

Serotonin transporter genotype interacts with paroxetine plasma levels to influence depression treatment response in geriatric patients

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Objective: To investigate whether variable antidepressant response may be influenced by an interaction between the serotonin transporter promoter polymorphism (5-HTTLPR) and antidepressant concentration. **Methods:** Elderly subjects with depression treated with paroxetine ($n = 110$) were genotyped and assessed with the Hamilton Rating Scale for Depression (HAMD). A mixed-effect analysis of repeated measures was used. **Results:** There was an interaction between early paroxetine concentration and 5-HTTLPR genotype on symptomatic improvement over 12 weeks ($F_{18,59.5} = 1.8, p < 0.05$), as well as main effects of both paroxetine concentration ($F_{68,55.3} = 2.4, p < 0.005$) and genotype ($F_{2,74.2} = 5.7, p < 0.005$). Paroxetine concentrations were correlated with change in HAMD scores after 2 weeks of treatment in subjects with the short (s) allele ($r = 0.31, p < 0.05$) but not in subjects homozygous for the long (l) allele. **Conclusion:** The results demonstrate a concentration–response relation for paroxetine in late-life depression and support the hypothesis for both a direct main effect and a moderating influence of 5-HTTLPR alleles on this concentration–response relation.

Objectif : Savoir si la réponse variable aux antidépresseurs peut être influencée par une interaction entre le polymorphisme du promoteur du transporteur de la sérotonine (5 HTTLPR) et la concentration d'antidépresseur. **Méthodes :** On a déterminé le génotype de sujets âgés atteints de dépression traitée à la paroxétine ($n = 110$) et on les a évalués au moyen de l'échelle de dépression de Hamilton (HAMD). On a utilisé une analyse à effets mixtes de mesures répétées. **Résultats :** On a constaté qu'une interaction entre la concentration de paroxétine au début et le génotype de la 5 HTTLPR était associée à une amélioration des symptômes en 12 semaines ($F_{18,59.5} = 1.8, p < 0,05$), et que la concentration de paroxétine ($F_{68,55.3} = 2.4, p < 0,005$) et le génotype ($F_{2,74.2} = 5.7, p < 0,005$) étaient associés à des effets principaux. On a établi une corrélation entre les concentrations de paroxétine et l'évolution des résultats du test HAMD après deux semaines de traitement chez les sujets qui avaient l'allèle (s) court ($r = 0,31, p < 0,05$), mais non chez les sujets homozygotes pour l'allèle long (l). **Conclusion :** Les résultats démontrent l'existence d'un lien concentration-réponse pour la paroxétine dans le cas de la dépression chez les personnes âgées et appuient l'hypothèse selon laquelle les allèles de la 5 HTTLPR ont à la fois un effet principal direct et une influence modératrice sur cette relation concentration-réponse.

Introduction

Many patients with depression do not achieve full remission with selective serotonin reuptake inhibitors (SSRIs) or else

improve very slowly.¹⁻³ Hypotheses for this response variability invoke differences in SSRI levels, because there can be a wide range of SSRI concentrations^{2,4} (hypothesis A), or genetic differences in pharmacodynamic sensitivity^{5,6} (hypothesis B),

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or both. In addition to having a main effect on response, genetic variability could also influence the concentration–response relation (hypothesis C), that is, affect SSRI potency and thus shift the concentration–response curve. In combination, all 3 hypotheses may potentially be clinically useful in guiding treatment approaches to SSRI nonresponders.

The following findings are consistent with hypothesis A (a role for differences in SSRI levels):

- Dropout rates for SSRIs can have dose–response relations.^{7,8}
- There is a concentration–response relation for SSRIs on acute neuroendocrine response.⁹
- Obese patients may have a poorer antidepressant response to fluoxetine.¹⁰
- There is evidence for a dose–response relation for the antidepressant effect of fluoxetine.¹¹
- In the treatment of neuropathic pain, a concentration–response relation has been described for paroxetine.¹²
- Brain paroxetine concentrations were found to be correlated with brain activity as assessed with the use of fluorine magnetic resonance spectroscopy.¹³
- In mice, there is an association between paroxetine concentration, brain serotonin transporter binding and changes in behaviour.¹⁴
- Finally, consistent with these various findings, a recent meta-analysis supported a dose–response relation for SSRIs.¹⁵

Nonetheless, most clinical trials have generally failed to observe a concentration dependence for SSRIs.^{16–19} One explanation is that there may be genetic heterogeneity that obscures evidence for it.⁷ It is plausible that different SSRI concentration–response curves can result from genetic differences in serotonin transporter (SERT) function (hypothesis C).

In addition to a possible main effect of concentration, a main effect of genetic differences (hypothesis B) has been observed. Genetic variation in the SERT promoter (5-HTTLPR) can influence response to SSRIs, as replicated in several studies²⁰ although certainly not in all.^{21,22} Specifically, in late-life depression, patients of European ethnicity with the short (s) allele in 5-HTTLPR responded less quickly to both paroxetine²³ and sertraline.²⁴

Consistent with this, the acute functional effect of SSRIs on various brain regions as determined by positron emission tomography is associated with genetic differences in 5-HTTLPR.²⁵ At a cellular level, cells with the short (s) allele can have 50% less SERT expression than cells that are homozygous for the long (l) allele,²⁶ and they also have lower maximal transport of serotonin.²⁷

A potential clinical implication of these findings is that, if the SSRI concentration is not at a maximally inhibiting level for a particular genotype, then an increased concentration could have a beneficial effect for individuals with that genotype. Some elderly subjects prescribed low dosages or who are poorly adherent to treatment may be in this category. Conversely, if the SSRI concentration is already achieving maximal inhibition of SERT for a given genotype, then increasing the concentration should have no additional benefit.

Supporting an interaction between SERT genotype and SSRI concentration (hypothesis C), we initially noted that

patients with low paroxetine plasma levels may have a slower antidepressant response if they have the 5-HTTLPR s allele.²⁸ Therefore, to explicitly test whether there is a concentration by genotype interaction, we combined 2 cohorts involving paroxetine treatment of older adults: a Maintenance Treatment of Late-Life Depression-2 (MTLD-2)²⁹ cohort and a cohort from a double-blind study of paroxetine and nortriptyline.³⁰ The specific hypothesis was that lower paroxetine plasma concentrations would more adversely affect subjects with the s allele, whereas subjects with the l/l genotype would be less affected by low concentrations. This would result in a concentration by genotype interaction. We also tested for main effects of both concentration and genotype. These results may have relevance for guiding the early (within the first few weeks) individualized treatment of major depression in those who are poor responders to SSRIs.

Methods

Subjects

Participants provided written informed consent, as approved by the University of Pittsburgh Medical Center Institutional Review Board, as well as similar additional consent for genotyping. The first cohort was limited to genotyped subjects from a clinical trial comparing paroxetine and nortriptyline³⁰ who had 2-week paroxetine levels available ($n = 47$). They were aged 60 years or older and started on 20 mg paroxetine. Paroxetine dosages were increased to 30 mg after 5 weeks in subjects who still had a score of 15 or above on the 17-item Hamilton Rating Scale for Depression (HAMD) or who had experienced a decrease in HAMD score of less than 50%. The patients in the second cohort, from the MTLD-2,²⁹ were started on open-label paroxetine treatment. The subset included in this study were those for whom genotyping was obtained, along with acute plasma paroxetine levels at either week 2 ($n = 49$) or week 3 ($n = 14$). Subjects without blood samples available between days 12 and 24 were not included in these analyses. These subjects, aged 70 years or older, were initially treated with paroxetine in dosages that were openly adjusted and augmented by weekly interpersonal psychotherapy.

All subjects met DSM-IV³¹ criteria for a major depressive episode and were without psychotic features or any unstable medical condition. Many had 4–6 chronic medical problems typical of this age group and scored 15 or higher on the 17-item HAMD as well as 18 or higher on the Folstein Mini-Mental State Examination. Benzodiazepines could be provided as needed.^{29,30}

Paroxetine concentration and genotyping

In both cohorts, plasma samples for paroxetine levels were planned and were obtained regardless of treatment response. Neither paroxetine levels nor genotypes were available to the clinicians involved in treating the patients and therefore did not influence treatment decisions. We assessed paroxetine concentrations from plasma, using high performance liquid chromatography with ultraviolet detection according to

methods previously described.³² Paroxetine levels were obtained weekly for all subjects in cohort 1, all of whom therefore had paroxetine levels available for week 2. In cohort 2, blood samples were obtained intermittently and variably during treatment. Of 63 subjects, 49 had week 2 (day 12–18 after the start of treatment) paroxetine levels available; for the other 14 subjects, week 3 levels (day 19–24 after the start of treatment) were used. Other than plasma levels, objective adherence assessments, such as electronic monitoring, were not routinely included in these studies.

For genotyping, high-molecular-weight DNA was isolated from blood lymphocytes with the PureGene kit (Gentra Systems, Minneapolis, MN) and amplified (polymerase chain reaction) with the flanking primers 5'-CTTGTTGGGGATTCTCCCGCCTGGCGT T-3' (forward) and 5'-TCGAGGCTGAGCGTCTAGAG-GGACTGAGCTGG-3' (reverse). Amplification products were resolved by electrophoresis on 2% agarose gels and visualized with ethidium bromide staining, as previously described.²³ Alleles were designated s (484 bp) and l (528 bp) by direct comparison with control samples run on the same gel. Both concentration measures and genotyping were performed blind to treatment and treatment outcome.

Statistics

Analyses used SPSS 13.0. The primary outcome was the change in baseline HAMD score over the course of 12 weeks of treatment for major depression. Sixty-three percent (29/47) of cohort 1 and 92% (58/63) of cohort 2 completed at least 10 weeks of treatment for major depression. Mixed-effect analyses of repeated measures were performed, with genotype and early exposure as fixed effects. Early exposure was defined as the plasma paroxetine concentration at week 2 (or week 3 when week 2 plasma levels were unavailable). Because of the repeated-measures design, a first-order antedependence covariance structure was used³³; according to Akaike's information criteria, it provided a better fit than either autoregressive or heterogeneous Toeplitz structures. The primary hypothesis was that there would be a genotype \times exposure interaction, although both main effects were also examined. We also included time as a factor, but to maximize statistical power, we did not assess interactions with time. The analysis controlled for multiple covariates: the cohort, baseline HAMD score, cumulative history of past antidepressant treatment obtained from the Antidepressant Treatment History Form (ATHF),³⁴ strength of prior antidepressant treatment obtained from the ATHF, use of benzodiazepines during the trial (yes or no), any use of estrogens and any use of thyroid hormone. The analysis was also performed without these covariates and then performed including them individually. Mixed-effect analyses of repeated measures were also used to examine whether any of the covariates individually influenced symptomatic improvement and also to compare drug levels and dosages between groups over time.

To help us interpret the results from these primary analyses, we then examined improvement in HAMD scores at week 2 for correlations with paroxetine levels during treat-

ment for major depression (defined as above). In this exploratory analysis, we elected to combine the s/s and s/l groups for consistency with prior findings in geriatric treatment response,^{23,24} and on the basis of power considerations (there are relatively few s/s subjects). We then created a receiver operating curve (ROC) for subjects with the s allele, using early paroxetine levels, and defining a HAMD score $<$ 7 by week 12 as categorical remission.

We used a χ^2 test to assess for Hardy–Weinberg equilibrium, and to compare demographic characteristics between groups. We also used *t* tests and analyses of variance to compare demographic characteristics between groups. Unless indicated otherwise, results are reported as mean and standard error of the mean (SEM).

Results

Demographics

The 5-HTTLPR was in Hardy–Weinberg equilibrium in both cohorts (19 l/l, 22 s/l, 6 s/s; and 21 l/l, 35 s/l, 7 s/s, respectively), and the frequencies of the s and l alleles were similar to those reported for populations of European ethnicity.³⁵ In both cohorts, the 2 genetic groups (those with an s allele and those without s alleles) did not significantly differ in age, weight, percentage of women or percentage of African Americans (Table 1), nor were there any demographic differences between groups with low paroxetine exposure ($<$ 60 ng/mL at week 2 or 3) and high paroxetine exposure ($>$ 60 ng/mL at week 2 or 3). Finally, there were no significant demographic differences between subjects in our study

Table 1: Demographic characteristics of cohort 1 and cohort 2 across either genotype (those with and without s allele) or paroxetine exposure (high v. low exposure at week 2 or 3)

Characteristic	No. of subjects (and %)*			
	No s allele	With s allele	Exposure	
			High†	Low‡
Cohort 1, <i>n</i>	19	28	9	36
African American	2 (10.5)	4 (14.3)	1 (11.1)	5 (13.9)
Female:male (% female)	14:5 (74)	23:5 (82)	8:1 (88.9)	26:10 (72.2)
Mean age (and SD), y	72.0 (7.8)	70.8 (7.4)	74.2 (6.9)	69.0 (7.2)
Mean weight (and SD), kg	71.5 (18.6)	75.7 (21.4)	72.0 (20.0)	72.9 (19.4)
Cohort 2, <i>n</i>	21	42	23	40
African American	2 (9.5)	2 (4.8)	1 (4.3)	3 (7.5)
Female:male (% female)	11:10 (52)	29:13 (69)	20:3 (87)	20:20 (50)
Mean age (and SD), y	78.1 (5.5)	76.9 (5.8)	77.3 (4.8)	77.1 (5.7)
Mean weight (and SD), kg	73.7 (15.0)	69.8 (15.3)	69.9 (14.6)	72.9 (15.0)

SD = standard deviation.

*Unless otherwise indicated.

† $>$ 60 ng/mL.

‡ $<$ 60 ng/mL.

compared with subjects from the 2 studies^{29,30} who had not been genotyped.

Paroxetine exposure

The average paroxetine levels in cohort 2 subjects did not significantly differ between the 2 genetic groups over time, although levels did rise over time (Table 2), similar to prior published observations in cohort 1.²³ This is consistent with paroxetine inhibiting its own metabolism. Notably, low-exposure subjects continued to have low paroxetine levels throughout the entire treatment period, differing from high-exposure subjects ($F_{1,60.4} = 159, p < 0.001$) (Table 2). Lower plasma levels were associated with lower dosages. At week 2, plasma levels also trended to being inversely associated with weight ($r = 0.2, p = 0.05$). The role of adherence (measured by electronic cap monitoring) or variability related to time of last dosage could not be explicitly assessed.

Improvement in depression symptoms

The mixed-effect analysis of repeated measures controlling for all the covariates (Table 3) supported the primary hypothesis of an interaction between early paroxetine exposure and genotype on symptomatic improvement ($F_{18,59.5} = 1.8, p < 0.05$). There was also a main effect of concentration ($F_{68,55.3} = 2.4, p < 0.005$) and of genotype ($F_{2,74.2} = 5.7, p < 0.005$). The results were not affected by excluding African Americans. Notably, the results were also not significantly affected by excluding the covariates or including them singly. Exploratory post hoc assessments of individual genotypes using the mixed-effect analyses indicated that early paroxetine exposure influenced symptomatic improvement in all 3 genotypes (l/l, s/l and s/s: $F_{33,6.37} = 4.3, p < 0.05$; $F_{43,18.1} = 4.6, p < 0.0005$; and $F_{10,31.1} = 6.3, p < 0.0005$, respectively), although the significant interaction was because of a larger effect size in those with the s allele, as hypothesized.

There were no significant differences between genotypes in any of the covariates examined. Examination of individual covariates as fixed factors in separate mixed-effect analyses indicated that neither cumulative antidepressant treatment history nor the strength of prior treatment influenced improvement in symptoms in these studies ($F_{12,126.98} = 0.8$ and $F_{4,129.28} = 1.2$, respectively). However, baseline HAMD score was associated with symptomatic improvement over 12 weeks ($F_{21,307.8} = 6.6, p < 0.005$). The use of benzodiazepines

was also favourably associated with treatment outcome ($F_{1,145.8} = 6.5, p < 0.05$).

Examination of early symptomatic improvement at week 2

Paroxetine concentration was significantly correlated with improvement in HAMD scores at week 2 in subjects with the s allele (intercept 4.2, slope 0.04, $r = 0.31, p < 0.05$) but not in subjects with l/l alleles (intercept = 7.9; slope = 0.01; $r = 0.12; p = 0.45$). The s/l and s/s subjects had fairly similar intercepts and slopes (for s/l subjects, 4.3 and 0.04, respectively; for s/s subjects, 3.6 and 0.04, respectively), supporting their inclusion together. Despite this post hoc evidence, because there were only 39 l/l subjects, there was only 52% power to detect a slope of 0.036 (given $\alpha = 0.05$ and the measured variability in l/l subjects). For the combined group of s allele subjects (given $\alpha = 0.05$ and the measured variability in s subjects),

Table 3: Covariates included in the repeated-measure analyses

Variable	High exposure*: mean (and SD)‡		Low exposure†: mean (and SD)‡	
	Without s allele (l/l)	With s allele (s/l or s/s)	Without s allele (l/l)	With s allele (s/l or s/s)
Early (wk 2 or 3) paroxetine concentration, ng/mL	95.1 (12.9)	97.7 (7.0)	26.8 (4.1)	28.7 (2.3)
Baseline HAMD score	22.8 (0.9)	20.8 (1.0)	21.3 (1.1)	19.4 (0.5)
Prior antidepressant strength in current episode§	1.7 (0.4)	1.8 (0.4)	1.3 (0.2)	1.5 (0.2)
Cumulative antidepressant strength over history of illness§	2.8 (0.9)	3.1 (0.9)	1.1 (0.3)	1.6 (0.3)
Used another antidepressant, %	7.7	4.8	7.7	0
Used benzodiazepines, %	46.2	19.0	38.5	26.1
Used estrogen, %	0	24.8	11.5	13.0
Used thyroid hormone, %	7.7	9.5	19.2	13.0

SD = standard deviation; HAMD = 17-item Hamilton Rating Scale for Depression.
 * > 60 ng/mL.
 † < 60 ng/mL.
 ‡ Unless otherwise indicated.
 § From the Antidepressant Treatment History Form, which rates prior drug trials from 0 to 4 (e.g., 0 = no prior treatment, 1 = less than 4 weeks of treatment, 4 = at least 4 weeks of high antidepressant dose).

Table 2: Cohort 2, paroxetine levels and doses given during the 12-week period

Subject group	Period; mean (and SEM)							
	Week 2 or 3		Week 4		Week 6		Week 10	
	Dose, mg	Level, ng/mL	Dose, mg	Level, ng/mL	Dose, mg	Level, ng/mL	Dose, mg	Level, ng/mL
l/l allele (n = 21)	16.1 (2.0)	43.9 (11.9)	21.8 (2.5)	77.9 (7.21)	24.5 (2.7)	89.7 (24.2)	30.0 (6.3)	154.6 (59.5)
s allele (n = 42)	19.2 (1.0)	55.6 (7.1)	23.5 (1.2)	100.8 (6.9)	26.4 (1.7)	110.7 (18.4)	36.7 (3.3)	185.7 (83.2)
Low exposure (n = 40)	17.7 (1.2)	28.8 (3.6)	20.9 (1.5)	46.6 (8.4)	23.5 (2.1)	62.8 (9.9)	25.0 (3.8)	92.4 (18.3)
High exposure (n = 23)	21.7 (2.1)	101.3 (10.1)	27.0 (2.1)	165.5 (20.5)	31.1 (2.6)	203.9 (21.2)	30.1 (3.7)	200.0 (29.6)

SEM = standard error of mean.

the power to detect a slope of 0.036 was estimated to be 72%. Moreover, with this type of regression analysis, to detect statistically different concentration–response slopes (0.036 v. 0.012) between the 2 genetic groups would require 202 1/1 subjects for 80% power. The differences in concentration–response at 2 weeks are illustrated in Figure 1.

ROC

Defining categorical remission as HAMD scores less than or equal to 7 by week 12, we generated an ROC to explore the subsequent predictive ability of early 2-week paroxetine levels. ROCs are plots of true positives versus false positives and are designed to examine the predictive ability of different values (e.g., early paroxetine concentrations) to determine a categorical outcome (e.g., remission by week 12). A relation

with zero predictive ability (where the percentages of true and false positives are always equal) will have an area under the curve (AUC) of 0.5. The ROC for subjects with the s allele had an AUC of 0.63 ($p < 0.05$, compared with 0.5). When the acute paroxetine level was < 60 ng/mL, there was about 88% sensitivity for identifying categorical nonremitters, albeit with a low specificity of 64% (Fig. 2). The AUC for 1/1 subjects did not differ significantly from 0.5. As illustrated in Figure 3, subjects with the s allele improved more slowly than 1/1 subjects when acute levels were < 60 ng/mL, consistent with the findings of the mixed-effect analysis. At higher concentrations, all genotypes responded to SSRI treatment similarly.

Discussion

The primary hypothesis of an interaction between genotype

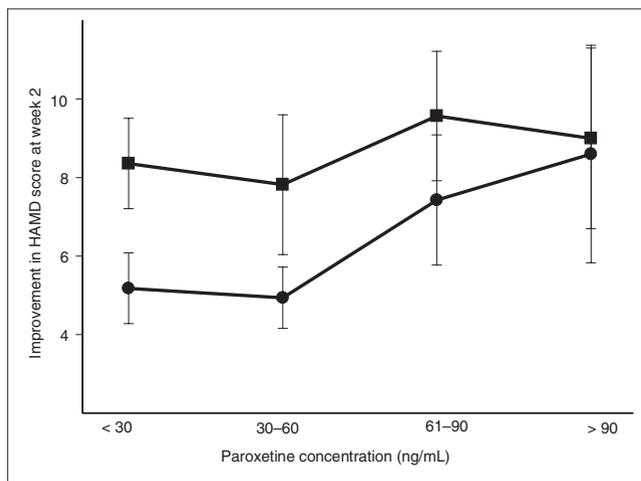


Fig. 1: Improvement in HAMD scores (mean and SEM) at week 2 of paroxetine treatment increases with acute paroxetine concentrations in s allele subjects (circles, linear $r = 0.31$ $p < 0.05$) but not 1/1 subjects (squares, $r = 0.12$ $p = 0.45$). HAMD = Hamilton Rating Scale for Depression; SEM = standard error of the mean.

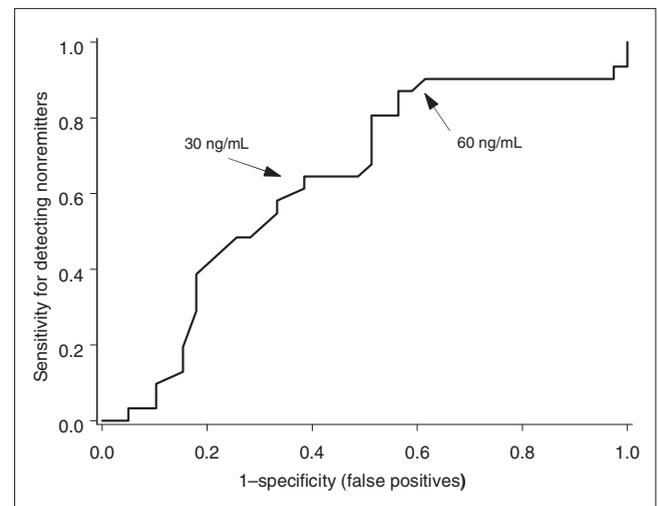


Fig. 2: Receiver operating curve for categorical remission (HAMD < 7), as predicted by early paroxetine levels for treatment of major depression ($n = 69$; area under the curve = 0.63; $p < 0.05$). HAMD = Hamilton Rating Scale for Depression.

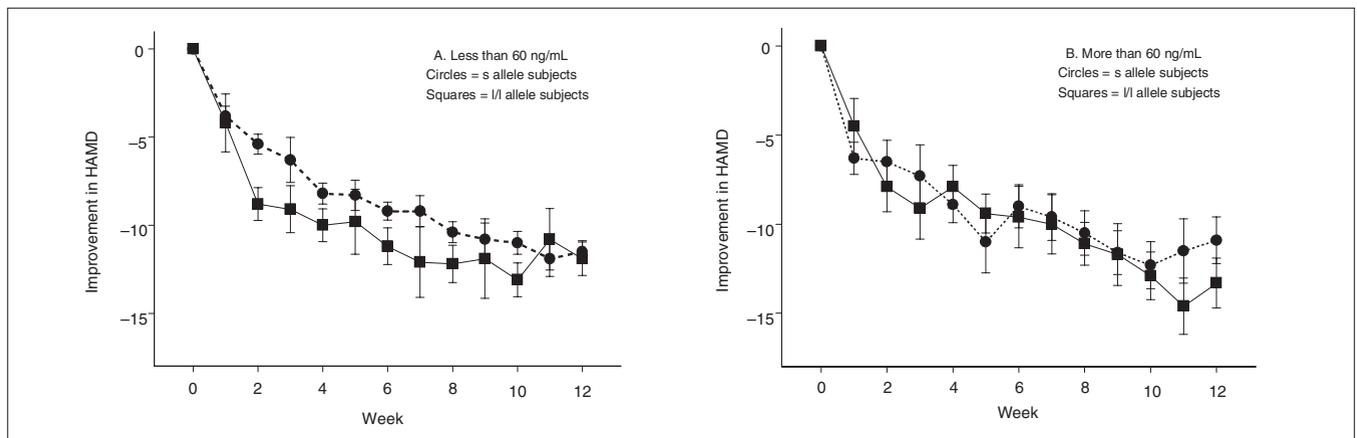


Fig. 3: The interaction between genotype and nondichotomized paroxetine levels was significant. To illustrate this finding, mean (and SEM) improvement in HAMD scores for s allele carriers (circles) and 1/1 homozygotes (squares) was dichotomized into 2 paroxetine exposure groups: (A) describes subjects with paroxetine concentrations < 60 ng/mL, and (B) describes subjects with concentrations > 60 ng/mL. HAMD = Hamilton Rating Scale for Depression.

and concentration was supported: the 5-HTTLPR genotype influenced the SSRI concentration–response relation in elderly patients with depression. Conversely, the SSRI concentration can influence whether 5-HTTLPR is associated with antidepressant outcome. Of clinical importance, these findings suggest that it may be feasible to rationally decide whether to increase dosage or change medication in poorly responding patients, particularly because early improvement is often predictive of later response.³⁶ At week 2, if the poorly responding patient has both the *s* allele and levels well below 60 ng/mL, the dosage may need to be increased or adherence problems addressed, or both. If the concentration is above 60 ng/mL or the patient has the *l/l* genotype, then increasing the concentration may be less effective. Additional studies are needed to confirm the utility of this hypothesis and to determine the optimal concentration threshold for clinical decision making. A value of about 60 ng/mL is very preliminary at this point. Moreover, before clinical implementation, these initial results will need to be replicated, in particular with other SSRIs and in other age groups.

We also confirmed that there is a main effect of early paroxetine concentration, an observation not always made in clinical trials.^{16–19} Notably, we explicitly focused on early (week 2) paroxetine concentrations rather than on later time points. The decision for this was multifold. First, these were not fixed-dose studies. Because dosages could be adjusted in reaction to adverse side effects or poor response, the clinical status of the subjects likely influenced concentrations at later time points rather than vice versa. Second, paroxetine is capable of inhibiting its own metabolism.³⁷ At later time points, most 5-HT transporters are likely to be occupied regardless of genotype,³⁸ and paroxetine may even start to inhibit norepinephrine reuptake.³⁹ The results of this study are therefore limited to concentrations in early treatment for major depression. We also did not fully explain the sources of variable paroxetine exposure. Electronic cap monitoring could have provided information regarding adherence but was not used. The dosage partially accounted for plasma levels, but poor adherence, higher weight and genetic differences in metabolism³⁷ could be additional reasons for low plasma levels.

We also replicated prior findings of a main effect of genotype on response.^{23,24,40–43} 5-HTTLPR is associated with functional differences in response to SSRIs used to treat major depression²⁵ despite the fact that these functional differences may not be the result of global changes in SERT expression.⁴⁴ Although the association with response has not always been observed,²¹ SSRI concentrations were not obtained in most studies. It is thus not clear whether past negative findings are the result of confounds associated with concentration differences. This is particularly salient in primary care settings, where adherence can be problematic.⁴⁵

However, there are some caveats that require comment. First, possible population stratification was not accounted for.⁴⁶ Although patients were from the same cohort (this is not a case–control study), which may mitigate against this prospect, population stratification is still a possibility that may confound the results. Because there were only 10 African-American subjects in this study, excluding them from the

analyses did not significantly affect the results. However, we cannot make any definitive statements about concentrations or genotype in African Americans. We must limit our conclusions to subjects of European ethnicity. In a cohort of Japanese subjects, the influence of 5-HTTLPR differed depending on whether paroxetine or fluvoxamine was used.⁴⁷ It is possible that P450 alleles can have variable effects on paroxetine concentrations in either Japanese⁴⁸ subjects or those of European ethnicity.⁴⁹ It is therefore conceivable that the 5-HTTLPR effects may vary among different ethnic groups.

Second, 5-HTTLPR may be in linkage disequilibrium with another polymorphism that may be causally responsible for the influence on concentration–response. In addition to other plausible linked and unlinked SERT polymorphisms, several other factors may influence SERT expression and function,²⁷ including kinases and cytokines. Moreover, there is evidence for a role of other genes in SSRI response.⁵⁰ It is likely that these other influences could explain some of the additional variability in response that was not explained by 5-HTTLPR, concentration, baseline HAMD and benzodiazepine use.

Third, our findings may specifically pertain to older patients treated in a closely monitored research setting with careful attention to identifying and treating potential side effects. In some clinical situations, higher SSRI levels may be counterproductive because of their side effects. Elevated SSRI levels may confound the HAMD score (e.g., by adversely influencing sleep and agitation) or result in poor adherence in some patients,^{51,52} or both; the *s* allele has been associated with more side effects in some studies.^{22,53} It is therefore plausible that, in some clinical settings, elevated paroxetine levels could “paradoxically” adversely affect response.⁵⁴ In 1 study, ideal paroxetine concentrations at 2 weeks were 43 ng/mL, with poorer outcomes at either higher or lower concentrations.⁵⁴ Other analyses have similarly suggested that paroxetine levels in treatment for chronic symptoms should be between 20 and 70 ng/mL.⁵⁵ Both of our cohorts were permitted to use benzodiazepines, the use of which was favourably associated with outcome. It is plausible that the role of 5-HTTLPR can be influenced by the clinical attention given to SSRI-induced side effects. Similarly, given potentially increased sensitivity to side effects in the elderly,² it is plausible that the role of 5-HTTLPR may differ depending on the patient’s age. Whether our results can be extended to other treatment situations (e.g., where benzodiazepines are not as permitted or in younger patients) will require further examination.

In summary, with the above considerations in mind, the results suggest that it may be possible to identify at week 2 those poorly responding patients who would simply benefit from increased paroxetine levels (achieved by targeting either increased dosage or improved adherence) and those for whom another intervention would be more beneficial. Replicating these results and then extending these findings to younger ages, other SSRIs, other patient populations and other clinical protocols will be an important next step. To date, few clinical studies have included regular and early antidepressant concentration measurements in their protocols. Therefore, an important implication of our findings is that the role of antidepressant concentrations should be included in

future antidepressant clinical trials, particularly those studies examining the role of pharmacogenetics and individualized treatment.

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Contributors: Dr. Lotrich designed the study. Drs. Pollock, Ferrell and Reynolds, and Ms. Kirshner acquired the data, which Drs. Lotrich, Pollock and Reynolds analyzed. Dr. Lotrich wrote the article, and Drs. Pollock, Ferrell and Reynolds, and Ms. Kirshner revised it. All authors gave final approval for the article to be published.

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