patients (75%) with the LA/LA genotype reached midbrain occupancies ≥ 80% after 6 weeks compared with 12 of 31 patients (39%) with other genotypes (Fisher’s exact; p= 0.112).

After exclusion of 2 potentially non-adherent patients, mean SERT occupancies in midbrain were significantly different between genotypes (S'/S’ 80.9% ±5.80, S'/L_A 65.8% ±5.17, L_A/L_A 91.6% ±6.07; p= 0.017). Moreover, 6 of 7 patients (86%) with the L_A/L_A genotype reached midbrain occupancies ≥80% after 6 weeks compared with 12 of 30 patients (40%) with the other genotypes (Fisher’s exact; p= 0.042).

Figure 8.1. Paroxetine serum concentration and SERT occupancy by paroxetine, stratified by SERT genotype.

Table 8.3. Ethnicity, clinical response and SERT occupancy by paroxetine stratified for SERT genotype.

<table>
<thead>
<tr>
<th>SERT genotype</th>
<th>Ethnicity - n (%)</th>
<th>S'/S, S'/L_A, L_A/L_A</th>
<th>L_A/L_A</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caucasian</td>
<td>7 (24.1)</td>
<td>15 (51.7)</td>
<td>7 (24.1)</td>
<td></td>
</tr>
<tr>
<td>Creole</td>
<td>3 (42.9)</td>
<td>3 (42.9)</td>
<td>1 (14.3)</td>
<td></td>
</tr>
<tr>
<td>Asian</td>
<td>2 (33.3)</td>
<td>4 (66.7)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Clinical</td>
<td>n = 12</td>
<td>n = 22</td>
<td>n = 8</td>
<td></td>
</tr>
<tr>
<td>Week 6 % decrease HDRS&lt;sub&gt;17&lt;/sub&gt;</td>
<td>39.4±8.18</td>
<td>22.2±5.34</td>
<td>30.4±7.97</td>
<td>0.183</td>
</tr>
<tr>
<td>Occupancy Diencephalon</td>
<td>n = 12</td>
<td>n = 22</td>
<td>n = 8</td>
<td>0.353</td>
</tr>
<tr>
<td>Baseline BP&lt;sub&gt;ND&lt;/sub&gt;</td>
<td>101.7±3.94</td>
<td>116.2±6.01</td>
<td>122.9±8.84</td>
<td></td>
</tr>
<tr>
<td>Mean occupancy (%)</td>
<td>58.3±6.94</td>
<td>62.7±4.25</td>
<td>62.0±5.76</td>
<td></td>
</tr>
<tr>
<td>Occupancy ≥80% (%)</td>
<td>2 (18.2)</td>
<td>4 (25.0)</td>
<td>0.888</td>
<td></td>
</tr>
<tr>
<td>Occupancy Midbrain</td>
<td>n = 11</td>
<td>n = 20</td>
<td>n = 8</td>
<td>0.879</td>
</tr>
<tr>
<td>Baseline BP&lt;sub&gt;ND&lt;/sub&gt;</td>
<td>60.1±5.20</td>
<td>61.9±5.03</td>
<td>57.8±4.52</td>
<td></td>
</tr>
<tr>
<td>Mean occupancy (%)</td>
<td>73.6±9.04</td>
<td>65.8±5.17</td>
<td>81.3±11.56</td>
<td></td>
</tr>
<tr>
<td>Occupancy ≥80% (%)</td>
<td>5 (45.5)</td>
<td>7 (35.0)</td>
<td>6 (75.0)</td>
<td></td>
</tr>
</tbody>
</table>

Continuous values represent means (±SEM). N= 42, including 2 non-adherent patients.
* Post-hoc LA/L_A vs. S'/S, S'/L_A, L_A/L_A, S'/L_A, L_A/L_A Fisher’s exact p= 0.112

Panels represent PSC-occupancy curves for S'/S’ vs. S'/L_A vs. L_A/L_A in midbrain (A) and diencephalon (B).
Equation fitted: OCC<sub>6 weeks</sub> = e<sup>-<sub>PSC</sub>/AIC</sup>.
Differences between curves were not significant (lower AIC for 1 fitted curve vs. three fitted curves)
PSC= Paroxetine Serum Concentration, OCC<sub>6 weeks</sub> = SERT occupancy after 6 weeks of paroxetine treatment.
**SERT occupancy and clinical response by genotype**

Despite no significant differences in mean proportional HDRS<sub>17</sub>-decrease across genotypes (Table 8.3), we found a significant genotype*SERT occupancy interaction in diencephalon and midbrain (Figure 8.2). Higher diencephalon SERT occupancy was associated with larger proportional HDRS<sub>17</sub>-decreases in L<sub>A</sub>/L<sub>A</sub> carriers (ANOVA; F<sub>2,5</sub> = 27.64; p = 0.002) (Figure 8.2B; corrected for age), and L<sub>S</sub>/L<sub>A</sub> and L<sub>S</sub>/L<sub>S</sub> carriers grouped together (ANOVA; F<sub>1,5</sub> = 8.380; p = 0.007) (Figure 8.2D). The genotype*SERT occupancy interaction in diencephalon was significant (ANOVA; F<sub>2,36</sub> = 3.273; p = 0.049, with correction for age) (Figure 2B). Higher midbrain SERT occupancy in L<sub>A</sub>/L<sub>A</sub> carriers was associated with larger proportional HDRS<sub>17</sub>-decreases when corrected for age (Figure 8.2C; ANOVA; F<sub>2,5</sub> = 56.918; p < 0.001). Introduction of baseline SERT availability in diencephalon (or midbrain) showed no significant improvement of the association between proportional HDRS<sub>17</sub> decrease and SERT occupancy (p>0.05). These results remained unchanged with absolute decrease in HDRS<sub>17</sub>-scores instead of proportional HDRS<sub>17</sub>-decreases. Performing the same analyses with the bi-allelic 5-HTTLPR classification found identical directions of the effects, but explained less of the variances (available on request).

*Figure 8.2. SERT occupancy by paroxetine and proportional decrease in HDRS<sub>17</sub>, stratified by SERT genotype.*

Panels A and C: Midbrain, panels B and D: Diencephalon.

In panel A and C (midbrain) a significant relation between SERT occupancy and % decrease in HDRS<sub>17</sub> (determined by the slopes of regression lines) existed for the L<sub>A</sub>/L<sub>A</sub> genotype when confounding by age was accounted for (F<sub>2,5</sub> = 56.92; p<0.001).

In panels B (diencephalon) this relation was significant for the L<sub>A</sub>/L<sub>A</sub> genotype (F<sub>1,6</sub> = 15.36; p = 0.008), and confounded by age (F<sub>2,5</sub> = 27.64; p = 0.002); for panel D this relation was significant for the S/L<sub>A</sub>, L<sub>S</sub>/L<sub>A</sub>, L<sub>A</sub>/L<sub>A</sub> genotypes (F<sub>1,38</sub> = 8.380; p = 0.007).

In panel A and C there was no significant interaction between the different genotype groups. In panel B and D a significant interaction existed between the genotype groups (F<sub>2,36</sub> = 5.273; p = 0.049 and F<sub>1,38</sub> = 6.293; p = 0.017 respectively).
**Discussion**

We quantified the relation between SERT occupancy in midbrain and diencephalon and clinical response after 6 weeks of paroxetine 20 mg/day. We found no significant relation between these occupancies and clinical response. Nor did we find significant differences in pretreatment SERT availability in patients with different 5-HTTLPR-genotypes. We found a significant modifying effect of the 5-HTTLPR genotype with respect to the association between SERT occupancy and the decrease in HDRS$_{17}$-score (proportional and absolute). In diencephalon, increased SERT occupancy was associated with larger decreases in HDRS$_{17}$ for L$_A$/L$_A$ and S’/L$_A$ carriers. In midbrain, increased SERT occupancy was associated with larger proportional decreases in HDRS$_{17}$ for L$_A$/L$_A$ carriers only. Age was a covariate in these associations. Unexpectedly, we found a trend of a modifying effect of the 5-HTTLPR genotype with respect to the association between paroxetine serum concentrations and occupancy.

**Critique of methods**

Some limitations might be addressed first. We observed a low 6 week response rate (24%), which may be attributable to 1) the inclusion of some patients with secondary co-morbid anxiety and/or substance abuse, who might show less and slower recovery, and 2) the relatively high pretreatment initial HDRS-scores. Nevertheless, the recent Sequential Treatment Alternatives to relieve Depression trial measured only 30% response rate after 6 weeks of citalopram as well.

Second, we included patients who were ethnically heterogeneous (Table 8.3). This raises the possibility of spurious results secondary to population stratification, which can be a problem when analyzing non-functional markers which are in linkage disequilibrium with the functional variants. Since we have analyzed the relation between a functional DNA sequence variant and transporter occupancy we do not expect effects by the heterogeneity of our sample.

Third, as recruitment of MDD patients willing to undergo two SPECT scans is difficult, we could only successfully scan 42 patients twice. This led to small genotype-subgroups, and replication in larger samples will be necessary. Nevertheless, these 42 patients scanned twice while treated with the same antidepressant (and dose) is the largest patient sample to date, and allowed us to address the relation between SERT occupancy and clinical response, and its modification by the 5-HTTLPR-genotype for the first time. Furthermore, we had low dropout rates and good adherence.

Fourth, in this open label study we did not control for placebo response (estimated to be 30%). Placebo-responses may potentially obscure the relation between occupancy and response. Nevertheless, our major finding of a significant relation between SERT occupancy and proportional response in L$_A$/L$_A$-carriers was found despite such potential placebo-effects. Additionally, a placebo comparator would have estimated test-retest variability in SERT availability, but this variability is known to be much lower than the observed 60-70% SERT occupancy.

Fifth, we used $^{[123]I}$β-CIT for SPECT imaging, which is a non-selective radioligand, and also binds to dopamine transporters (DAT; e.g. midbrain substantia nigra). Nevertheless, uptake in midbrain and diencephalon is considered to reflect predominantly SERT, as these structures are rich of SERT relative to DAT. Therefore, although some additional DAT-binding might have concealed SERT occupancy, we think the change in $^{[123]I}$β-CIT-binding in diencephalon and midbrain mainly reflects SERT occupancy. Nevertheless, it would be challenging to replicate our study with selective radioligands like $[^{11}C]$DASB or $^{[123]I]}$ADAM.

Finally, we did not further explore whether 5-HTTLPR (or other polymorphisms) also influenced transporter site kinetics. Rausch et al. showed that initial serotonin kinetic conditions of SERT in platelets could predict treatment response in MDD patients, which was modified by SERT polymorphism. Patients with an L-allele were more responsive to fluoxetine than patients with an S-allele, with the initial affinity constant $K_m$ and dose being significant covariates for treatment outcome. It is unlikely that $K_m$ is directly affected by the SERT promoter polymorphism that primarily regulates expression. Because the S and L variants actually represent 10 subtypes,
other subtypes or an unknown other polymorphism could very well be associated with differences in $K_m$, which may better explain the inter-individual variation between PSC and SERT occupancy. Recently, Smeraldi et al. investigated the relation between clinical response to fluvoxamine and different L and S subtypes (identified by Nakamura et al.). They confirmed better responses in depressed patients bearing the L-allele, but also found significant differences in response among L-allele carriers according to the subtype of the L-allele, accounting for 0.6% of the variance. Because these subtypes are relatively uncommon, it would be very interesting to specifically study these L-allele subtypes in future neuroimaging studies using a case-control design.

**SERT occupancy and clinical response**

Previous studies postulated that a SERT occupancy of at least 80% would be associated with clinical response to SSRIs. Our data did not show significant associations between pretreatment SERT availability or SERT occupancy and clinical response to paroxetine, and neither suggested an 80% SERT occupancy threshold with increased response rates. We could not replicate a relation between SERT occupancy and decrease of symptoms. Receiver operating characteristic curves relating SERT occupancy to response (available on request) showed that SERT occupancy had no diagnostic value in the prediction of response.

The relation between SERT occupancy and clinical response is modified by 5-HTTLPR genotype

We found that 5-HTTLPR-polymorphisms modify the relation between SERT occupancy and clinical response. Previous clinical studies and meta-analyses found superior treatment effects in L-allele carriers. Despite a low response rate in our sample, we found that in LA/L-A carriers higher SERT occupancy was associated with more improvement. We put forward two possible explanations for this finding.

First, the LA/L-A genotype is associated with a more than two-fold higher serotonin uptake in human lymphoblasts. If we assume that serotonergic cells maintain a certain tonus in neurotransmission, in LA/L-A carriers more serotonin must be released as this is more effectively evacuated. When SERTs are blocked with paroxetine, this may increase serotonin neurotransmission more in LA/L-A carriers than in other genotypes, which may result in larger postsynaptic effects.

Second, 5-HTTLPR also modifies the development and synaptic plasticity of neural networks critically involved in MDD. Our findings might thus point to differences in postsynaptic effects (evoked by increased serotonin neurotransmission) in neuronal networks that have been developed differently as a result of different SERT genotypes. Pezawas et al. showed that healthy subjects with an S/S polymorphism demonstrated relative uncoupling of the amygdala from the anterior perigenual cingulate. S/S carriers were also associated with increased anxiety traits, increased amygdala reactivity, decreased mood after tryptophan depletion and increased risk for MDD. In summary, the limbic-cortical network and the serotonergic innervations appear to be more flexible in non S/S carriers. Our results may be supportive of the hypothesis that in LA/L-A carriers the significant association between higher SERT occupancy and increased reduction of symptoms could be indicative for a broader range for regulation of the serotonergic system. Higher SERT occupancy might then result in more effects of serotonergic antidepressants in LA/L-A carriers.

Interestingly, Pollock et al. found quicker response in L/L carriers when treated with paroxetine for 12 weeks, but no difference when treated with the noradrenergic antidepressant nortriptyline. Additionally better treatment outcomes and fewer adverse events in S/S genotypes were found when treated with mirtazapine (a postsynaptic 5-HT$_{2A}$, 5-HT$_{2C}$ antagonist and noradrenergic agonist), which were contrary to the effects of paroxetine. Future pharmacogenomic studies should investigate whether non S'/S' carriers will benefit more from serotonergic drugs while S'/S' carriers may benefit from noradrenergic antidepressants. Furthermore, since the mechanisms of action of antidepressants may be influenced by polymorphisms of other genes, future research should investigate additive effects of multiple candidate gene polymorphism combinations.
Are paroxetine serum concentrations and SERT occupancy modified by 5-HTTLPR genotype?

Six of 8 patients (75%) with the LA/LA genotypereached midbrain occupancies ≥80% after 6 weeks compared with 12 of 31 patients (39%) with other genotypes, although this was statistically not significant (p = 0.112, Fisher’s exact). Which factor determines the maximum SERT occupancy remains unclear. 5-HTTLPR-polymorphisms primarily affect gene transcription, and as a consequence affect SERT expression. Therefore, we a priori did not expect differences in SERT occupancy for different genotypes, as occupancy is expected to be independent of available SERTs. As such, our results could represent an epiphenomenon: e.g. a different SNP in the SERT gene, in linkage disequilibrium with the 5-HTTLPR-polymorphism (e.g. rs2228673; www.ncbi.nlm.nih.gov/SNP?SNP_ref.cgi?rs=2228673) may have affected SERT occupancy. Contrary, the suggested modifying properties of the 5-HTTLPR-genotype for the relation between PSC and occupancy may also lack power, since the PSC-occupancy curves in diencephalon show a clear trend of modification by 5-HTTLPR. Therefore, this needs further exploration in larger samples.

A rationale for a modifying role of the 5-HTTLPR polymorphism in the occupancy of the SERT by paroxetine and clinical response could be postulated as follows. SERT imaging assesses the in-vivo binding to SERTs. After 6 weeks of treatment with paroxetine, the availability of SERTs to bind to the radiotracer is lower, presumably due to SERT blockade by the SSRI. However, it is not possible to assess whether in addition to blockade, secondary effects occur such as down-regulation of the SERT. This might also result in lowering of the availability of SERTs to bind with the radiotracer. Indeed, in rats down-regulation of SERTs after prolonged exposure to paroxetine has been reported.57-58 Said differently, we are unable to discriminate between direct (i.e., blockade of SERTs by paroxetine) and potential indirect pharmacological effects (i.e., down-regulation). Importantly, Benmansour et al., suggested that SSRI-induced down-regulation of the SERT may be a key component for the clinical response to SSRIs.57-58 Down-regulation of SERTs in rats was not caused by decreased gene transcription, but is presumably caused by increased (posttranslational) internalization of SERTs.57 Although speculative, the level of down-regulation of SERTs might be diverse for different 5-HTTLPR-genotypes, with faster or greater down-regulation in LA/LA versus other genotypes. This hypothesis could be studied in future imaging studies with antidepressant-exposed primates (having 5-HTTLPR-polymorphisms) in which abrupt discontinuation is possible.

Conclusion

In conclusion, we find that SERT genotype modifies the relation between SERT occupancy and clinical response, with more improvement at higher midbrain and diencephalon SERT occupancy in LA/LA carriers. Although this SERT promoter-polymorphism presumably does not influence SERT occupancy at given PSCs, our data may point to a more flexible serotonergic system in LA/LA carriers, which is more easily influenced by serotonergic antidepressants. This hypothesis could serve as a starting point for future pharmacogenetic studies. This might provide the necessary data to guide the choice of antidepressant treatment for individual patients, reducing the patient’s suffering, and lowering healthcare costs.

Acknowledgements

The authors wish to thank the patients in this study for their participation, and willingness to participate in the SPECT-study. We thank all participating general practitioners in the area of Amsterdam Oost and Zuidoost, Hoofddorp, Nieuw Vennep, and Abcoude for their inclusions and referrals for the study. Mrs. E. Miedema M.D. and Mrs. M.C. ten Doesschate M.D. were indispensable for their help in rating questionnaires. This study was financed by a grant from the Netherlands Organisation for Health Research and Development (ZonMw), program Mental Health, education of investigators in mental health to H.G. Ruhé (OOG; #100-002-002).
Conflicts of interest

None

References


169


