Modification of the association between paroxetine serum concentration and SERT-occupancy by *ABCB1* (P-glycoprotein) polymorphisms in major depressive disorder

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Background **Selective serotonin reuptake inhibitors (SSRIs) exert substantial variability in effectiveness in patients with major depressive disorder (MDD), with up to 50–60% not achieving adequate response. Elucidating pharmacokinetic factors that explain this variability is important to increase treatment effectiveness.**

Objectives **To examine potential modification of the relationship between paroxetine serum concentration (PSC) and serotonin transporter (SERT)-occupancy by single nucleotide polymorphisms (SNPs) of the *ABCB1* gene, coding for the P-glycoprotein (P-gp) pump, in MDD patients. To investigate the relationship between *ABCB1* SNPs and clinical response.**

Methods **Patients had MDD and received paroxetine 20 mg/day. We measured PSC after 6 weeks. We quantified SERT-occupancy with SPECT imaging (n=38) and measured 17-item Hamilton Depression Rating Scale ($HDRS_{17}$)-scores at baseline and after 6 weeks (n=81). We genotyped *ABCB1* at rs1045642 [3435C>T], rs1128503 [1236C>T], rs2032582 [2677G>T/A] and rs2235040 [2505G>A]. For our primary aim, we modeled mean SERT-occupancy in an E_{max} nonlinear regression model with PSC and assessed whether the model improved by genetic subgrouping. For our secondary aim, we used multivariate linear regression analysis.**

Results **The rs1128503 and rs2032582 SNPs modified the relationship between PSC and SERT-occupancy in both our intention-to-treat and sensitivity analyses at the carriership level. However, we could not detect significant differences in clinical response between any of the genetic subgroups.**

Conclusion **Pharmacokinetic influences of the *ABCB1* rs1128503 and rs2032582 represent a potentially relevant pharmacogenetic mechanism to consider when evaluating paroxetine efficacy. Future studies are needed to support the role of *ABCB1* genotyping for individualizing SSRI pharmacotherapy. *Psychiatr Genet* 30: 19–29**

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Introduction

Selective serotonin reuptake inhibitors (SSRIs) are among the most frequently prescribed classes of drugs for treatment of major depressive disorder (MDD) (Chen *et al.*, 2008; Noordam *et al.*, 2015; Stephenson *et al.*, 2013). They exert their antidepressant effect by occupying the serotonin transporter (SERT), thereby blocking presynaptic reuptake of serotonin (Rominger *et al.*, 2015; Yeh *et al.*, 2015). Unfortunately, SSRIs show substantial variability in their effectiveness. Up to 50–60% of MDD patients do not achieve a clinically relevant response (Fava, 2003; Trivedi *et al.*, 2006). Although many factors such as age, sex, body weight, genetics and comedication are related to this variability (Dechant and Clissold, 1991; Sindrup *et al.*, 1992; Bagby *et al.*, 2002; Kugaya *et al.*, 2004; Serretti *et al.*, 2005; Feng *et al.*, 2006; Kato *et al.*, 2006; Domschke *et al.*, 2014), more specifically pharmacodynamic and pharmacokinetic factors may be important to understand variations in SSRI response rates. If such factors are elucidated, treatment with SSRIs may be optimized by personalizing drug choices and dosing. In this study, we focus on the pharmacokinetic mechanisms of MDD treatment with the SSRI paroxetine.

Systemic and brain availability of paroxetine is influenced by the permeability glycoprotein (P-gp) efflux pump as reported in in-vitro and in-vivo studies (O'Brien *et al.*,

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2012). P-gp is located in, among others, the blood–brain barrier and protects the brain against potentially toxic substances by clearing its substrates out of the brain at the blood–brain barrier. In fact, P-gp is the primary drug efflux mechanism, and thus responsible for drug concentrations within the brain (Cordon-Cardo *et al.*, 1989). P-gp is encoded by the ATP binding cassette subfamily B member 1 (*ABCB1*; or *MDR1*) gene (Linnet and Ejsing, 2008).

Research on the influence of *ABCB1* polymorphisms on treatment outcomes during SSRI treatment has yielded mixed results (Kato *et al.*, 2006; Gex-Fabry *et al.*, 2008; Kato *et al.*, 2008; Mihaljevic Peles *et al.*, 2008; Uhr *et al.*, 2008; Menu *et al.*, 2010; Sarginson *et al.*, 2010; Kato *et al.*, 2015; Ray *et al.*, 2015). Two recent meta-analyses found no associations between six *ABCB1* SNPs and SSRI treatment outcomes (Niitsu *et al.*, 2013; Breitenstein *et al.*, 2015), except for rs2032582 in one meta-analysis: patients with GT and TT genotypes showed better remission rates than those with GG (Niitsu *et al.*, 2013). Of note, one out of three unique rs2032582 studies investigated paroxetine specifically (Kato *et al.*, 2008). Furthermore, the rs2235040 variant A-allele has been associated with shorter time to remission in paroxetine-treated patients (Sarginson *et al.*, 2010). The rs1045642C-rs2032582G–rs1128503T-haplotype has been associated with poor paroxetine response, while other haplotypes showed no association with response (Kato *et al.*, 2008). Therefore, no definite conclusions can be drawn concerning the involvement of *ABCB1* polymorphisms in the treatment effects of SSRIs in general or paroxetine in particular.

At a pharmacokinetic level, several studies on involvement of P-gp in paroxetine treatment have been performed using paroxetine serum concentration (PSC) (Yasui-Furukori *et al.*, 2007; Gex-Fabry *et al.*, 2008; O'Brien *et al.*, 2012). Unfortunately, PSC cannot be used to predict clinical response and as such is not a measure for treatment outcome. Furthermore, investigation of the relationship between P-gp and PSC might not address the expected differences in intracerebral levels of paroxetine as determined by P-gp, for which SERT-occupancy is a better measure (Ruhé *et al.*, 2013). SERT-occupancy can be visualized and calculated *in vivo* using radioligands and PET or single-photon emission computed tomography (SPECT) imaging. In general, SERT-occupancy plateaus at low SSRI serum levels, both in healthy and MDD subjects (Meyer *et al.*, 2004; Ruhé *et al.*, 2013). It has been suggested that a SERT-occupancy level of >80% is necessary for clinical response (Meyer *et al.*, 2001; Suhara *et al.*, 2003; Meyer *et al.*, 2004), although response might also occur at lower levels (Ruhé *et al.*, 2009a). Differences in curves describing serum concentrations and SERT-occupancy for different *ABCB1* polymorphisms might therefore explain the variability between SSRI serum concentrations and SERT-occupancy on the one hand and clinical response on the other hand. To the best of our knowledge, the association between PSC and SERT-occupancy stratified by *ABCB1* polymorphisms has not been investigated before.

We hypothesized that the *ABCB1* polymorphisms with lower P-gp expression or activity or an association with favorable treatment outcomes or two or all of these three phenotypic presentations, would (1) also influence the nonlinear relationship between PSC and SERT-occupancy in the midbrain, with higher SERT-occupancy in these variant allele groups because of higher paroxetine concentrations in the brain and (2) be associated with higher response rates during paroxetine use (Ruhé *et al.*, 2009a; Sarginson *et al.*, 2010; Brambila-Tapia, 2013).

Our primary aim was to evaluate whether the three most studied *ABCB1* SNPs (rs1045642 [3435C>T], rs1128503 [1236C>T] and rs2032582 [2677G>T/A]) and the aforementioned rs2235040 [2505G>A] modified the relationship between PSC and SERT-occupancy in paroxetine-treated MDD patients. As a secondary aim, we investigated the relationship of these SNPs and the rs1045642C-rs2032582G–rs1128503T-haplotype with clinical response in a larger sample of paroxetine-treated MDD patients.

Methods

Design, setting and study population

Data and DNA-samples in this study were from the first six weeks of the 'Dose-Escalation Legitimate? Pharmacology and Imaging studies in depression' (DELPHI)-trial and the nested neuroimaging substudy DELPHI-SPECT (ISRCTN register no. ISRCTN44111488) described earlier (Ruhé et al., 2009a,b). We previously reported on modification by SERT-polymorphisms of the association between SERT-occupancy and clinical response in the same sample (Ruhé *et al.*, 2009a). The study was approved by the Academic Medical Centre (AMC) medical ethical committee and all participants provided written informed consent. In short, patients aged 17–70 years [25–55 years for the SPECT-sample to reduce variability in SERT-measurements by age (van Dyck *et al.*, 2000)] diagnosed with a major depressive disorder and drug-free (SPECT-sample; washout >5 half-lives of previous treatments if any) or who had undergone no more than one antidepressant treatment (other than paroxetine) for the present MDD-episode were eligible for the study. Patients were treated with paroxetine 20 mg/day for six weeks; only short-acting benzodiazepines were allowed as incidental comedication. More detailed information about the design, setting and study population is described elsewhere (Ruhé et al., 2009a,b) and can be found in the Supplemental Methods, Supplemental Digital Content 1, *http://links.lww.com/PG/A230*.

Primary outcome: serotonin transporter-occupancy

Primary outcome was the SERT-occupancy by paroxetine in the midbrain. We a priori chose to use only the midbrain SPECT-data, as midbrain SERT-occupancy

had previously been shown to be most reliably associated with PSC (Ruhé *et al.*, 2009a), and to avoid the need for power-lowering corrections for multiple testing in our limited SPECT sample. SPECT imaging for in-vivo assessment of SERT availability was performed at study-entry and after six weeks of paroxetine treatment between 2 and 10 pm according to previously described procedures (de Win *et al.*, 2005). All scans were made 230 ± 18 (SD) minutes after intravenous injection of 100 MBq [^{123}I]methyl 3ß-(4-iodophenyl) tropane-2 ß –carboxylate ([123I] β-CIT), when the radioligand is at equilibrium for SERT binding in brain areas expressing high densities of SERTs, such as the midbrain (Pirker *et al.*, 2000). We measured the SERT-occupancy in the midbrain as a proxy for cortical SERT-occupancy. The definitions of the regions of interest for midbrain and cerebellum (reference) has been described previously (de Win *et al.*, 2005; Ruhé et al., 2009a,b). Using activity in the cerebellum as indicator of nondisplaceable activity (nonspecific binding and free radioactivity) in calculating the nondisplaceable binding potential (BP_{ND}) of the radioligand to SERT as described previously (Ruhé *et al.*, 2009a), we calculated SERT-occupancy at 6 weeks relative to the untreated SERT BP_{ND} (study-entry) as

$$OCC_{6weeks} = \frac{(BP_{NDstudy\text{-}entry} - BP_{ND6weeks})}{BP_{NDstudy\text{-}entry}}$$

Secondary outcomes: 17-item Hamilton Depression Rating Scale score

Secondary clinical outcomes were the absolute decrease in 17-item Hamilton Depression Rating Scale ($HDRS_{17}$) score (Hamilton, 1960), and the proportion of patients achieving response (≥50% decrease in $HDRS_{17}$-score). The $HDRS_{17}$ is a well validated instrument to measure the severity of MDD (Hamilton, 1960). The $HDRS_{17}$ was administered at study-entry and after six weeks of paroxetine treatment.

Permeability glycoprotein-genotyping procedures and analysis

Genomic DNA was isolated out of blood using a filter-based method (QIAamp DNA Mini Kit; Qiagen Ltd, Manchester, UK). *ABCB1* genetic polymorphisms rs1045642 [3435C>T], rs1128503 [1236C>T], rs2032582 [2677G>T/A] and rs2235040 [2505G>A] were determined with allelic discrimination on an ABI 7500 Thermal Cycler using validated Drug Metabolizing Enzyme assays C-7586657-20 (C3435C>T), C-7586662-10 (1236C>T), C-11711720C-30 and C-11711720D-40 (2677G>T/A) and C-15951386-20 (2505G>A) (ThermoFisher Scientific, Waltham, Massachusetts, USA).

Paroxetine serum concentrations

Blood for paroxetine trough serum concentration (PSC; therapeutic range 10–75 µg/L) was collected after six weeks of treatment, immediately before SPECT scanning. For subjects who did not participate in the SPECT study, blood for PSC could only be obtained in subjects treated at the AMC ($n = 15$), and was collected immediately after the study visit at week 6. Storage and measurement of PSC have been described before (Ruhé *et al.*, 2009a).

Statistical analysis

We performed descriptive and statistical analyses using IBM SPSS (version 24 for Windows; IBM Corp., Armonk, New York, USA) and GraphPad Prism (version 5.0 for Windows; GraphPad Software Inc., La Jolla, California, USA). For comparison of differences between groups in dichotomous and categorical variables, we used Chi square tests or Fisher's exact tests as appropriate. For comparison of differences in continuous variables, we used independent t-tests or ANOVAs. We report medians and used Mann–Whitney U tests for non-normally distributed continuous variables. Differences were considered statistically significant when $P < 0.05$.

To investigate the potential modification of the PSC-SERT-occupancy relationship by *ABCB1* polymorphisms, we modeled SERT-occupancy after six weeks (OCC_{6weeks}) in an E_{max} model as OCC_{6weeks}=aPSC(b+PSC), in which *a* represents maximal SERT-occupancy in the model (OCC_{max}) and *b* the PSC with 50% SERT-occupancy (EC_{50}) (Meyer *et al.*, 2001; Kent *et al.*, 2002; Suhara *et al.*, 2003; Catafau *et al.*, 2006; Takano *et al.*, 2006). We calculated *a* and *b* by fitting a nonlinear regression model that minimizes the sum of squares of the residuals in GraphPad Prism and SPSS. To assess whether PSC-SERT-occupancy curves improved by subgrouping (genetic subgroups), we fitted one curve, two curves (carriership) or three curves (genotypes) and determined whether the 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.

To investigate the relationship between *ABCB1* polymorphisms (genotype and carrier groups) and clinical response, we performed multivariate linear regression analysis for the absolute decrease in $HDRS_{17}$-score corrected for baseline $HDRS_{17}$-score (analysis of covariance) and multivariate logistic regression analysis for the number of responders (patients with ≥50% decrease in $HDRS_{17}$-score). We investigated the data for potential confounding by age, sex and PSC. These variables were included in the models if they were univariately associated with the outcome (using analysis of covariance) at a significance level of $P < 0.20$ (Maldonado and Greenland, 1993).

All data were analyzed on an intention-to-treat basis. One responder and four nonresponders were potentially nonadherent [PSC < 5 µg/L, or reported to not have taken

most or all of the dosages or answered 'yes' to three or four questions of the Morisky scale after 6 weeks (Morisky *et al.*, 1986)]. We performed a sensitivity analysis to investigate the influence of nonadherent cases on both analyses (SERT-occupancy and clinical response). We performed another sensitivity analysis to investigate the influence of the non-Caucasian subjects on both analyses (SERT-occupancy and clinical response).

Results

Participants

Of 278 patients referred for assessment of eligibility, 107 started treatment with paroxetine 20 mg/day in the DELPHI-study. Eighty-one patients finished the six weeks of paroxetine treatment and the $HDRS_{17}$-measurements at baseline and after 6 weeks. Of these, 46 patients with analyzable baseline scans of the midbrain were included in the current SPECT substudy. For the analyses of the PSC-SERT-occupancy models, three patients were excluded, because the $OCC_{6\ weeks}$ in the midbrain could not be calculated due to unanalyzable (repeated) scans. Moreover, five patients dropped out due to adverse effects, leaving a sample size of 38 SPECT-patients.

At study-entry, no significant clinical, demographic, imaging or genetic differences were found at baseline between responders ($n=25$) and nonresponders ($n=56$) in the total study population except for alcohol use (≤/>7 units/week $P=0.02$, all other $P\geq 0.08$; Table 1). No significant differences were found between the SPECT-sample ($n=38$) and other patients in the total study population

Table 1 Characteristics of the total study population ($n=81$) stratified by response after 6 weeks of paroxetine 20 mg/day

	Responders[a,b] ($n=25$)	Nonresponders[a,b] ($n=56$)	P value[c]
Age at baseline (years)	44.8 ± 1.8	43.0 ± 1.3	0.43
Sex (female)	17 (68.0)	37 (66.1)	0.87
Ethnicity			0.73
Caucasian	14 (56.0)	34 (60.7)	
Surinamese-Creole	2 (8.0)	4 (7.1)	
Surinamese-Hindu	2 (8.0)	3 (5.4)	
Antillian-Aruban	2 (8.0)	6 (10.7)	
Other	5 (20.0)	9 (16.1)	
Level of education			0.72
Low	6 (24.0)	14 (25.5)	
Middle	13 (52.0)	32 (58.2)	
High	6 (24.0)	9 (16.4)	
Current smoker	10 (43.5)	28 (50.0)	0.60
Alcohol use			**0.02**
≤7 units/week	16 (66.7)	51 (91.1)	
>7 units/week	8 (33.3)	5 (8.9)	
$HDRS_{17}$ at baseline	22.9 ± 0.7	24.8 ± 0.6	0.08
First episode	12 (48.0)	35 (62.5)	0.22
No of episodes [median (range)]	2 (1–10)	1 (1–10)	0.16
Melancholic	17 (89.5)	38 (88.4)	1.00
Duration of episode			0.55
<5 months	7 (28.0)	13 (23.6)	
5 months–2 years	14 (56.0)	37 (67.3)	
≥2 years	4 (16.0)	5 (9.1)	
Psychiatric comorbidity	12 (50.0)	18 (32.1)	0.13
Drug naïve	14 (62.5)	38 (67.9)	0.64
Used psychotropic drugs in current episode	4 (16.7)	7 (12.5)	0.73
SERT-availability midbrain at baseline ($n=38$)	0.60 ± 0.09 ($n=8$)	0.61 ± 0.03 ($n=30$)	0.83
P-gp genotype rs1045642			0.36
CC	5 (20.0)	17 (30.4)	
CT	9 (36.0)	23 (41.1)	
TT	11 (44.0)	16 (28.6)	
P-gp genotype rs1128503			0.83
CC	7 (28.0)	18 (32.1)	
CT	13 (52.0)	25 (44.6)	
TT	5 (20.0)	13 (23.2)	
P-gp genotype rs2032582			0.92
GG	11 (44.0)	22 (39.3)	
GT or GA	9 (36.0)	22 (39.3)	
AA or TT or TA	5 (20.0)	12 (21.4)	
P-gp genotype rs2235040			1.00
GG	20 (80.0)	43 (76.8)	
GA	4 (16.0)	9 (16.1)	
AA	1 (4.0)	4 (7.1)	
rs1045642 C -rs2032582 G- rs1128503			0.67
T-haplotype			
Present	9 (36.0)	23 (41.1)	

$HDRS_{17}$, Hamilton Depression Rating Scale; SERT, serotonin transporter.
[a]Data are given as number (percentage) or mean ± SEM unless stated otherwise.
[b]Responders defined as patients with ≥50% decrease in baseline $HDRS_{17}$-score.
[c]P values <0.05 are shown in bold.

($n=43$) (all $P \geq 0.05$; Supplemental Table 1, Supplemental Digital Content 1, *http://links.lww.com/PG/A230*).

Difference in paroxetine serum concentration, nondisplaceable binding potential and serotonin transporter-occupancy by ABCB1 genotype after 6 weeks of treatment

We found no differences in mean PSC, BP_{ND} or SERT-occupancy between the various genotype groups in the SPECT-sample ($n=38$, all $P>0.12$; Supplemental Table 2/inlays in Supplemental Fig. 1, Supplemental Digital Content, Supplemental Digital Content 1, *http://links.lww.com/PG/A230*) or between the carriership groups for the four SNPs (Table 2/inlays in Fig. 1), except for rs2235040: carriers of the variant A-allele ($n=10$) had lower PSC than noncarriers ($n=28$; $P<0.01$, all other $P>0.06$).

Relationship between serotonin transporter-occupancy and paroxetine serum concentration by ABCB1 genotype

The PSC-SERT-occupancy curve in the midbrain was curvilinear ($F_{2,36}=263.8$, $P<0.0001$; AIC = –120.0; see Supplemental Fig. 1, Supplemental Digital Content 1, *http://links.lww.com/PG/A230*). The EC_{50} and E_{max} values for the unstratified and all stratified models are shown in Supplemental Table 3, Supplemental Digital Content 1, *http://links.lww.com/PG/A230*. The nonlinear regression models were significant throughout all stratifications for genotype (all $F_{6,32}>44.1$; all $P<0.0001$) and carriership (all $F_{4,34}>90.4$; all $P<0.0001$). Stratification of the PSC-SERT-occupancy curve by *ABCB1* genotype did not indicate an improvement of the model for any of the four SNPs under study, as the models with three curves per SNP (Supplemental Fig. 1, Supplemental Digital Content 1, *http://links.lww.com/PG/A230*) resulted in higher AICs than the model with one curve fitting the data (AIC increase 27.4 for rs1045642, 19.5 for rs1128503, 14.8 for rs2032582 and 19.5 for rs2235040, respectively).

When we analyzed the data for *ABCB1* genotype carriership of the wildtype allele rs1128503 (AIC = –121.8) and rs2032582 (AIC = –123.7) and the variant allele for rs1045642 (AIC = –120.2) and rs2235040 (AIC = –104.9; Fig. 1), we observed decreases in AIC when fitting two curves for rs1128503 (AIC decrease 1.8) and rs2032582 (AIC decrease 3.8) and rs1045642 (AIC decrease 0.2), indicating improved fit of the models for these SNPs, but not for rs2235040 (AIC increase 15.0).

In our first sensitivity analysis, leaving out nonadherent cases, again no better fit of the data was found when stratifying for *ABCB1* genotypes ($n=33$; AIC for the unstratified model = –101.8, all AIC increases > 0.6; data not shown). However, stratification for *ABCB1* carriership improved fitting for rs1128503, rs2032582 and rs2235040 (AIC decreases 1.9, 4.3 and 1.6, respectively) but deteriorated the model fit for rs1045642 (AIC increase 1.4; Supplemental Table 4, Supplemental Digital Content 1, *http://links.lww.com/PG/A230*).

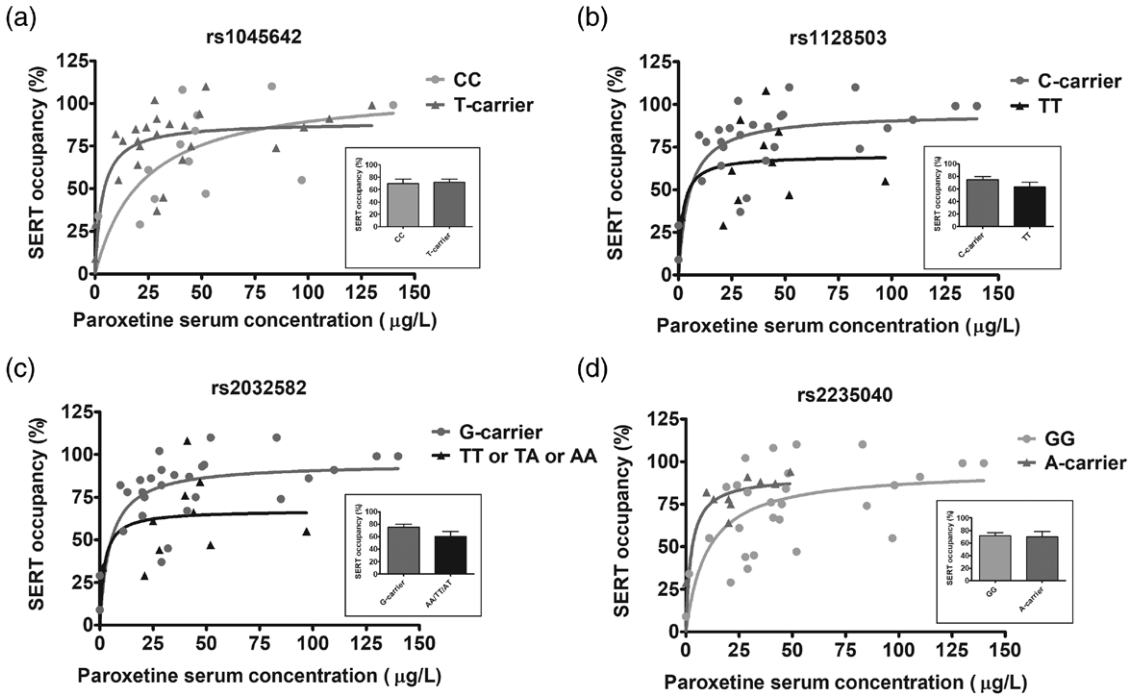
Our second sensitivity analysis, leaving out non-Caucasian cases, did not change the results compared with the original intention-to-treat analysis: stratification for genotype did not improve the model for any of the four SNPs ($n=25$; AIC for the unstratified model = –78.2, all AIC increases >0.6 compared to the unstratified model

Table 2 Mean paroxetine serum concentration (μg/L), mean baseline nonspecific binding ratio and mean serotonin transporter-occupancy (%) by *ABCB1* single nucleotide polymorphism allele carriership in the single-photon emission computed tomography-sample ($n=38$) after 6 weeks of paroxetine 20 mg/day

A. Mean PSC (μg/L) by ABCB1 SNP allele carriership[a]			
SNP	Carrier (genotype; *n*)	Noncarrier (genotype; *n*)	*P* value[b]
rs1045642 (variant allele)	38.9 ± 6.7 (CT/TT; *n*=25)	51.3 ± 10.1 (CC; *n*=13)	0.30
rs1128503 (wildtype allele)	45.0 ± 7.3 (CC/CT; *n*=27)	38.7 ± 7.2 (TT; *n*=11)	0.62
rs2032582 (wildtype allele)	44.4 ± 7.1 (GG/GA/GT; *n*=28)	39.7 ± 7.9 (AA/AT/TT; *n*=10)	0.72
rs2235040 (variant allele)	23.87 ± 4.8 (GA/AA; *n*=10)	50.0 ± 7.0 (GG; *n*=28)	**<0.01**
B. Mean baseline nondisplaceable binding potential by ABCB1 SNP allele carriership[a]			
SNP	Carrier (genotype; *n*)	Noncarrier (genotype; *n*)	*P* value[b]
rs1045642 (variant allele)	0.62 ± 0.04 (CT/TT; *n*=25)	0.59 ± 0.05 (CC; *n*=13)	0.70
rs1128503 (wildtype allele)	0.63 ± 0.04 (CC/CT; *n*=27)	0.57 ± 0.05 (TT; *n*=11)	0.47
rs2032582 (wildtype allele)	0.63 ± 0.04 (GG/GA/GT; *n*=28)	0.57 ± 0.05 (AA/AT/TT; *n*=10)	0.45
rs2235040 (variant allele)	0.67 ± 0.07 (GA/AA; *n*=10)	0.59 ± 0.04 (GG; *n*=28)	0.25
C. Mean SERT-occupancy (%) by ABCB1 SNP allele carriership[a]			
SNP	Carrier (genotype; *n*)	Noncarrier (genotype; *n*)	*P* value[b]
rs1045642 (variant allele)	74.8 ± 4.8 (CT/TT; *n*=25)	69.6 ± 7.6 (CC; *n*=13)	0.55
rs1128503 (wildtype allele)	77.1 ± 4.7 (CC/CT; *n*=27)	63.1 ± 7.4 (TT; *n*=11)	0.12
rs2032582 (wildtype allele)	77.6 ± 4.5 (GG/GA/GT; *n*=28)	60.4 ± 7.6 (AA/AT/TT; *n*=10)	0.06
rs2235040 (variant allele)	76.7 ± 6.0 (GA/AA; *n*=10)	71.7 ± 5.1 (GG; *n*=28)	0.60

PSC, paroxetine serum concentration; SERT, serotonin transporter; SNP, single nucleotide polymorphism; SPECT, single-photon emission computed tomography.
[a]Data are given as mean ± SEM.
[b]*P* values <0.05 are shown in bold.

Fig. 1



Paroxetine serum concentration and SERT-occupancy by paroxetine, stratified by *ABCB1* gene carriership of the mutant allele at rs1045642 and rs2235040 and carriership of the wildtype allele at rs1128503 and rs2032582. PSC and SERT-occupancy after 6 weeks of 20 mg/day paroxetine ($OCC_{6\,weeks}$) stratified by *ABCB1* gene carriership of the mutant allele at rs1045642 (CC n=13/38, T-carrier n=25/38; panel a), carriership of the wildtype allele at rs1128503 (C-carrier n=27/38, TT n=11/38; panel b), carriership of the wildtype allele at rs2032582 (G-carrier n=28/38, AA/AT/TT n=10/38; panel c) and carriership of the mutant allele at rs2235040 (GG n=28/38, A-carrier n=10/38; panel d). Equation fitted: $\mathrm{OCC}_{6\,\mathrm{weeks}} = a * \frac{\mathrm{PSC}}{(b + \mathrm{PSC})}$, in which a represents maximal SERT-occupancy in the model (OCC_{max}) and b the PSC with 50% SERT-occupancy (EC_{50}). The corresponding EC_{50} and E_{max} values for all models shown are reported in Supplemental Table 3, Supplemental Digital Content 1, *http://links.lww.com/PG/A230*. All fitted models were significant throughout all stratifications for carriership (all $F_{4,34}$>90.4; all P<0.0001). Models fit for two curves were improved relative to no stratification for rs1045642, rs1128503 and rs2032582 (AIC decrease for one fitted curve vs two fitted curves 0.2, 1.8 and 3.8, respectively) but not for rs2235040 (AIC increase 15.0). AIC, Akaike Information Criterion; PSC, paroxetine serum concentration; SERT, serotonin transporter.

in the Caucasian sample; data not shown) and after stratification for carriership as described above, we observed decreases in AIC when fitting two curves for rs2032582 (AIC decrease 9.8) and rs1045642 (AIC decrease 6.3) and rs1128503 (AIC decrease 5.3), indicating improved fit of the models for these SNPs, but not for rs2235040 (AIC increase 0.5; Supplemental Table 4, Supplemental Digital Content 1, *http://links.lww.com/PG/A230*), similar to our initial analyses.

Relationship between 17-item Hamilton Depression Rating Scale-score and ABCB1 genotype

No associations were found between the *ABCB1* genotypes or the rs1045642C-rs2032582G-rs1128503T-haplotype and clinical response to six weeks of paroxetine treatment. Neither decrease in $HDRS_{17}$-score (corrected for baseline $HDRS_{17}$-score; all $P \geq 0.08$, Supplemental Table 5, Supplemental Digital Content 1, *http://links.lww.com/PG/A230*), nor the number of responders (≥50% decrease in $HDRS_{17}$-score; all $P \geq 0.37$; Supplemental Table 5, Supplemental Digital Content 1, *http://links.lww.com/PG/A230*) showed significant associations in the regression models.

For analyses based on carriership, also neither decrease in $HDRS_{17}$-score (corrected for baseline $HDRS_{17}$-score; all $P \geq 0.13$, Table 3), nor the number of responders (all $P \geq 0.34$; Table 3) showed significant associations in any of the regression models for genotype, carrier or haplotype groups.

Exclusion of the five potentially nonadherent patients (one responder and four nonresponders) or the non-Caucasian patients in our separate sensitivity analyses did not change the results on baseline-adjusted $HDRS_{17}$-score or response-rate for genotype, carrier or haplotype groups [all $P \geq 0.06$ after exclusion of the potentially nonadherent patients (n = 76) and all $P \geq 0.08$ after exclusion of the non-Caucasian patients (n = 48); data not shown].

Table 3 Clinical response after 6 weeks of paroxetine 20 mg/day stratified by permeability glycoprotein carriership at four single nucleotide polymorphisms (n=81)

	n	Decrease in $HDRS_{17}$-score[a]	P value[b]
P-gp genotype rs1045642			
CC	22	5.9±0.05	0.13
T-carrier	59	8.4±0.04	
P-gp genotype rs1128503			
C-carrier	63	8.16±0.03	0.28
TT	18	6.3±0.06	
P-gp genotype rs2032582			
G-carrier	64	7.8±0.02	0.83
AA or AT or TT	17	7.5±0.03	
P-gp genotype rs2235040			
GG	63	8.3±0.02	0.20
A-carrier	18	5.9±0.05	
rs1045642 C -rs2032582 G- rs1128503 T-haplotype			
Absent	49	7.8±0.00	0.72
Present	32	7.4±0.00	
	n	Number of responders[c]	P value[d]
P-gp genotype rs1045642			
CC	22	5 (22.7)	0.34
T-carrier	59	20 (33.9)	
P-gp genotype rs1128503			
C-carrier	63	20 (31.7)	0.75
TT	18	5 (27.8)	
P-gp genotype rs2032582			
G-carrier	64	20 (31.3)	0.88
AA or AT or TT	17	5 (29.4)	
P-gp genotype rs2235040			
GG	63	20 (31.7)	0.75
A-carrier	18	5 (27.8)	
rs1045642 C -rs2032582 G- rs1128503 T-haplotype			
Absent	49	16 (32.7)	0.67
Present	32	9 (28.1)	

$HDRS_{17}$, Hamilton Depression Rating Scale; P-gp, permeability glycoprotein.
[a]Data are given as mean decrease in $HDRS_{17}$ after correction for baseline $HDRS_{17}$-score ± SEM.
[b]From linear regression analysis.
[c]Data are given as number of patients with ≥50% decrease in baseline $HDRS_{17}$-score (percentage).
[d]From logistic regression analysis.

Data were not confounded by age, sex or PSC in any of the regression analyses, except for the sensitivity analyses of response-rate for the Caucasian patients. Sex confounded the response-rate in this subgroup (P=0.149 for the univariate model) and was therefore included in the regression models for response rate.

Discussion

In this study, we quantified that two of four previously studied *ABCB1* gene polymorphisms (rs1128503, rs2032582) modify the association between PSC and SERT-occupancy in the midbrain (n=38) but none of the four polymorphisms of interest were associated with clinical response after six weeks of paroxetine treatment (n=81).

ABCB1 and serotonin transporter-occupancy

To the best of our knowledge, this is the first study to investigate whether the association between SSRI serum concentration and SERT-occupancy is modified by *ABCB1* polymorphisms. We expected that *ABCB1* polymorphisms associated with lower P-gp expression or activity or with higher response-rate or shorter time to remission or associated with more than one of these four phenotypic presentations, would also influence the non-linear relationship between PSC and SERT-occupancy in the midbrain, with higher SERT-occupancy in these variant allele groups because of higher paroxetine concentrations at the target site (Ruhé *et al.*, 2009a; Brambila-Tapia, 2013). However, the evidence on the associations between *ABCB1* polymorphisms and P-gp expression, activity or expected (in-vivo) effects is limited and mostly coming from in-vitro studies. The available literature is therefore insufficient to make definite statements about the expected effects in our study. Nevertheless, we summarize the available study results per SNP hereafter.

rs1045642

For rs1045642, we confirmed our hypothesis – after having certified that the results were not due to mean differences in SERT-occupancy between carriership groups. Our intention-to-treat analysis showed higher SERT occupancies at lower PSC for the rs1045642 TT genotype, which is in agreement with studies showing that this genotype is associated with decreased P-gp gene expression, decreased mRNA stability and a diminished function (Hitzl *et al.*, 2001; Wang *et al.*, 2005; Salama *et al.*, 2006; Hemauer *et al.*, 2010). However, after leaving out the potentially nonadherent patients, stratification for carriership of the variant T allele did not improve the model anymore. As this sensitivity analysis may better reflect the relationship of PSC and SERT-occupancy, this result suggests that if rs1045642 modifies the PSC-SERT-occupancy relationship, the effect may be small. This might be explained by the fact that it is a synonymous SNP, which does not alter the amino acid sequence of the P-gp protein.

rs1128503 and rs2032582

Stratification for carriership of the wildtype alleles for both rs1128503 and rs2032582 showed a significant modification of the PSC-SERT-occupancy curve without differences in PSC or SERT-occupancy between the carriership groups. However, higher SERT occupancies were found for carriers of the wildtype C-/G-alleles at all levels of PSC (Table 2b and Fig. 1b and c), while we expected the opposite from studies that reported decreased gene expression and diminished function with the rs1128503 variant T-allele (Salama *et al.*, 2006; Hemauer *et al.*, 2010) and reduced protein expression and diminished function for the rs2032582 variant T(/A)-allele (Salama *et al.*, 2006; Hemauer *et al.*, 2010). One explanation for this counter-intuitive finding may be that the evidence for effects of these SNPs on P-gp expression and activity is limited, based on a few small studies while results are often contradictory (Brambila-Tapia, 2013). Another explanation may be that the exact role of P-gp in paroxetine in general is not yet fully understood. Most studies agree on paroxetine being a P-gp substrate, but paroxetine has also been identified as a weak inhibitor (Uhr *et al.*, 2003; Mihaljevic

Peles *et al.*, 2008; O'Brien *et al.*, 2012) or even a (strong) inhibitor instead of a substrate (Weiss *et al.*, 2003; Maines *et al.*, 2005). However, if paroxetine is an inhibitor of the P-gp, our results may only be explained by increased function of P-gp with the variant T/A-alleles for these two SNPs or decreased P-gp function with the wildtype alleles. In the former case, the increased P-gp function would be at least partially undone by P-gp inhibition by paroxetine, while in the latter situation, P-gp inhibition leads to an even larger dysfunction of the P-gp-enzyme in wildtype carriers, both resulting in higher SERT-occupancy for the G/C-carriers compared to the variant T/A-alleles. To confirm these possible explanations, P-gp expression/activity patterns and measurements of paroxetine concentration within the brain would be necessary.

rs2235040

Only in our sensitivity analysis, rs2235040 was also associated with a modified relationship between PSC and SERT-occupancy at the carriership level with – confirming our hypothesis – higher SERT occupancies for carriers of the variant A-allele compared to the GG genotype. However, carriers of the variant A-allele had lower PSC than noncarriers in both our intention-to-treat and sensitivity analysis (both $P = 0.004$). This may be the result of fewer subjects with the A-allele (Table 2), but limits the straightforward interpretation for this SNP. Replication of this study in a larger sample size is warranted to confirm whether the genotype at rs2235040 explains some of the variability in the relationship between PSC and SERT-occupancy.

ABCB1 and clinical response

rs1045642 and rs1128503

Our results showed no association with *ABCB1* genotypes at rs1045642 and rs1128503, in line with previous studies and two meta-analyses (Gex-Fabry *et al.*, 2008; Kato *et al.*, 2008; Mihaljevic Peles *et al.*, 2008; Menu *et al.*, 2010; Niitsu *et al.*, 2013; Breitenstein *et al.*, 2015). As for the variant T-allele of both SNPs inconsistent effects on P-gp gene and protein expression and activity were reported, our nonsignificant results may at best be indicative of a small, clinically irrelevant effect on P-gp activity (Brambila-Tapia, 2013). However, we think a relevant association of these SNPs with clinical outcomes is unlikely since none of the individual studies using paroxetine included in the two meta-analyses found an effect of rs1045642 on response. Furthermore, our sensitivity analyses of nonadherence also pointed to a lack of modification of the PSC-SERT-occupancy curves by these SNPs.

rs2032582

For rs2032582, our results are in agreement with most studies including a second meta-analysis by Breitenstein *et al.* (2015), showing that this SNP is not associated with clinical response (Gex-Fabry *et al.*, 2008; Mihaljevic Peles *et al.*, 2008; Kato *et al.*, 2015;). Previous studies have found contradictory results on the effect of this polymorphism on P-gp expression and activity. In contrast to our SERT-occupancy and response analyses, one meta-analysis by Niitsu *et al.* (2013) including 1252 subjects showed a weak evidence of worse response in the GG genotype group compared to the TT genotype [odds ratio (OR) = 0.75, 95% confidence interval (CI) 0.58–0.97]. Although three of the four studies included in that meta-analysis focused on paroxetine, pooled efficacy stratified by *ABCB1* genotype was only given for all antidepressants together, limiting firm conclusions regarding paroxetine specifically. In the studies in patients using SSRI's including paroxetine ($n = 1176$), the meta-analyzed remission rate for patients with GG genotype was worse than in patients with the TT genotype (OR = 0.70, 95% CI 048–0.98), which is in contrast with our SERT-occupancy results (Niitsu *et al.*, 2013). However, we were unable to subdivide the homozygous mutant group based on presence of A- or T-alleles (instead of the G allele) in our sample, and thus we were unable to replicate findings specifically related to the T-allele.

rs22305040

For rs22305040, no evidence is available on the effects of this polymorphism on P-gp expression or activity. While one study reported shorter time to remission during paroxetine treatment for geriatric depression in A-allele carriers for rs2235040, we found no association of the genotype for this SNP with response after six weeks of paroxetine treatment and neither did a recent meta-analysis of *ABCB1* gene polymorphisms and antidepressant treatment (Sarginson *et al.*, 2010; Breitenstein *et al.*, 2015).

rs1045642C-rs2032582G-rs1128503T haplotype

The rs1045642C-rs2032582G-rs1128503T-haplotype has been shown to be associated with lower $HDRS_{21}$-change to paroxetine in 68 Japanese MDD-patients followed for six weeks (Kato *et al.*, 2008). Although our SERT-occupancy results are suggestive of effects in this direction, we found no significant association with efficacy. Comparison of our results with the Japanese sample might be complicated by potential effect modification by ethnicity, a known source of bias in (*ABCB1*) pharmacogenomics (Brambila-Tapia, 2013).

Strengths and limitations

Strengths of this study are the combination of variability in the *ABCB1* gene and a better quantifiable measure of the possible interacting effect of the genotypes, namely, SERT-occupancy. This is an innovative approach to investigate possible factors for personalizing medicine. Nevertheless, some limitations need to be considered when interpreting the results of this study. First, although the largest SPECT treatment study to date (Ruhé et al., 2009a,b), only 38 patients were analyzed for the effects of genotype on SERT-occupancy. Despite the resulting low

power to find effects of genotypes, we found modification of the relationship between PSC and SERT occupancy for at least two *ABCB1* polymorphisms. Nevertheless, replication of our findings in larger samples is warranted. Also, our analyses of treatment outcome with 81 participants are powered to distinguish effect sizes of 0.7 only. Therefore, our study might have resulted in nonsignificant findings for smaller effects for different genotypes instead of carriership. Moreover, our clinical results are skewed to non-responders, which we could partially address by using the continuous decrease in $HDRS_{17}$-score. Second, we used [^{123}I]β-CIT for SPECT imaging, a nonselective radioligand that also binds to dopamine transporters (DATs; e.g., midbrain substantia nigra) and norepinephrine transporter (NET; e.g., locus coeruleus) (Neumeyer et al., 1991, 1996; Innis *et al.*, 2007). Nevertheless, uptake in the midbrain is considered to reflect predominantly SERT, as this structure is relatively rich of SERT compared with DAT and NET (Laruelle *et al.*, 1993). Moreover, we measured SERT-occupancy with SPECT four hours after injection of the radioligand. At that time point, the radioligand is at equilibrium for SERT binding, while the equilibrium for DAT binding is reached after 24 hours (Pirker *et al.*, 2000). Therefore, we believe the change in [^{123}I]β-CIT-binding in the midbrain reflects SERT-occupancy in particular (Ruhé *et al.*, 2009a). Unfortunately, PET data on [^{11}C]DASB SERT-occupancy after exposure to different SSRIs (Meyer et al., 2001, 2004) in combination with *ABCB1* polymorphisms are unavailable (J.H. Meyer, personal communication). Third, we measured SERT-occupancy in the midbrain as a proxy for SERT-occupancy in the cortex, where therapeutic effects occur. However, there are no SPECT ligands available to measure cortical SERT occupancy. Fourth, we previously demonstrated (in the present sample) that the 5-HTTLPR promoter polymorphism modified the association between SERT occupancy and clinical response: in the patients with the L_A/L_A genotype higher SERT occupancy was associated with increased response on the Hamilton scale (Ruhé *et al.*, 2009b). Although not our primary aim of investigation, due to our modest sample size, we could not investigate the combined effect of these two factors of clinical outcome. In addition, although a different aim too, changing effects in combination with cytochrome P450 2D6 polymorphisms could not be examined. Fifth, our sample had no homogenous ethnicity (Table 1), which might have confounded our results. Therefore, we performed a sensitivity analysis in which we excluded all non-Caucasian patients; this did not change the results. Sixth, a recent study reported significant, ethnicity independent, associations of the rs10245483 G/G homozygotes with the SSRIs escitalopram and sertraline (Schatzberg *et al.*, 2015), while in an elderly population (Sarginson *et al.*, 2010), this SNP did not affect efficacy of paroxetine. As we choose our variants before this positive result was published, we did not determine the same SNPs in our analysis. Seventh, although blocking SERT is considered the mechanism of action of SSRIs, an easier explanation for the absence of a significant relationship with response might be that the direct relationship between SERT-occupancy and response to paroxetine treatment is at least questionable (Ruhé *et al.*, 2009a). This suggests that our findings of modified PSC-SERT-occupancy relationships by P-gp polymorphisms are the most important points of the present study, indicating modified intracerebral pharmacokinetics due to P-gp polymorphisms. Because response to SSRI will presumably not be determined by SERT-occupancy only, it is possible that the different SERT-occupancies by the SNPs under study may be a contributing factor to final response and must be investigated in combination with other factors. Given our sample size, this was not possible in our modest study population, which warrants further research. Finally, we only addressed four (well studied) SNPs of the *ABCB1* gene. In addition, we only considered therapeutic effects of paroxetine, while the influence on side effects could be interesting as well (Bet *et al.*, 2016). A genome wide association study, for example, would provide more insight in other *ABCB1* gene SNPs potentially associated with effects and side effects of paroxetine or SSRI treatment in general. This information is additionally required.

Conclusion

We found evidence that at least two previously studied *ABCB1* gene polymorphisms (rs1128503 and rs2032582) are associated with a modified relationship between PSC and SERT-occupancy in the midbrain. As such, pharmacokinetic influences of the *ABCB1* polymorphisms rs1128503 and rs2032582 might have a potentially relevant pharmacogenetic effect in SSRI efficacy, although those are not likely to be the only factor. However, none of the four studied SNPs nor the rs1045642C-rs2032582G-rs1128503T-haplotype were significantly associated with clinical response after six weeks of paroxetine treatment, but power to detect differences in efficacy was low with our moderate sample size. Future studies are needed to support the role of *ABCB1* genotyping to aid in individualizing SSRI pharmacotherapy.

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Conflicts of interest

There are no conflicts of interest.

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