Somatic monitoring of patients with mood and anxiety disorders

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

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ABSTRACT

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

1. 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* (or *MDR1)* gene, coding for the P-glycoprotein efflux pump (P-gp), in MDD patients.
2. 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 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 modelled 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 paroxetine serum concentration 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.

Conclusions

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.

INTRODUCTION

Selective serotonin reuptake inhibitors (SSRIs) are among the most frequently prescribed classes of drugs for treatment of major depressive disorder (MDD).[1-3] They exert their antidepressant effect by occupying the serotonin transporter (SERT), thereby blocking presynaptic reuptake of serotonin.[4,5] Unfortunately, SSRIs show substantial variability in their effectiveness. Up to 50-60% of MDD patients do not achieve a clinically relevant response.[6,7] Although many factors such as age, sex, body weight, genetics and co-medication are related to this variability[8-15], 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 are influenced by the permeability glycoprotein (P-gp) efflux pump as reported in *in vitro* and *in vivo* studies.[16] P-gp is located in, amongst 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.[17] P-gp is encoded by the ATP binding cassette subfamily B member 1 (*ABCB1;* or *MDR1*) gene.[18]

Research on the influence of *ABCB1* polymorphisms on treatment outcomes during SSRI treatment has yielded mixed results.[9,19-26] Two recent meta-analyses found no associations between six *ABCB1* SNPs and SSRI treatment outcomes[27,28], except for rs2032582 in one meta-analysis: patients with GT and TT genotypes showed better remission-rates than those with GG.[28] Of note, one out of three unique rs2032582 studies investigated paroxetine specifically.[25] Furthermore, the rs2235040 variant A-allele has been associated with shorter time to remission in paroxetine-treated patients.[20] The rs1045642C-rs2032582G–rs1128503T-haplotype has been associated with poor paroxetine response, while other haplotypes showed no association with response.[25] 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).[16,19,29] 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.[30] SERT-occupancy can be visualized and calculated *in vivo* using radioligands and Positron Emission Tomography (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.[30,31] It has been suggested that a SERT-occupancy level of >80% is necessary for clinical

response[31-33], although response might also occur at lower levels.[34] 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 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 and/or activity and/or an association with favourable treatment outcomes, 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.[20,34,35]

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 sub-study DELPHI-SPECT (ISRCTN register no. ISRCTN44111488) described earlier.[34,36] We previously reported on modification by SERT-polymorphisms of the association between SERT-occupancy and clinical response in the same sample.[34] 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[37]) diagnosed with a major depressive disorder and drug-free (SPECT-sample; washout more than five 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 20mg/day for six weeks; only short-acting benzodiazepines were allowed as incidental co-medication. More detailed information about the design, setting and study population is described elsewhere[34,36] and can be found in the Supplemental methods.

Primary outcome: SERT-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[34], and to avoid the need for power-lowering

corrections for multiple testing in our limited SPECT sample. Single photon emission computed tomography (SPECT) imaging for *in-vivo* assessment of SERT availability was performed at study-entry and after six weeks of paroxetine treatment between 2 to 10 pm according to previously described procedures.[38] All scans were made 230±18 (SD) minutes after intravenous injection of 100 MBq [^{123}I]methyl 3β-(4-iodophenyl) tropane-2 β –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.[39] We measured the SERT-occupancy in the midbrain as a proxy for cortical SERT-occupancy. The definitions of the regions of interest (RoIs) for midbrain and cerebellum (reference) has been described previously.[34,36,38] Using activity in the cerebellum as indicator of non-displaceable activity (non-specific binding and free radioactivity) in calculating the non-displaceable binding potential (BP_{ND}) of the radioligand to SERT as described previously[34], we calculated SERT-occupancy at six weeks relative to the untreated SERT BP_{ND} (study-entry) as

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

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Secondary outcomes: $HDRS_{17}$-score

Secondary clinical outcomes were the absolute decrease in 17-item Hamilton Depression Rating Scale($HDRS_{17}$)-score[40], 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.[40] The $HDRS_{17}$ was administered at study-entry and after six weeks of paroxetine treatment.

P-gp-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). *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 (DME) 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 MA, 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.[34]

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 potential modification of the PSC-SERT-occupancy relationship by *ABCB1* polymorphisms, we modelled SERT-occupancy after six weeks ($OCC_{6\ weeks}$) in an E_{max} model as $OCC_{6weeks} = a\frac{PSC}{(b+PSC)}$, in which *a* represents maximal SERT-occupancy in the model (OCC_{max}) and *b* the PSC with 50% SERT-occupancy (EC_{50}).[32,33,41-43] 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 sub-grouping (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 $p<0.20$.[44]

One responder and four non-responders were potentially non-adherent (PSC<5μg/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 six weeks[45]). All data were analysed on an intention-to-treat basis. We performed a sensitivity analysis to investigate the influence of non-adherent cases 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 six weeks. Of these, 46 patients with analysable baseline scans of the midbrain were included in the current

SPECT sub-study. 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 unanalysable (repeated) scans. Moreover, 5 patients dropped out due to adverse effects, leaving a sample size of 38 SPECT-patients.

At study-entry, no significant differences were found at baseline between responders (n=25) and non-responders (n=56) in the total study population except for alcohol use (≤/>7 units/week p=0.02, all other p≥0.08; Table 1). No significant differences were found between the SPECT-sample (n=38) and other patients in the total study population (n=43) (all p≥0.05; Supplemental table 1).

Difference in PSC, BP_{ND} and SERT-occupancy by *ABCB1* genotype

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 figure 1) or between the carriership groups for the four SNPs (Table 2/inlays in Figure 1), except for rs2235040: carriers of the variant A-allele (n=10) had lower PSC than non-carriers (n=28; p<0.01, all other p>0.06).

Relationship between SERT-occupancy and PSC by *ABCB1* genotype

The PSC-SERT-occupancy curve in the midbrain was curvilinear ($F_{2,36}$=263.8, p<0.0001; AIC=-120.0). The EC_{50} and E_{max} values for the unstratified and all stratified models are shown in Supplemental table 3. 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 figure 1) 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 analysed 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; Figure 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 sensitivity analysis, leaving out non-adherent cases, again no better fit of the data was found when stratifying for *ABCB1* genotypes (AIC for the unstratified model=-101.8, all AIC increases>0.6; data not shown). However, stratification for *ABCB1* carriership t 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; data available on request).

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)	Non-responders[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	11 (44.0%)	32 (57.1%)	
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	8 (32.0%)	11 (19.6%)	
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 co-morbidity	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%)	

Table 1. (continued)

	Responders[a,b] (n=25)	Non-responders[a,b] (n=56)	p-value[c]
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 T-haplotype present	9 (36.0%)	23 (41.1%)	0.67

[a] Data are given as number (percentage) or mean ± standard error of the mean 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.

Table 2. Mean paroxetine serum concentration (PSC; µg/L), mean baseline non-specific binding ratio (BPND) and mean SERT-occupancy (%) by *ABCB1* SNP allele carriership in the SPECT-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)	Non-carrier (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 non displaceable binding potential (BP_{ND}) by *ABCB1* SNP allele carriership[a]			
SNP	Carrier (genotype; n)	Non-carrier (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)	Non-carrier (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

[a] Data are given as mean ± standard error of the mean
[b] p-values<0.05 are shown in bold

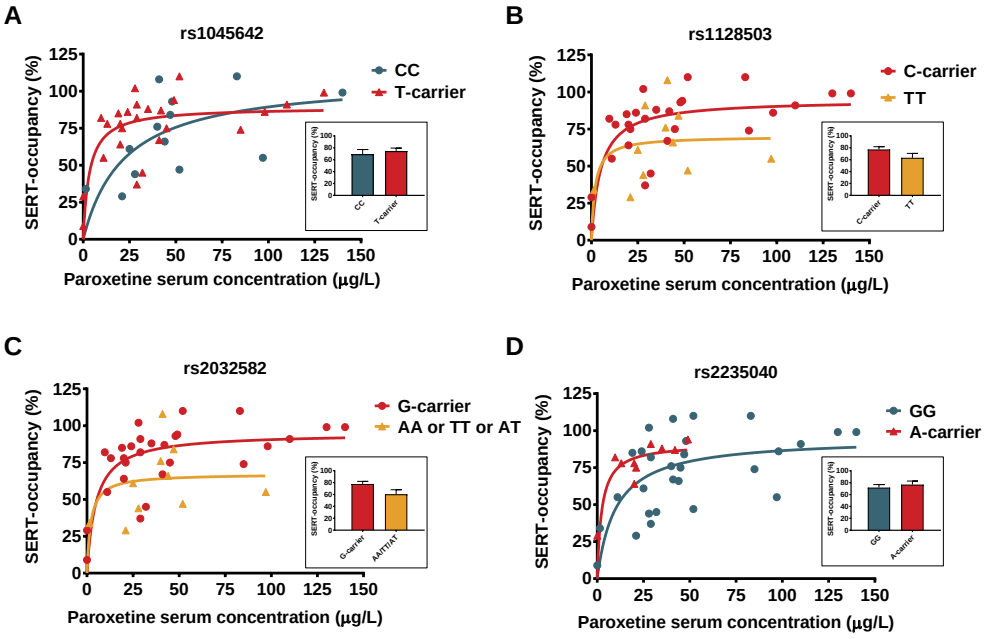

Figure 1. Paroxetine serum concentration and SERT-occupancy by paroxetine, stratified by *ABCB1* gene carriership of the mutant allele at rs1045642 and rs2235040 and carriership of the wildtype allele at rs1128503 and rs2032582. PSC and SERT-occupancy after 6 weeks of 20 mg/day paroxetine ($OCC_{6\ weeks}$) 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: $OCC_{6\ weeks} = a * \frac{PSC}{(b+PSC)}$, 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 in the Supplemental Digital Content. 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).

Relationship between $HDRS_{17}$-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≥0.08, Supplemental table 4A), nor the number of responders (≥50% decrease in $HDRS_{17}$-score; all p≥0.37; Supplemental table 4B) 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≥0.13, Table 3A), nor the number of responders (all p≥0.34; Table 3B) showed significant associations in any of the regression models for genotype, carrier or haplotype groups.

Table 3. Clinical response after 6 weeks of paroxetine 20 mg/day stratified by P-gp carriership at four SNPs (n=81)

A.	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	
B.	**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%)	

[a] Data are given as mean decrease in $HDRS_{17}$ after correction for baseline $HDRS_{17}$-score ± standard error of the mean

[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

Exclusion of the five potentially non-adherent patients (one responder and four non-responders) in our sensitivity analysis did not change these results on baseline-adjusted $HDRS_{17}$-score or response-rate for genotype, carrier or haplotype groups (all $p \geq 0.06$; data not shown). Data were not confounded by age, sex or PSC in any of the regression analyses.

DISCUSSION

In this study, we quantified that two of four previously studied *ABCB1* gene polymorphisms (rs1128503, rs2032582) modify the association between paroxetine serum concentration (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 SERT-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 and/or activity and/or with higher response-rate and/or shorter time to remission, would 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 at the target site.[34,35] 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.[46-49] However, after leaving out the potentially non-adherent 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 (see Table 2B and Figure 1B/C), while we expected the opposite from studies that reported decreased gene expression and diminished function with the rs1128503 variant T-allele[46,49] and reduced protein expression and diminished function for the rs2032582 variant T(/A)-allele[46,49]. 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.[35] 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[16,24,50] or even a (strong) inhibitor instead of a substrate[51,52]. 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 – conform 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 non-carriers 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 (see 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.[19,22,24,25,27,28] As for the variant T-allele of both SNPs inconsistent effects on P-gp gene and protein expression and activity were reported, our non-significant results may at best be indicative of a small, clinically

irrelevant effect on P-gp activity.[35] 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 non-adherence 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.*, showing that this SNP is not associated with clinical response.[19,24,26,27] 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.* including 1252 subjects showed weak evidence of worse response in the GG genotype group compared to the TT genotype (OR=0.75, 95%CI 0.58-0.97).[28] 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-analysed 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.[28] 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.[20,27]

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.[25] 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.[35]

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[34,36] only 38 patients were analysed 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 non-selective radioligand that also binds to dopamine transporters (DAT; e.g. midbrain substantia nigra) and norepinephrine transporter (NET; e.g. locus coeruleus).[53-55] Nevertheless, uptake in the midbrain is considered to reflect predominantly SERT, as this structure is relatively rich of SERT compared to DAT and NET.[56] 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.[39] Therefore, we believe the change in [^{123}I]β-CIT-binding in the midbrain reflects SERT-occupancy in particular.[34] Unfortunately, PET data on [^{11}C]DASB SERT-occupancy after exposure to different SSRIs[31,33] 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.[34] 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, which might have confounded our results.

Sixth, a recent study reported significant, ethnicity-independent, associations of the rs10245483 G/G homozygotes with the SSRIs escitalopram and sertraline[57], while in an elderly population[20] 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.[34] 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. Since 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.[58] A genome wide association (GWAS) 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 paroxetine serum concentration 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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REFERENCES

1. Chen Y, Kelton CM, Jing Y, Guo JJ, Li X, Patel NC. Utilization, price, and spending trends for antidepressants in the US Medicaid Program. Res Social Adm Pharm 2008 Sep;4(3):244-257.
2. Stephenson CP, Karanges E, McGregor IS. Trends in the utilisation of psychotropic medications in Australia from 2000 to 2011. Aust N Z J Psychiatry 2013 Jan;47(1):74-87.
3. Noordam R, Aarts N, Verhamme KM, Sturkenboom MC, Stricker BH, Visser LE. Prescription and indication trends of antidepressant drugs in the Netherlands between 1996 and 2012: a dynamic population-based study. Eur J Clin Pharmacol 2015 Mar;71(3):369-375.
4. Rominger A, Cumming P, Brendel M, Xiong G, Zach C, Karch S, et al. Altered serotonin and dopamine transporter availabilities in brain of depressed patients upon treatment with escitalopram: A [123 I]beta-CIT SPECT study. Eur Neuropsychopharmacol 2015 Jun;25(6):873-881.
5. Yeh YW, Ho PS, Kuo SC, Chen CY, Liang CS, Yen CH, et al. 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. Int J Neuropsychopharmacol 2015 Jan 7;18(7):pyu120.
6. Fava M. Diagnosis and definition of treatment-resistant depression. Biol Psychiatry 2003 Apr 15;53(8):649-659.
7. Trivedi MH, Rush AJ, Wisniewski SR, Nierenberg AA, Warden D, Ritz L, et al. Evaluation of outcomes with citalopram for depression using measurement-based care in STAR*D: implications for clinical practice. Am J Psychiatry 2006 Jan;163(1):28-40.
8. Kugaya A, Sanacora G, Staley JK, Malison RT, Bozkurt A, Khan S, et al. Brain serotonin transporter availability predicts treatment response to selective serotonin reuptake inhibitors. Biol Psychiatry 2004 Oct 1;56(7):497-502.
9. Kato M, Fukuda T, Wakeno M, Fukuda K, Okugawa G, Ikenaga Y, et al. Effects of the serotonin type 2A, 3A and 3B receptor and the serotonin transporter genes on paroxetine and fluvoxamine efficacy and adverse drug reactions in depressed Japanese patients. Neuropsychobiology 2006;53(4):186-195.
10. Bagby RM, Ryder AG, Cristi C. Psychosocial and clinical predictors of response to pharmacotherapy for depression. J Psychiatry Neurosci 2002 Jul;27(4):250-257.
11. Serretti A, Benedetti F, Zanardi R, Smeraldi E. The influence of Serotonin Transporter Promoter Polymorphism (SERTPR) and other polymorphisms of the serotonin pathway on the efficacy of antidepressant treatments. Prog Neuropsychopharmacol Biol Psychiatry 2005 Jul;29(6):1074-1084.
12. Dechant KL, Clissold SP. Paroxetine. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential in depressive illness. Drugs 1991 Feb;41(2):225-253.
13. Feng Y, Pollock BG, Ferrell RE, Kimak MA, Reynolds CF,3rd, Bies RR. Paroxetine: population pharmacokinetic analysis in late-life depression using sparse concentration sampling. Br J Clin Pharmacol 2006 May;61(5):558-569.
14. Sindrup SH, Brosen K, Gram LF, Hallas J, Skjelbo E, Allen A, et al. The relationship between paroxetine and the sparteine oxidation polymorphism. Clin Pharmacol Ther 1992 Mar;51(3):278-287.
15. Domschke K, Tidow N, Schwarte K, Deckert J, Lesch KP, Arolt V, et al. Serotonin transporter gene hypomethylation predicts impaired antidepressant treatment response. Int J Neuropsychopharmacol 2014 Aug;17(8):1167-1176.
16. O'Brien FE, Dinan TG, Griffin BT, Cryan JF. Interactions between antidepressants and P-glycoprotein at the blood-brain barrier: clinical significance of in vitro and in vivo findings. Br J Pharmacol 2012 Jan;165(2):289-312.

17. Cordon-Cardo C, O'Brien JP, Casals D, Rittman-Grauer L, Biedler JL, Melamed MR, et al. Multidrug-resistance gene (P-glycoprotein) is expressed by endothelial cells at blood-brain barrier sites. Proc Natl Acad Sci U S A 1989 Jan;86(2):695-698.
18. Linnet K, Ejsing TB. A review on the impact of P-glycoprotein on the penetration of drugs into the brain. Focus on psychotropic drugs. Eur Neuropsychopharmacol 2008 Mar;18(3):157-169.
19. Gex-Fabry M, Eap CB, Oneda B, Gervasoni N, Aubry JM, Bondolfi G, et al. CYP2D6 and ABCB1 genetic variability: influence on paroxetine plasma level and therapeutic response. Ther Drug Monit 2008 Aug;30(4):474-482.
20. Sarginson JE, Lazzeroni LC, Ryan HS, Ershoff BD, Schatzberg AF, Murphy GM,Jr. ABCB1 (MDR1) polymorphisms and antidepressant response in geriatric depression. Pharmacogenet Genomics 2010 Aug;20(8):467-475.
21. Uhr M, Tontsch A, Namendorf C, Ripke S, Lucae S, Ising M, et al. Polymorphisms in the drug transporter gene ABCB1 predict antidepressant treatment response in depression. Neuron 2008 Jan 24;57(2):203-209.
22. Menu P, Gressier F, Verstuyft C, Hardy P, Becquemont L, Corruble E. Antidepressants and ABCB1 gene C3435T functional polymorphism: a naturalistic study. Neuropsychobiology 2010 Aug;62(3):193-197.
23. Ray A, Tennakoon L, Keller J, Sarginson JE, Ryan HS, Murphy GM, et al. ABCB1 (MDR1) predicts remission on P-gp substrates in chronic depression. Pharmacogenomics J 2015 Aug;15(4):332-339.
24. Mihaljevic Peles A, Bozina N, Sagud M, Rojnic Kuzman M, Lovric M. MDR1 gene polymorphism: therapeutic response to paroxetine among patients with major depression. Prog Neuropsychopharmacol Biol Psychiatry 2008 Aug 1;32(6):1439-1444.
25. Kato M, Fukuda T, Serretti A, Wakeno M, Okugawa G, Ikenaga Y, et al. ABCB1 (MDR1) gene polymorphisms are associated with the clinical response to paroxetine in patients with major depressive disorder. Prog Neuropsychopharmacol Biol Psychiatry 2008 Feb 15;32(2):398-404.
26. Kato M, Serretti A, Nonen S, Takekita Y, Wakeno M, Azuma J, et al. Genetic variants in combination with early partial improvement as a clinical utility predictor of treatment outcome in major depressive disorder: the result of two pooled RCTs. Transl Psychiatry 2015 Feb 24;5:e513.
27. Breitenstein B, Bruckl TM, Ising M, Muller-Myhsok B, Holsboer F, Czamara D. ABCB1 gene variants and antidepressant treatment outcome: A meta-analysis. Am J Med Genet B Neuropsychiatr Genet 2015 Jun;168B(4):274-283.
28. Niitsu T, Fabbri C, Bentini F, Serretti A. Pharmacogenetics in major depression: a comprehensive meta-analysis. Prog Neuropsychopharmacol Biol Psychiatry 2013 Aug 1;45:183-194.
29. Yasui-Furukori N, Saito M, Niioka T, Inoue Y, Sato Y, Kaneko S. Effect of itraconazole on pharmacokinetics of paroxetine: the role of gut transporters. Ther Drug Monit 2007 Feb;29(1):45-48.
30. Ruhe HG, Visser AKD, Frokjaer V, Haarman BCM, Klein HC, Booij J. Chapter 5. Molecular imaging of depressive disorders. In: Dierckx RAJO, Otte A, de Vries EFJ, Waarde A, den Boer JA, editors. PET and SPECT in psychiatry: Springer Berlin Heidelberg; 2013. p. 93-171.
31. Meyer JH, Wilson AA, Sagrati S, Hussey D, Carella A, Potter WZ, et al. Serotonin transporter occupancy of five selective serotonin reuptake inhibitors at different doses: an [11C]DASB positron emission tomography study. Am J Psychiatry 2004 May;161(5):826-835.
32. Suhara T, Takano A, Sudo Y, Ichimiya T, Inoue M, Yasuno F, et al. High levels of serotonin transporter occupancy with low-dose clomipramine in comparative occupancy study with fluvoxamine using positron emission tomography. Arch Gen Psychiatry 2003 Apr;60(4):386-391.
33. Meyer JH, Wilson AA, Ginovart N, Goulding V, Hussey D, Hood K, et al. Occupancy of serotonin transporters by paroxetine and

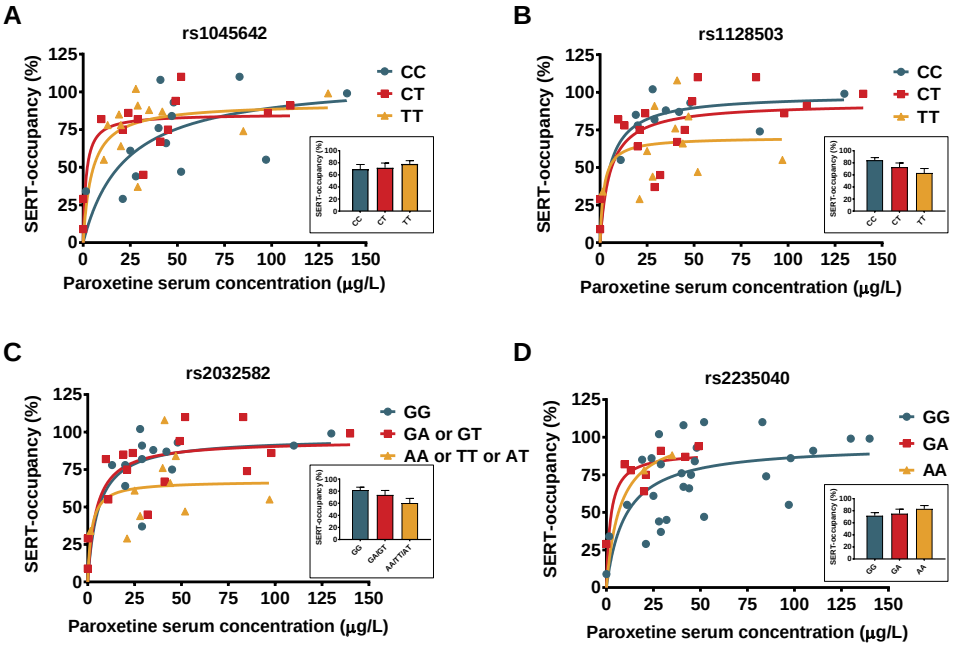
citalopram during treatment of depression: a [(11)C]DASB PET imaging study. Am J Psychiatry 2001 Nov;158(11):1843-1849.

34. Ruhe HG, Ooteman W, Booij J, Michel MC, Moeton M, Baas F, et al. Serotonin transporter gene promoter polymorphisms modify the association between paroxetine serotonin transporter occupancy and clinical response in major depressive disorder. Pharmacogenet Genomics 2009 Jan;19(1):67-76.
35. Brambila-Tapia AJ. MDR1 (ABCB1) polymorphisms: functional effects and clinical implications. Rev Invest Clin 2013 Sep-Oct;65(5):445-454.
36. Ruhe HG, Booij J, v Weert HC, Reitsma JB, Franssen EJ, Michel MC, et al. Evidence why paroxetine dose escalation is not effective in major depressive disorder: a randomized controlled trial with assessment of serotonin transporter occupancy. Neuropsychopharmacology 2009 Mar;34(4):999-1010.
37. van Dyck CH, Malison RT, Seibyl JP, Laruelle M, Klumpp H, Zoghbi SS, et al. Age-related decline in central serotonin transporter availability with [(123)I]beta-CIT SPECT. Neurobiol Aging 2000 Jul-Aug;21(4):497-501.
38. de Win MM, Habraken JB, Reneman L, van den Brink W, den Heeten GJ, Booij J. Validation of [(123)I]beta-CIT SPECT to assess serotonin transporters in vivo in humans: a double-blind, placebo-controlled, crossover study with the selective serotonin reuptake inhibitor citalopram. Neuropsychopharmacology 2005 May;30(5):996-1005.
39. Pirker W, Asenbaum S, Hauk M, Kandlhofer S, Tauscher J, Willeit M, et al. Imaging serotonin and dopamine transporters with 123I-beta-CIT SPECT: binding kinetics and effects of normal aging. J Nucl Med 2000 Jan;41(1):36-44.
40. Hamilton M. A rating scale for depression. J Neurol Neurosurg Psychiatry 1960 Feb;23:56-62.
41. Takano A, Suzuki K, Kosaka J, Ota M, Nozaki S, Ikoma Y, et al. A dose-finding study of duloxetine based on serotonin transporter occupancy. Psychopharmacology (Berl) 2006 Apr;185(3):395-399.
42. Kent JM, Coplan JD, Lombardo I, Hwang DR, Huang Y, Mawlawi O, et al. Occupancy of brain serotonin transporters during treatment with paroxetine in patients with social phobia: a positron emission tomography study with 11C McN 5652. Psychopharmacology (Berl) 2002 Dec;164(4):341-348.
43. Catafau AM, Perez V, Plaza P, Pascual JC, Bullich S, Suarez M, et al. Serotonin transporter occupancy induced by paroxetine in patients with major depression disorder: a 123I-ADAM SPECT study. Psychopharmacology (Berl) 2006 Dec;189(2):145-153.
44. Maldonado G, Greenland S. Simulation study of confounder-selection strategies. Am J Epidemiol 1993 Dec 1;138(11):923-936.
45. Morisky DE, Green LW, Levine DM. Concurrent and predictive validity of a self-reported measure of medication adherence. Med Care 1986 Jan;24(1):67-74.
46. Hemauer SJ, Nanovskaya TN, Abdel-Rahman SZ, Patrikeeva SL, Hankins GD, Ahmed MS. Modulation of human placental P-glycoprotein expression and activity by MDR1 gene polymorphisms. Biochem Pharmacol 2010 Mar 15;79(6):921-925.
47. Hitzl M, Drescher S, van der Kuip H, Schaffeler E, Fischer J, Schwab M, et al. The C3435T mutation in the human MDR1 gene is associated with altered efflux of the P-glycoprotein substrate rhodamine 123 from CD56+ natural killer cells. Pharmacogenetics 2001 Jun;11(4):293-298.
48. Wang D, Johnson AD, Papp AC, Kroetz DL, Sadee W. Multidrug resistance polypeptide 1 (MDR1, ABCB1) variant 3435C>T affects mRNA stability. Pharmacogenet Genomics 2005 Oct;15(10):693-704.
49. Salama NN, Yang Z, Bui T, Ho RJ. MDR1 haplotypes significantly minimize intracellular uptake and transcellular P-gp substrate transport in recombinant LLC-PK1 cells. J Pharm Sci 2006 Oct;95(10):2293-2308.
50. Uhr M, Grauer MT, Holsboer F. Differential enhancement of antidepressant penetration

into the brain in mice with abcb1ab (mdr1ab) P-glycoprotein gene disruption. Biol Psychiatry 2003 Oct 15;54(8):840-846.

51. Maines LW, Antonetti DA, Wolpert EB, Smith CD. Evaluation of the role of P-glycoprotein in the uptake of paroxetine, clozapine, phenytoin and carbamazapine by bovine retinal endothelial cells. Neuropharmacology 2005 Oct;49(5):610-617.
52. Weiss J, Dormann SM, Martin-Facklam M, Kerpen CJ, Ketabi-Kiyanvash N, Haefeli WE. Inhibition of P-glycoprotein by newer antidepressants. J Pharmacol Exp Ther 2003 Apr;305(1):197-204.
53. Innis RB, Cunningham VJ, Delforge J, Fujita M, Gjedde A, Gunn RN, et al. Consensus nomenclature for in vivo imaging of reversibly binding radioligands. J Cereb Blood Flow Metab 2007 Sep;27(9):1533-1539.
54. Neumeyer JL, Wang SY, Milius RA, Baldwin RM, Zea-Ponce Y, Hoffer PB, et al. 123I]-2 beta-carbomethoxy-3 beta-(4-iodophenyl) tropane: high-affinity SPECT radiotracer of monoamine reuptake sites in brain. J Med Chem 1991 Oct;34(10):3144-3146.
55. Neumeyer JL, Tamagnan G, Wang S, Gao Y, Milius RA, Kula NS, et al. N-substituted analogs of 2 beta-carbomethoxy-3 beta-(4'-iodophenyl)tropane (beta-CIT) with selective affinity to dopamine or serotonin transporters in rat forebrain. J Med Chem 1996 Jan 19;39(2):543-548.
56. Laruelle M, Baldwin RM, Malison RT, Zea-Ponce Y, Zoghbi SS, al-Tikriti MS, et al. SPECT imaging of dopamine and serotonin transporters with [123I]beta-CIT: pharmacological characterization of brain uptake in nonhuman primates. Synapse 1993 Apr;13(4):295-309.
57. Schatzberg AF, DeBattista C, Lazzeroni LC, Etkin A, Murphy GM,Jr, Williams LM. ABCB1 Genetic Effects on Antidepressant Outcomes: A Report From the iSPOT-D Trial. Am J Psychiatry 2015 Aug 1;172(8):751-759.
58. Bet PM, Verbeek EC, Milaneschi Y, Straver DB, Uithuisje T, Bevova MR, et al. A common polymorphism in the ABCB1 gene is associated with side effects of PGP-dependent antidepressants in a large naturalistic Dutch cohort. Pharmacogenomics J 2016 Apr;16(2):202-208.

SUPPLEMENTAL MATERIALS



Supplemental figure 1. Paroxetine serum concentration (PSC) and SERT-occupancy by paroxetine, stratified by *ABCB1* genotype. PSC and SERT-occupancy after 6 weeks of 20 mg/day paroxetine ($OCC_{6\ weeks}$) stratified by *ABCB1* genotype at rs1045642 (CC n=13/38, CT n=13/38, TT n=12/38; panel A), rs1128503 (CC n=10/38, CT n=17/38, TT n=11/38; panel B), rs2032582 (GG n=13/38, GA/GT n=15/38, AA/AT/TT n=10/38; panel C) and rs2235040 (GG n=28/38, GA n=8/38, AA n=2/38; panel D). Equation fitted: $OCC_{6\,weeks} = a * \frac{PSC}{(b+PSC)}$, 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 Supplementary Table S3. All fitted models were significant throughout all stratifications for genotype (all $F_{6,32}$>44.1; all p<0.0001). Models fit for three curves were not improved relative to no stratification (lower AIC for one fitted curve vs. three fitted curves).

Supplemental methods

Design and setting

Between October 2003 and February 2007, patients were recruited from primary care, the AMC Program for Mood Disorders, and public psychiatric settings. Patients were treated by their referring physician or were referred to the AMC outpatient department. All eligible patients were treated open-label with paroxetine 20 mg/day for six weeks. When severe adverse effects occurred, the dosage was reduced to 10 mg/day and again increased to 20 mg/day after one week. Adherence was checked by pill-counts and medical history.[1] Benzodiazepines (temazepam 10-20 mg/day or oxazepam 10-30 mg/day) were allowed if necessary.

Study population

General inclusion criteria for eligibility for the DELPHI trial were age between 18 and 70 years, major depressive disorder (MDD) determined by the structured clinical interview for DSM-IV (SCID)[2], and a 17-item Hamilton Depression Rating Scale score ($HDRS_{17}$) score above 18.[3] All patients were either drug-free and/or had undergone no more than one antidepressant treatment (other than paroxetine) at an effective dose for ≥6 weeks for the present MDD-episode. Patient aged between 25 and 55 years and drug-free (for >4 weeks and ≥5 half-lives of a previous antidepressant, when treated previously) were asked to additionally participate in DELPHI the SPECT-study. The age restriction was used to reduce variability in SERT-measurements by age.[4]

Exclusion criteria were pregnancy (or wish to become pregnant), bipolar disorder, psychotic features, neurological cognitive impairments (i.e. dementia), primary anxiety and/or substance abuse disorders and acute, severe suicidal ideation. Contrary, secondary co-morbid anxiety and/or substance abuse were allowed to increase applicability of the findings.[5]

Single photon emission computed tomography (SPECT) imaging

Single photon emission computed tomography (SPECT) imaging for in-vivo assessment of SERT availability was performed according to previously described procedures.[6] All scans were made 230±18 (SD) minutes after intravenous injection of 100 MBq iodine-123-labeled 2β-carbomethoxy-3β-(4-iodophenyl)-tropane ([123I]β-CIT), when the radioligand is at equilibrium for SERT binding in brain areas expressing high densities of SERTs, such as the midbrain.[7] To prevent thyroid uptake of [123I], all subjects received oral potassium iodide solution. SPECT imaging was performed 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 (http://www.neurophysics.com). Each acquisition consisted of 15 slices with 3 min scanning time per slice, acquired in a 64 x 64 matrix.[6] After attenuation correction, images were reconstructed in 3D mode (http://www.neurophysics.com).

References

1. Saunders K, Simon G, Bush T, Grothaus L. Assessing the feasibility of using computerized pharmacy refill data to monitor antidepressant treatment on a population basis: a comparison of automated and self-report data. J Clin Epidemiol 1998 Oct;51(10):883-890.
2. First MB, Spitzer RL, Gibbon M, Williams JBW. Structured Clinical Interview for DSM-IV Axis I Disorders Patient Edition (SCID-I/P version 2.0). Translated in Dutch by: Groenestijn, M.A.C., Akkerhuis, G.W., Kupka, R.W., Schneider, N., and Nolen, W.A. Lisse, The Netherlands: Swets&Zeitlinger B.V.; 1999.
3. Hamilton M. A rating scale for depression. J Neurol Neurosurg Psychiatry 1960 Feb;23:56-62.
4. van Dyck CH, Malison RT, Seibyl JP, Laruelle M, Klumpp H, Zoghbi SS, et al. Age-related

decline in central serotonin transporter availability with [(123)I]beta-CIT SPECT. Neurobiol Aging 2000 Jul-Aug;21(4):497-501.

5. Ruhe HG, Booij J, v Weert HC, Reitsma JB, Franssen EJ, Michel MC, et al. Evidence why paroxetine dose escalation is not effective in major depressive disorder: a randomized controlled trial with assessment of serotonin transporter occupancy. Neuropsychopharmacology 2009 Mar;34(4):999-1010.
6. de Win MM, Habraken JB, Reneman L, van den Brink W, den Heeten GJ, Booij J. Validation of [(123)I]beta-CIT SPECT to assess serotonin transporters in vivo in humans: a double-blind, placebo-controlled, crossover study with the selective serotonin reuptake inhibitor citalopram. Neuropsychopharmacology 2005 May;30(5):996-1005.
7. Pirker W, Asenbaum S, Hauk M, Kandlhofer S, Tauscher J, Willeit M, et al. Imaging serotonin and dopamine transporters with 123I-beta-CIT SPECT: binding kinetics and effects of normal aging. J Nucl Med 2000 Jan;41(1):36-44.

Supplemental table 1. Comparison of the characteristics of the study population included in the analyses of SPECT-based SERT-occupancy (n=38) and the patient sample not included in the SPECT-sample (n=43). Both groups were combined for the analyses of clinical response after 6 weeks of paroxetine 20 mg/day

	DELPHI-SPECT population[a] (n=38)	Non-SPECT DELPHI population[a](n=43)	p-value
Age at baseline (years)	41.7±1.4	45.1±1.5	0.10
Sex (female)	25 (65.8%)	29 (67.4%)	0.88
Ethnicity:			0.57
Dutch	22 (57.9%)	21 (48.8%)	
Surinamese-Creole	4 (10.5%)	2 (4.7%)	
Surinamese-Hindu	3 (7.9%)	2 (4.7%)	
Antillean-Aruban	2 (5.3%)	6 (14.0%)	
Other	7 (18.4%)	12 (27.8%)	
Level of education:			0.06
Low	5 (13.2%)	15 (35.7%)	
Middle	24 (63.2%)	21 (50.0%)	
High	9 (23.7%)	6 (14.3%)	
Current smoker	22 (57.9%)	16 (39.0%)	0.09
Alcohol use:			0.05
≤ 7 units/week	35 (92.1%)	32 (76.2%)	
> 7 units/week	3 (7.9%)	10 (23.8%)	
$HDRS_{17}$ at baseline	24.5±0.7	23.9±0.6	0.50
Responders[b]	8 (21.1%)	17 (39.5%)	0.07
First episode	21 (55.3%)	26 (60.5%)	0.64
No of episodes (median (range))	1 (1-10)	1 (1-10)	0.29
Melancholic	28 (96.6%)	27 (81.8%)	0.11
Duration of episode			0.50
<5 months	12 (31.6%)	8 (19.0%)	
5 months – 2 years	22 (57.9%)	29 (69.0%)	
≥ 2 years	4 (10.5%)	5 (11.9%)	

Supplemental table 1. (continued)

	DELPHI-SPECT population[a] (n=38)	Non-SPECT DELPHI population[a](n=43)	p-value
Psychiatric co-morbidity	11 (28.9%)	19 (45.2%)	0.13
Drug-naïve	24 (64.9%)	29 (67.4%)	0.81
Used psychotropic drugs in current episode	3 (7.9%)	8 (18.6%)	0.16
P-gp genotype rs1045642			0.39
CC	13 (34.2%)	9 (20.9%)	
CT	13 (34.2%)	19 (44.2%)	
TT	12 (31.6%)	15 (34.9%)	
P-gp genotype rs1128503			0.37
CC	10 (26.3%)	15 (34.9%)	
CT	17 (44.7%)	21 (48.8%)	
TT	11 (28.9%)	7 (16.3%)	
P-gp genotype rs2032582			0.42
GG	13 (34.2%)	20 (46.5%)	
GT or GA	15 (39.5%)	16 (37.2%)	
AA or TT or TA	10 (26.3%)	7 (16.3%)	
P-gp genotype rs2235040			0.59
GG	28 (73.7%)	35 (81.4%)	
GA	8 (21.1%)	5 (11.6%)	
AA	2 (5.3%)	3 (7.0%)	
rs1045642 C -rs2032582 G-rs1128503 T-haplotype present	14 (36.8%)	18 (41.9%)	0.65

[a] Data are given as number (percentage) or mean ± standard error of the mean unless stated otherwise
[b] Responders defined as patients with ≥50% decrease in baseline $HDRS_{17}$-score

Supplemental table 2. Mean paroxetine serum concentration (PSC; µg/L), mean baseline non-specific binding ratio (BP_{ND}) and mean SERT-occupancy (%) by *ABCB1* SNP genotype in the SPECT-sample (n=38) after 6 weeks of paroxetine 20 mg/day

A. Mean PSC (µg/L) by *ABCB1* SNP genotype[a]

SNP	Homozygote wildtype (genotype; n)	Heterozygote (genotype; n)	Homozygote variant (genotype; n)	p-value
rs1045642	51.3±10.1 (CC; n=13)	39.3±9.3 (CT; n=13)	38.4±10.0 (TT; n=12)	0.58
rs1128503	44.7±11.5 (CC; n=10)	45.1±9.8 (CT; n=17)	38.7±7.2 (TT; n=11)	0.88
rs2032582	44.5±9.8 (GG; n=13)	44.3±10.5 (GA/GT; n=15)	39.7±8.0 (AA/AT/TT; n=10)	0.91
rs2235040	50.0±7.0 (GG; n=28)	23.0±5.8 (GA; n=8)	27.5±7.5 (AA; n=2)	0.12

B. Mean baseline non-displaceable binding potential (BP_{ND}) by *ABCB1* SNP genotype[a]

SNP	Homozygote wildtype (genotype; n)	Heterozygote (genotype; n)	Homozygote variant (genotype; n)	p-value
rs1045642	0.59±0.05 (CC; n=13)	0.63±0.06 (CT; n=13)	0.60±0.05 (TT; n=12)	0.87
rs1128503	0.62±0.04 (CC; n=10)	0.63±0.06 (CT; n=17)	0.57±0.05 (TT; n=11)	0.77

Supplemental table 2. (continued)

SNP	Homozygote wildtype (genotype; n)	Heterozygote (genotype; n)	Homozygote variant (genotype; n)	p-value
rs2032582	0.58±0.05 (GG; n=13)	0.66±0.06 (GA/GT; n=15)	0.57±0.05 (AA/AT/TT; n=10)	0.42
rs2235040	0.59±0.04 (GG; n=28)	0.66±0.09 (GA; n=8)	0.71±0.02 (AA; n=2)	0.51

C. Mean SERT-occupancy (%) by *ABCB1* SNP genotype[a]

SNP	Homozygote wildtype (genotype; n)	Heterozygote (genotype; n)	Homozygote variant (genotype; n)	p-value
rs1045642	69.6±7.6 (CC; n=13)	71.8±7.8 (CT; n=13)	78.1±5.4 (TT; n=12)	0.69
rs1128503	84.3±4.3 (CC; n=10)	72.8±6.9 (CT; n=17)	63.1±7.4 (TT; n=11)	0.15
rs2032582	81.9±4.7 (GG; n=13)	73.8±7.4 (GA/GT; n=15)	60.4±7.6 (AA/AT/TT; n=10)	0.12
rs2235040	71.7±5.1 (GG; n=28)	75.1±7.4 (GA; n=8)	83.0±5.5 (AA; n=2)	0.81

[a] Data are given as mean ± standard error of the mean

Supplemental table 3. EC_{50} and E_{max} values and 95% CI for the unstratified model of paroxetine serum concentration (PSC) and SERT-occupancy by paroxetine, and for all models stratified by *ABCB1* genotype and carriership (n=38)

Stratification of the model	EC_{50}	95% confidence interval EC_{50}	EC_{max}	95% confidence interval EC_{max}
None	4.0	-1.4-9.5	87.1	72.6-101.6
rs1045642 – CC genotype	22.3	-19.8-64.3	109.2	44.53-174.0
rs1045642 – CT genotype	1.6	-5.8-9.0	85.3	63.0-107.6
rs1045642 – TT genotype	4.7	-4.9-14.3	92.7	62.4-123.0
rs1128503 – CC genotype	5.0	-1.6-11.7	98.7	79.3-118.0
rs1128503 – CT genotype	5.3	-4.1-14.7	93.0	68.5-117.5
rs1128503 – TT genotype	3.0	-4.7-8.8	70.11	47.1-93.12
rs2032582 – GG genotype	5.6	-4.9-16.0	96.3	68.6-124.0
rs2032582 – GT or GA genotype	4.5	-3.2-12.2	94.5	72.1-116.8
rs2032582 – TT or AA or TA genotype	2.1	-5.2-9.3	67.3	43.4-91.3
rs2235040 – GG genotype	10.1	-2.7-22.9	95.2	70.79-119.7
rs2235040 – GA genotype	2.2	-6.0-10.4	90.5	55.4-125.5
rs2235040 – AA genotype	7.2	N/A	106.1	N/A
rs1045642 –carriership of variant T-alelle	3.4	-2.2-8.9	89.2	72.4-106.1
rs1128503 – carriership of wildtype C-allele	5.0	-0.8-10.8	94.8	78.8-110.7
rs2032582 – carriership of wildtype G-allele	4.9	-0.7-10.5	95.0	79.4-110.5
rs2235040 – carriership of variant A-allele	2.5	-4.2-9.1	91.3	64.4-118.3

The formula for the PSC-SERT-occupancy curve is: SERT-occupancy. The E_{max} is the maximum occupancy that can be reached and the EC_{50} represents the PSC at which 50% occupancy is reached. PSC paroxetine serum concentration; SERT serotonin transporter.

Supplemental table 4. Clinical response after 6 weeks of paroxetine 20 mg/day stratified by P-gp genotype at four SNPs (n=81)

A.	n	Decrease in $HDRS_{17}$-score[a]	p-value[b]
P-gp genotype rs1045642			
CC	22	5.9±0.03	(reference)
CT	32	7.7±0.03	0.32
TT	27	9.0±0.03	0.08 (0.37 vs. CT)
P-gp genotype rs1128503			
CC	25	8.2±0.02	(reference)
CT	38	7.9±0.02	0.99
TT	18	6.3±0.02	0.36 (0.32 vs. CT)
P-gp genotype rs2032582			
GG	33	7.3±0.01	(reference)
GA or GT	31	8.0±0.01	0.84
AA or AT or TT	17	7.5±0.01	0.92 (0.78 vs. GT/GA)
P-gp genotype rs2235040			
GG	63	8.3±0.00	(reference)
GA	13	5.1±0.01	0.25
AA	5	6.0±0.01	0.48 (0.97 vs. GA)
B.	**n**	**Number of responders[c]**	**p-value[d]**
P-gp genotype rs1045642			0.37
CC	22	5 (22.7)	
CT	32	9 (28.1)	
TT	27	11 (40.7)	
P-gp genotype rs1128503			0.83
CC	25	7 (28.0)	
CT	38	13 (34.2)	
TT	18	5 (27.8)	
P-gp genotype rs2032582			0.92
GG	33	11 (33.3)	
GA or GT	31	9 (29.0)	
AA or AT or TT	17	5 (29.4)	
P-gp genotype rs2235040			0.86
GG	63	20 (31.7)	
GA	13	4 (30.8)	
AA	5	1 (20.0)	

[a] Data are given as mean decrease in $HDRS_{17}$ after correction for baseline $HDRS_{17}$-score ± standard error of the mean

[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