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ORIGINAL ARTICLE

Interaction between polymorphisms in serotonin transporter (*SLC6A4*) and serotonin receptor 2A (*HTR2A*) genes predict treatment response to venlafaxine XR in generalized anxiety disorder

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Variation in genes involved in serotonergic signaling is thought to be associated with antidepressant treatment response in generalized anxiety disorder (GAD). We examined a possible interaction between the serotonin transporter gene (*SLC6A4*) 5-HTTLPR/rs25531 haplotype and the serotonin 2A receptor gene (*HTR2A*) single-nucleotide polymorphism (SNP) rs7997012 in antidepressant treatment outcome in GAD. Patients diagnosed with GAD received venlafaxine XR treatment as part of an 18-month relapse prevention study. Genotypes obtained for the 5-HTTLPR/rs25531 (La/La, La/S or S/S) haplotype and rs7997012 SNP (G or A) in the European American population ($n = 112$) were used for pharmacogenetic analysis. Our data show that subjects with genotypes La/La + G/G or La/La + G/A ($n = 28$) had significantly lower Hamilton Anxiety Scale (HAM-A) scores than those with genotypes La/S + A/A or S/S + A/A ($n = 12$) at 6 months (HAM-A difference = 10.7; $P < 0.0001$). Single-marker analysis only showed HAM-A differences of 4.3 (5-HTTLPR/rs25531: La/La versus La/S + S/S) and 4.8 (rs7997012: G/G + G/A versus A/A), showing for the first time a significant gene–gene interaction between these markers.

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INTRODUCTION

Generalized anxiety disorder (GAD) is a highly prevalent chronic psychiatric disorder with significant morbidity and mortality. Antidepressant drugs are widely used for both acute and chronic treatment of GAD; studies have shown antidepressants such as escitalopram and venlafaxine (VEN) are both well tolerated and effective. However, treatment response among patients is often variable, with some responding well to antidepressants while others fail treatment.[1–3] Only a third of patients achieve remission in the acute phase of treatment,[4,5] and many often suffer relapse.[6,7] The majority of pharmacogenetic studies of antidepressant drugs have been studied in major depressive disorder (MDD)[8] while only a few reports exist for anxiety disorders.[9,10]

As the underlying neurobiological mechanisms of treatment response are unknown, all current pharmacotherapy for GAD is determined by the clinician based on the patient's history. Evidence has shown that genetic factors may influence treatment response and tolerability to medication. Genes in the serotonergic system have been widely studied in antidepressant pharmacogenetic studies, given that the serotonergic system has been the main target of selective serotonin reuptake inhibitors (SSRIs) and selective serotonin–norepinephrine reuptake inhibitors used to treat both GAD and MDD. Two promoter polymorphisms in the serotonin transporter (*5-HTT*) gene (*SLC6A4*) have been studied extensively in treatment response in MDD. The 5-HTTLPR is an insertion/deletion polymorphism, with the long (L) allele being associated with better response to antidepressants in European Americans (EA).[11] The 5-HTTLPR was thought to be functionally bi-allelic, with the L allele having higher transcription of *SLC6A4* than the S allele.[12,13] This finding has been supported *in vivo*, with LL homozygotes having higher levels of 5-HTT than S carriers.[14]

However, recent studies have shown that the presence of an a/g single-nucleotide polymorphism (SNP; rs25531) in the 5-HTT promoter region forms a functional haplotype with the 5-HTTLPR, with La carriers associated with the highest transcription and all other alleles being low-activity alleles.[15] It is possible that functional differences between these alleles could result in differential treatment response to antidepressants.[16] A meta-analysis by Serretti *et al.*[17] in depression found 5-HTTLPR L carriers had better response rates to various antidepressants; however, a more recent meta-analysis that included new studies within the past few years did not confirm this finding.[18] One study in depression showed that in the presence of the g SNP, the L allele of 5-HTTLPR is associated with nonresponse to antidepressant treatment.[19]

Although there have been many pharmacogenetic studies of the 5-HTTLPR in depression with unclear findings, few studies have looked at the effect of the 5-HTTLPR on antidepressants in anxiety disorders. One study in obsessive-compulsive disorder found no association with 5-HTTLPR genotype and response to

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antidepressants,[20] although another study in panic disorder did find an association with the 5-HTTLPR and response to paroxetine.[21] Stein *et al.*[22] found an association between the 5-HTTLPR genotype and SSRI treatment response in social anxiety disorder. They also saw a trend, although not significant, in the 5-HTTLPR/rs25531 haplotype and SSRI treatment response.[22] One recent study by Lenze *et al.*[23] found a dominant effect of La carriers and escitalopram treatment response in GAD. Together, it appears the 5-HTTLPR/rs25531 haplotype may have a role in GAD antidepressant treatment outcome.

The serotonin receptor 2A gene (*HTR2A*) has likewise been implicated in antidepressant pharmacogenetic studies in MDD, particularly the robust association of SNP rs7997012 and treatment outcome in the STAR*D sample.[24] Since then, several other groups have replicated the finding. Peters *et al.*[25] used the same sample and confirmed the association of the A allele and better treatment outcome. Although others confirmed the association of rs7997012 in independent samples, they found an association with the G allele and better treatment response.[26,27] We recently reported a significant association of the G allele and better treatment response to VEN in our GAD sample.[28]

The rs7997012 is an intronic SNP, with no clear biological function, although it might be in linkage disequilibrium with another functional variant in the *HTR2A* gene. Three other coding SNPs in the *5HTR2A* gene, rs6311 (102T/C), rs6313 (1438 A/G) and rs6314 have also been associated with antidepressant treatment response in MDD (see Kato and Serretti[8] for review and meta-analysis). Together, these studies indicate a strong role for variants in *SLC6A4* and *HTR2A* in antidepressant response in MDD and anxiety disorders.

Although several pharmacogenetic studies have investigated single polymorphisms or several polymorphisms within a single gene, few studies have looked at gene–gene interactions in antidepressant treatment response. Lin *et al.*[29] found a significant gene–gene interaction with markers in the *SLC6A4* and *HTR2A* genes and antidepressant treatment response in MDD; however, they did not analyze either the 5-HTTLPR or the rs7997012 in their study. Other studies have found significant interactions between the *HTR1A* gene and the 5-HTTLPR,[30] the *HTR2A* and *CYP2D6* genes,[31] and the *HTR2A*, the *FKBP5* and the *GRIK4* genes[27] in MDD. To our knowledge, no one has investigated any gene–gene interactions in the pharmacogenetics of anxiety disorders.

In this study, we investigated a possible gene–gene interaction between the *SLC6A4* 5-HTTLPR/rs25531 haplotype and the *HTR2A* SNP rs7997012 in antidepressant treatment response to VEN in GAD. We show for the first time a significant interaction between the 5-HTTLPR/rs25531 haplotype and SNP rs7997012 in antidepressant treatment outcome in GAD.

MATERIALS AND METHODS

Patients in this study participated in an 18-month relapse prevention study that comprised three treatment phases:[32] a 6-month open-label VEN XR flexible-dose treatment phase (75–225 mg day^{-1}; Phase I), a 6-month, randomized, double-blind, placebo-controlled relapse phase (Phase II), and a final 6-month, randomized, double-blind, placebo-controlled relapse phase (Phase III). Primary pharmacogenetic analysis was conducted for the first phase (Phase I). A brief summary of methodology is included here.[32]

Subjects had to be 18 years or older and meet the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition criteria for GAD, which was determined by the Structured-Clinical Interview and psychiatric evaluation. Subjects also needed to have a score of $\geqslant$20 on the Hamilton Anxiety Scale (HAM-A) at screen and at baseline, and a score of $\geqslant$4 on the Clinical Global Impression (CGI)-Severity scale. Exclusion criteria included any current anxiety spectrum Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition diagnosis at the threshold but not sub-threshold level, current or past history of bipolar disorder, schizophrenia, other psychotic disorders or dementia. Severely depressed subjects were excluded with a HAM-D cutoff score of $\leqslant$18; a cutoff score of $<$2 on the suicide item of the HAM-D was used to exclude suicidal patients. Subjects with an episode of major depression and substance abuse or dependence in the past 6 months were also excluded. Occasional recreational drug or alcohol use was not an exclusion criterion, thus allowing our sample to represent GAD patients in the community. Patients on a low daily dose of benzodiazepine anxiolytics or hypnotics were allowed to enter the trial and were tapered off their benzodiazepine during the first 12 weeks of VEN treatment.

Patients were recruited at the University of Pennsylvania Medical Center with approval and oversight by the Institutional Review Board of the University of Pennsylvania. Written informed consent was obtained before performing any study procedures. After a 4- to 28-day screening period, eligible patients were started on VEN 37.5 mg for 1 week followed by 75 mg day^{-1} for the second week. After the second week, flexible dosing was used in a range of 75–225 mg day^{-1}. Every attempt was made to raise the patient's daily dose to 225 mg by week 8, unless adverse events prevented this increase or the patient was in remission. Pill counts were used as a measure of adherence.

In total, 156 patients (EA $n=112$; African-Americans $n=41$; others $n=3$) were assessed for treatment response. However, only the EA population ($n=112$) was used in the pharmacogenetic analysis to reduce population stratification owing to ethnic differences in allele frequencies. The HAM-A was used as a primary outcome measure, with response defined as HAM-A reduction of $\geqslant$50% and remission was defined as HAM-A$\leqslant$7. The CGI of Improvement (CGI-I) score at 6 months was used as a secondary outcome measure. Improvement was defined as CGI-I of 1 and 2, remission was defined as CGI-I of 1. The last observation carried forward imputation method was used for this study to account for missing data (patients who were unresponsive, with a CGI-I$\geqslant$4, were discontinued).

Genotypes for the 5-HTTLPR and rs25531 were obtained using standard procedures.[12] Briefly, the 5-HTTLPR genotypes (L or S alleles) were obtained using a standard PCR procedure, followed by a restriction endonuclease digest to obtain the haplotype genotypes (La or Lg alleles). Individuals with an Lg allele were considered equivalent to having an S allele (Lg = S), similar to previous reports.[23] Genotyping of the *HTR2A* variant rs7997012 was performed as described previously.[28] In all, 10% duplicates of subjects were genotyped for quality control. Genotype and allele frequencies were compared between groups using χ^2 contingency analysis and Fisher's exact test (where applicable). One-way analysis of variance (ANOVA) was used to compare mean HAM-A scores between groups. A two-tailed type I error rate of 5% was chosen for the analysis.

RESULTS

We previously reported genotype information for rs7997012 in our GAD population.[28] Genotype distributions for rs7997012 were in accordance with Hardy–Weinberg Equilibrium, and the concordance rate was 100% with respect to the 10% of samples that were genotyped twice for quality control. Allelic frequencies for the Caucasian samples were consistent with those reported in the HapMap database for Utah residents with Northern and Western Europe ancestry from the CEPH collection. Genotype distributions for the 5-HTTLPR/rs25531 haplotype were also in accordance with Hardy–Weinberg Equilibrium. The concordance rate was 100% for the 10% duplicates that were genotyped for quality control. Genotype frequencies for the 5-HTTLPR and rs25531 were consistent with those previously reported.[15,33]

Treatment response to VEN over time based on the 5-HTTLPR/rs25531 haplotype (La, Lg, S, where Lg = S) for the EA population ($n=112$) using the HAM-A outcome measure is shown in Figure 1. Subjects who were homozygous La/La showed a significant reduction in HAM-A compared with S carriers (La/S + S/S; Lg = S) at 6 months (La/La group mean HAM-A: 4.35 s.e.: 0.54; the La/S + S/S group mean HAM-A: 8.67 s.e.: 0.91; HAM-A difference = 4.32; one-way ANOVA: $F=9.207$; $P=0.003$). Exploratory analyses showed a trend in association as early as week 12 (one-way ANOVA: $F=3.343$; $P=0.07$), with significant associations in week 16 (one-way ANOVA: $F=4.733$; $P=0.03$) and week 20 (one-way ANOVA: $F=4.608$; $P=0.03$).

Results for the EA sample using a categorical outcome measure of response and remission (using HAM-A and CGI-I) at 6 months for the haplotype is shown in Table 1. We found a genotypic

association for HAM-A response ($P = 0.01$) and remission ($P = 0.02$) and CGI-I improvement ($P = 0.003$) and remission ($P = 0.003$). Again, we saw a dominant effect of S carriers (La/S + S/S; Lg = S) and worse treatment outcomes. Subjects who were homozygous La/La showed better treatment response (HAM-A response: $P = 0.003$; $OR = 12.16$; CGI-I improvement: $P = 0.0008$) and remission (HAM-A remission: $P = 0.007$; $OR = 4.03$; CGI-I remission: $P = 0.0008$) than La/S or S/S individuals.

Similarly, we have previously shown that EA patients ($n = 112$) with at least one G allele for *HTR2A* SNP rs7997012 showed a significant reduction in HAM-A score when compared with the A/A group at the main study endpoint of 6 months (HAM-A difference between A/A and G/A + G/G groups: 4.80; see Figure 1 in Lohoff *et al*[28]). Exploratory analysis showed that the difference between groups was statistically significant as early as week 12.[28]

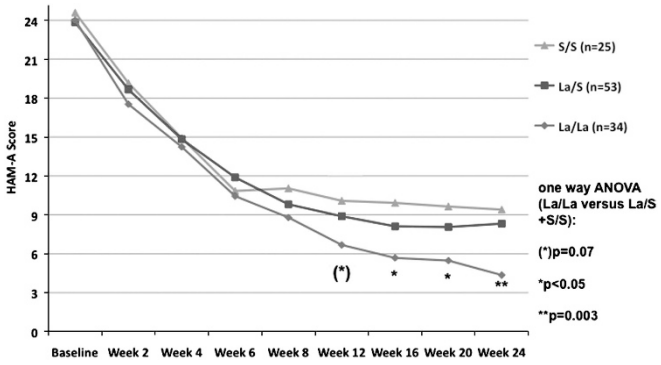

Figure 1. Treatment response to VEN based on 5-HTTLPR/rs25531 haplotype is shown (EA $n = 112$). Individuals with the Lg allele were considered equivalent to S (La/S = La/Lg + La/S). Primary analysis at week 24 (6 months) showed a significant dominant effect of the S allele and worse response, with a HAM-A difference of 4.32 between La/La and La/S + S/S groups. There were no significant differences between La/S and S/S groups.

Based on the positive findings with these variants, we sought to investigate a possible interactive effect between *SLC6A4* and *HTR2A*. We considered a dominant model for both markers (La/La versus La/S + S/S for the 5-HTTLPR/rs25531 marker and G/G + G/A versus A/A for rs7997012).

We labeled the 5-HTTLPR/rs25531 haplotype as marker 1 (M1) and rs7997012 as marker 2 (M2). Genotypes that were associated with better antidepressant response were labeled as positive (+) for each marker (La/La = M1(+) for 5-HTTLPR/rs25531 and G/A + G/G = M2(+) for rs7997012). Genotypes associated with worse antidepressant response were labeled as negative (−) for each marker (La/S + S/S = M1(−) for 5-HTTLPR/rs25531 and A/A = M2(−) for rs7997012). We then investigated if there was a significant difference in antidepressant treatment response between patients with two positive markers (M1(+)/M2(+) = La/La + G/G or La/La + G/A), patients with only one positive marker (M1(+)/M2(−) = La/La + A/A or M1(−)/M2(+) = La/S + G/G or La/S + G/A or S/S + G/G or S/S + G/A) and patients with no positive markers (M1(−)/M2(−) = La/S + A/A or S/S + A/A).

Treatment response to VEN in the EA population ($n = 112$) based on 5-HTTLPR/rs25531 and rs7997012 interaction is shown in Figure 2. Subjects who had two positive markers (M1(+)/M2(+) $n = 28$) showed highly significant reduction in HAM-A compared with subjects with no positive markers (M1(−)/M2(−) $n = 12$) at 6 months M1(+)/M2(+) group mean HAM-A: 4.43 s.e.: 0.66; M1(−)/M2(−) group mean HAM-A: 15.08 s.e.: 2.23; HAM-A difference = 10.7; one-way ANOVA: $F = 36.91$; $P < 0.0001$). Exploratory analysis showed a trend in association as early as week 8 (one-way ANOVA: $F = 3.05$; $P = 0.089$) with significant associations in week 12 (one-way ANOVA: $F = 8.92$; $P = 0.0049$), week 16 (one-way ANOVA: $F = 16.76$; $P = 0.0002$) and week 20 (one-way ANOVA: $F = 13.54$; $P = 0.0007$).

There was also a significant difference between patients with just one positive marker ((M1(+)/M2(−) or M1(−)/M2(+) $n = 72$) and patients with no positive markers (M1(−)/M2(−) $n = 12$) at 6 months (HAM-A difference: 7.9; one-way ANOVA: $F = 11.83$; $P = 0.001$), with significant associations seen as early as week 12 (one-way ANOVA: $F = 4.87$; $P = 0.03$). There were no

Table 1. 5-HTTLPR/rs25531 6 months outcome data

5-HTTLPR/rs25531	*Sample*	n	*Genotype frequency*			P	*Allele frequency*	P	*OR*	*OR 95% confidence interval*
			La/La	*La/S*	*S/S*		*f (La)*			
Response HAM-A LOCF	Responders	90	0.37 (33)	0.44 (40)	0.19 (17)	0.05	0.59	0.015	2.77	1.39–5.52
	Non-responders	22	0.05 (1)	0.59 (13)	0.36 (8)		0.34			
			La/La versus La/S + S/S					0.015	12.16	1.56-94.6
Improvement CGI LOCF	Improvers	91	0.37 (34)	0.42 (38)	0.21 (19)	0.015	0.58	0.04	2.51	1.25–5.04
	Non-Improvers	21	0.00 (0)	0.71 (15)	0.29 (6)		0.36			
			La/La versus La/S + S/S					0.004	25.8	1.513-439.9
Remission HAM-A LOCF	Remitters	75	0.45 (34)	0.39 (29)	0.16 (12)	0.10	0.60	0.05	2.08	1.18–3.66
	Non-remitters	37	0.24 (9)	0.49 (18)	0.27 (10)		0.42			
			La/La versus La/S + S/S					0.035	4.03	1.41–11.54
Remission CGI LOCF	Remitters	91	0.37 (34)	0.42 (38)	0.21 (19)	0.015	0.58	0.04	2.51	1.25–5.04
	Non-remitters	21	0.00 (0)	0.71 (15)	0.29 (6)		0.36			
			La/La versus La/S + S/S					0.004	25.8	1.513-439.9

Abbreviations: CGI, Clinical Global Impression; HAM-A, Hamilton Anxiety Scale; LOCF, last observation carried forward; OR, odds ratio; VEN, venlafaxine. 5-HTTLPR/rs25531 genotypes La/La, La/S and S/S and categorical outcomes HAM-A response/remission and CGI-I Improvement/remission to VEN treatment. χ^2 or Fisher's exact test was used to calculate all *P*-values.

statistically significant differences between patients with two positive markers (M1(+)/M2(+) $n=28$) and individuals with one positive marker ((M1(+)/M2(−) or M1(−)/M2(+) $n=72$). All data presented are based on the last observation carried forward data; results based on completers (EA $n=88$) showed similar significant differences (data not shown). Drug doses did not differ statistically significantly between groups (data not shown).

Results for the EA sample ($n=112$) using a categorical outcome measure of response and remission (using HAM-A and CGI-I) at 6 months for the interaction analysis is shown in Table 2. We found a significant association between all three groups for HAM-A response ($P=0.0005$) and remission ($P=0.0025$) and CGI-I improvement ($P=0.000009$) and remission ($P=0.0048$). Interestingly, we saw a dominant effect of individuals who had at least one positive marker (M1(+)/M2(+) or (M1(+)/M2(−) or M1(−)/M2(+) $n=100$) and better treatment response (HAM-A response: $P=0.0019$ $OR=7.93$; CGI-I improvement: $P=0.0001$ $OR=13.38$) and remission (HAM-A remission: $P=0.0021$ $OR=7.71$; CGI-I remission: $P=0.0055$ $OR=6.00$).

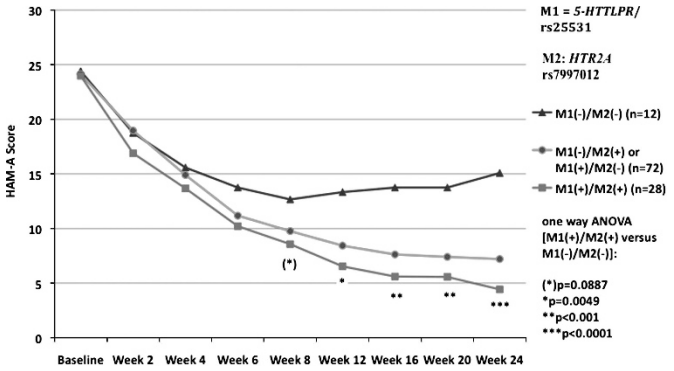

Figure 2. Treatment response to VEN based on 5-HTTLPR/rs25531 (M1) and *HTR2A* rs7997012 (M2) interaction is shown (EA $n=112$). Each group contained the following genotypes: M1(−)/M2(−) = La/S + A/A or S/S + A/A; M1(−)/M2(+) = La/S + G/G or La/S + G/A or S/S + G/G or S/S + G/A; M1(+)/M2(−) = La/La + A/A; M1(+)/M2(+) = La/La + G/G or La/La + G/A. Primary analysis at week 24 (6 months) outcome showed a significant HAM-A difference (10.7) between M1(+)/M2(+) and M1(−)/M2(−) groups. There was also a significant HAM-A difference (7.9) between M1(+)/M2(−) or M1(−)/M2(+) and M1(−)/M2(−) groups at week 24. There were no significant differences between M1(+)/M2(+) and M1(+)/M2(−) or M1(−)/M2(+) groups.

DISCUSSION

In this study, we report for the first time a significant interaction between markers in the *SLC6A4* and *HTR2A* genes in predicting antidepressant treatment response in GAD. Using a dominant model for both the 5-HTTLPR/rs25531 (La/La versus La/S + S/S) and rs7997012 (G/G + G/A versus A/A) markers, we show that individuals who carry two positive markers (M1(+)/M2(+) $n=28$) for both genes have significantly lower HAM-A scores than individuals who carry no positive markers (M1(−)/M2(−) $n=12$) for either gene at 6 months treatment outcome (HAM-A difference = 10.7). This pharmacogenetic effect was increasingly statistically significant over time (see Figure 2) and was confirmed using categorical outcome measures (see Table 2).

Given that most double-blind placebo-controlled clinical trials of antidepressant drugs in GAD show on average a HAM-A score difference between drug and placebo of about 3–4,[34] our data is highly clinical relevant as it indicates that inter-individual differences in *SLC6A4* and *HTR2A* variants contribute largely to treatment response (HAM-A mean difference 10.7). In fact, based on our data, individuals with M1(−)/M2(−) genotypes should perhaps be given a different course of treatment (that is, benzodiazepines). Interestingly, mean HAM-A differences for single-marker analyses in each gene were 4.3 (5-HTTLPR/rs25531: La/La versus La/S + S/S) and 4.8 (*HTR2A* rs7997012: G/

Table 2. *5-HTTLPR* and *HTR2A* gene–gene interaction 6 months outcome data

5-HTTLPRxHTR2A	*Sample*	n	*Genotype frequency*			P	*OR*	*OR 95% confidence interval*
			M1(+)/ M2(+)	*M1(+)/M2(−) or M1(−)/ M2(+)*	*M1(−) / M2(−)*			
Response HAM-A LOCF	Responders	90	0.30 (27)	0.64 (58)	0.06 (5)	0.0005	—	—
	Non-responders	22	0.04 (1)	0.64 (14)	0.32 (7)			
	M1(+)/M2(+) and M1(+)/M2(−) and M1(−)/M2(+) versus M1(−)/M2(−)					0.0019	7.93	2.22-28.31
Improvement CGI LOCF	Improvers	91	0.31 (28)	0.65 (59)	0.04 (4)	9×10^{-6}	—	—
	Non-improvers	21	0.00 (0)	0.62 (13)	0.38 (8)			
	M1(+)/M2(+) and M1(+)/M2(−) and M1(−)/M2(+) versus M1(−)/M2(−)					0.0001	13.38	3.53-50.82
Remission HAM-A LOCF	Remitters	75	0.31 (23)	0.65 (49)	0.04 (3)	0.0025	—	—
	Non-remitters	37	0.14 (5)	0.62 (23)	0.24 (9)			
	M1(+)/M2(+) and M1(+)/M2(−) and M1(−)/M2(+) versus M1(−)/M2(−)					0.0021	7.71	1.95-30.59
Remission CGI LOCF	Remitters	79	0.30 (24)	0.65 (51)	0.05 (4)	0.0048	—	—
	Non-remitters	33	0.12 (4)	0.64 (21)	0.24 (8)			
	M1(+)/M2(+) and M1(+)/M2(−) and M1(−)/M2(+) versus M1(−)/M2(−)					0.0055	6.00	1.66-21.64

Abbreviations: CGI, Clinical Global Impression; HAM-A, Hamilton Anxiety Scale; LOCF, last observation carried forward; OR, odds ratio; VEN, venlafaxine. Interaction between 5-HTTLPR/rs25531 genotypes La/La, La/S and S/S and *HTR2A* SNP rs7997012 G/A in categorical outcomes HAM-A response/remission and CGI-I Improvement/remission to VEN treatment. M1(+)/M2(+) = La/La + G/G or La/La + G/A; M1(+)/M2(−) = La/La + A/A; M1(−)/M2(+) = La/S + G/G or S/S + G/G or La/S + G/A or S/S + G/A; M1(−)/M2(−) = La/S + A/A or S/S + A/A. Fisher's exact test was used to calculate all *P*-values.

G + G/A versus A/A), documenting an additive effect of the gene–gene interaction in antidepressant treatment response.

In contrast to most pharmacogenetic studies of antidepressant treatment outcome in either MDD or anxiety disorders that are typically 8–12 weeks in length,[8,9] our study is the longest pharmacogenetic study of antidepressant treatment response today, with some patients completing 18 months of treatment. Similar to our previous work, which show that chronic GAD patients treated long-term with VEN do better over time,[32] our pharmacogenetic analysis documents that long-term treatment outcome is also influenced by genetic variation. Individuals who carry at least one positive marker (M1(+)/M2(+) or M1(+)/M2(−) or M1(−)/M2(+)) should continue antidepressant treatment beyond 8–12 weeks to maximize their potential for response, while individuals that reach remission and are M1(+)/M2(+) are at low risk for relapse.

The 5-HTTLPR/rs25531 has some documented functional effect on *SLC6A4* expression, with the La allele is associated with higher transcription of *SLC6A4*, which may underlie its association with better antidepressant treatment response.[15] Several studies reported an association between the 5-HTTLPR and anxiety-related traits, like neuroticism, with S carriers having higher anxiety symptoms.[12,35,36] Knockout mice for 5-HTT show increased anxiety and depression symptoms, implying a functional role of this transporter in both anxiety and depression. Imaging studies based on the 5-HTTLPR showed higher activity in the amygdala in S carriers.[37] As antidepressants are efficacious in anxiety disorders as well, it is possible that functional differences between the 5-HTTLPR alleles could predict variability in antidepressant response in anxiety disorders.[16]

Although no clear data exist on the biological role of the *HTR2A* rs7997012, there is much evidence suggesting a role of the HTR2A receptor (HTR2A-R) in mood and anxiety disorders.[38] Several antidepressants downregulate HTR2A-Rs in rodent and primate forebrain, which correlate with the timeframe of therapeutic onset in humans.[39–41] Null-mutant mice for the HTR2A-R have reduced inhibition in conflict anxiety, without affecting fear-conditioned or depression-related behaviors.[42] Thus, the downregulation of HTR2A-Rs under chronic SSRI therapy may also be correlated to the anxiolytic effect of SSRIs. It is possible that variation in the *HTR2A* gene might contribute to the effectiveness of antidepressants by having functional effects on HTR2A-Rs.

Our results should be considered in light of the limitations of this study. First, it should be noted that DNA samples were collected retrospectively after patients participated in the treatment trial; thus, there may be an ascertainment bias. Future studies should use a prospective clinical trial design to address this concern adequately. Furthermore, future studies should also include a placebo arm. In addition, we only had one measure of adherence, so it is possible some of our patients were not adhering to their medication. Future studies should more carefully monitor this, possibly with plasma drug levels.

As most GAD patients are no longer treatment-naive, prior treatment history may also be a confounding factor to consider in future studies. Co-morbidity of anxiety disorders and depression is common and should be considered carefully when interpreting results. Although our sample was a primary GAD sample, with current MDD as exclusion criterion, past episodes of MDD were allowed, and patients may have had some moderate depressive symptoms at baseline. Also, our sample size was small once we separated based on ethnicity (EA $n = 112$), requiring replication of our results in a larger sample, particularly with a placebo arm. Although there were no significant differences in drug dosage between genotype groups in our study, future studies should also look at plasma drug levels to ensure variable plasma drug concentrations do not confound the results.

In summary, we show for the first time a gene–gene interaction between markers in *SLC6A4* and *HTR2A* genes and antidepressant treatment outcome in GAD. Our results suggest genotyping the 5-HTTLPR/rs25531 haplotype and *HTR2A* SNP rs7997012 could be clinically effective in predicting treatment response to VEN in GAD. Replication using a prospective clinical trial design in a larger population is necessary to confirm this finding.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

1 Baldwin DS, Nair RV. Escitalopram in the treatment of generalized anxiety disorder. *Expert Rev Neurother* 2005; **5**: 443–449.
2 Gelenberg AJ, Lydiard RB, Rudolph RL, Aguiar L, Haskins JT, Salinas E. Efficacy of venlafaxine extended-release capsules in nondepressed outpatients with generalized anxiety disorder: a 6-month randomized controlled trial. *JAMA 2000; **283**: 3082–3088.*
3 Rickels K, Downing R, Schweizer E, Hassman H. Antidepressants for the treatment of generalized anxiety disorder. A placebo-controlled comparison of imipramine, trazodone, and diazepam. *Arch Gen Psychiatry* 1993; **50**: 884–895.
4 Rickels K, Rynn M. Pharmacotherapy of generalized anxiety disorder. *J Clin Psychiatry* 2002; **63**(Suppl 14): 9–16.
5 Rickels K, Rynn M, Iyengar M, Duff D. Remission of generalized anxiety disorder: a review of the paroxetine clinical trials database. *J Clin Psychiatry* 2006; **67**: 41–47.
6 Andrews G, Sanderson K, Slade T, Issakidis C. Why does the burden of disease persist? Relating the burden of anxiety and depression to effectiveness of treatment. *Bull World Health Organ* 2000; **78**: 446–454.
7 Pollack MH. Refractory generalized anxiety disorder. *J Clin Psychiatry* 2009; **70**(Suppl 2): 32–38.
8 Kato M, Serretti A. Review and meta-analysis of antidepressant pharmacogenetic findings in major depressive disorder. *Mol Psychiatry* 2010; **15**: 473–500.
9 Tiwari AK, Souza RP, Muller DJ. Pharmacogenetics of anxiolytic drugs. *J Neural Transm* 2009; **116**: 667–677.
10 Narasimhan S, Lohoff FW. Pharmacogenetics of antidepressant drugs: current clinical practice and future directions. *Pharmacogenomics* 2012; **13**: 441–464.
11 Serretti A, Artioli P, Quartesan R. Pharmacogenetics in the treatment of depression: pharmacodynamic studies. *Pharmacogenet Genomics* 2005; **15**: 61–67.
12 Lesch KP, Bengel D, Heils A, Sabol SZ, Greenberg BD, Petri S *et al.* Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science* 1996; **274**: 1527–1531.
13 Heils A, Teufel A, Petri S, Stober G, Riederer P, Bengel D *et al.* Allelic variation of human serotonin transporter gene expression. *J Neurochem* 1996; **66**: 2621–2624.
14 Heinz A, Jones DW, Mazzanti C, Goldman D, Ragan P, Hommer D *et al.* A relationship between serotonin transporter genotype and *in vivo* protein expression and alcohol neurotoxicity. *Biol Psychiatry* 2000; **47**: 643–649.
15 Hu XZ, Lipsky RH, Zhu G, Akhtar LA, Taubman J, Greenberg BD *et al.* Serotonin transporter promoter gain-of-function genotypes are linked to obsessive-compulsive disorder. *Am J Hum Genet* 2006; **78**: 815–826.
16 Lotrich FE, Pollock BG, Ferrell RE. Polymorphism of the serotonin transporter: implications for the use of selective serotonin reuptake inhibitors. *Am J Pharmacogenomics* 2001; **1**: 153–164.
17 Serretti A, Kato M, De Ronchi D, Kinoshita T. Meta-analysis of serotonin transporter gene promoter polymorphism (5-HTTLPR) association with selective serotonin reuptake inhibitor efficacy in depressed patients. *Mol Psychiatry* 2007; **12**: 247–257.
18 Taylor MJ, Sen S, Bhagwagar Z. Antidepressant response and the serotonin transporter gene-linked polymorphic region. *Biol Psychiatry* 2010; **68**: 536–543.
19 Kraft JB, Slager SL, McGrath PJ, Hamilton SP. Sequence analysis of the serotonin transporter and associations with antidepressant response. *Biol Psychiatry* 2005; **58**: 374–381.
20 Billett EA, Richter MA, King N, Heils A, Lesch KP, Kennedy JL. Obsessive compulsive disorder, response to serotonin reuptake inhibitors and the serotonin transporter gene. *Mol Psychiatry* 1997; **2**: 403–406.
21 Perna G, Favaron E, Di Bella D, Bussi R, Bellodi L. Antipanic efficacy of paroxetine and polymorphism within the promoter of the serotonin transporter gene. *Neuropsychopharmacology* 2005; **30**: 2230–2235.

22 Stein MB, Seedat S, Gelernter J. Serotonin transporter gene promoter polymorphism predicts SSRI response in generalized social anxiety disorder. *Psychopharmacology* 2006; **187**: 68–72.
23 Lenze EJ, Goate AM, Nowotny P, Dixon D, Shi P, Bies RR *et al*. Relation of serotonin transporter genetic variation to efficacy of escitalopram for generalized anxiety disorder in older adults. *J Clin Psychopharmacol* 2010; **30**: 672–677.
24 McMahon FJ, Buervenich S, Charney D, Lipsky R, Rush AJ, Wilson AF *et al*. Variation in the gene encoding the serotonin 2A receptor is associated with outcome of antidepressant treatment. *Am J Hum Genet* 2006; **78**: 804–814.
25 Peters EJ, Slager SL, Jenkins GD, Reinalda MS, Garriock HA, Shyn SI *et al*. Resequencing of serotonin-related genes and association of tagging SNPs to citalopram response. *Pharmacogenet Genomics* 2009; **19**: 1–10.
26 Lucae S, Ising M, Horstmann S, Baune BT, Arolt V, Muller-Myhsok B *et al*. HTR2A gene variation is involved in antidepressant treatment response. *Eur Neuropsychopharmacol* 2010; **20**: 65–68.
27 Horstmann S, Lucae S, Menke A, Hennings JM, Ising M, Roeske D *et al*. Polymorphisms in GRIK4, HTR2A, and FKBP5 show interactive effects in predicting remission to antidepressant treatment. *Neuropsychopharmacology* 2010; **35**: 727–740.
28 Lohoff FW, Aquino TD, Narasimhan S, Multani PK, Etemad B, Rickels K. Serotonin receptor 2A (HTR2A) gene polymorphism predicts treatment response to venlafaxine XR in generalized anxiety disorder. *Pharmacogenomics J*; advance online publication, 18 October 2011 (e-pub ahead of print).
29 Lin E, Chen PS, Chang HH, Gean PW, Tsai HC, Yang YK *et al*. Interaction of serotonin-related genes affects short-term antidepressant response in major depressive disorder. *Prog Neuropsychopharmacol Biol Psychiatry* 2009; **33**: 1167–1172.
30 Arias B, Catalan R, Gasto C, Gutierrez B, Fananas L. Evidence for a combined genetic effect of the 5-HT(1A) receptor and serotonin transporter genes in the clinical outcome of major depressive patients treated with citalopram. *J Psychopharmacol* 2005; **19**: 166–172.
31 Suzuki Y, Sawamura K, Someya T. Polymorphisms in the 5-hydroxytryptamine 2A receptor and CytochromeP4502D6 genes synergistically predict fluvoxamine-induced side effects in japanese depressed patients. *Neuropsychopharmacology* 2006; **31**: 825–831.
32 Rickels K, Etemad B, Khalid-Khan S, Lohoff FW, Rynn MA, Gallop RJ. Time to relapse after 6 and 12 months' treatment of generalized anxiety disorder with venlafaxine extended release. *Arch Gen Psychiatry* 2010; **67**: 1274–1281.
33 Wendland JR, Martin BJ, Kruse MR, Lesch KP, Murphy DL. Simultaneous genotyping of four functional loci of human SLC6A4, with a reappraisal of 5-HTTLPR and rs25531. *Mol Psychiatry* 2006; **11**: 224–226.
34 Reinhold JA, Mandos LA, Rickels K, Lohoff FW. Pharmacological treatment of generalized anxiety disorder. *Expert Opin Pharmacother* 2011; **12**: 2457–2467.
35 Greenberg BD, Li Q, Lucas FR, Hu S, Sirota LA, Benjamin J *et al*. Association between the serotonin transporter promoter polymorphism and personality traits in a primarily female population sample. *Am J Med Genet* 2000; **96**: 202–216.
36 Katsuragi S, Kunugi H, Sano A, Tsutsumi T, Isogawa K, Nanko S *et al*. Association between serotonin transporter gene polymorphism and anxiety-related traits. *Biol Psychiatry* 1999; **45**: 368–370.
37 Hariri AR, Drabant EM, Weinberger DR. Imaging genetics: perspectives from studies of genetically driven variation in serotonin function and corticolimbic affective processing. *Biol Psychiatry* 2006; **59**: 888–897.
38 Norton N, Owen MJ. HTR2A: association and expression studies in neuropsychiatric genetics. *Ann Med* 2005; **37**: 121–129.
39 Strome EM, Clark CM, Zis AP, Doudet DJ. Electroconvulsive shock decreases binding to 5-HT2 receptors in nonhuman primates: an *in vivo* positron emission tomography study with [18F]setoperone. *Biol Psychiatry* 2005; **57**: 1004–1010.
40 Drevets WC. Neuroimaging studies of mood disorders. *Biol Psychiatry* 2000; **48**: 813–829.
41 Peremans K, Audenaert K, Hoybergs Y, Otte A, Goethals I, Gielen I *et al*. The effect of citalopram hydrobromide on 5-HT2A receptors in the impulsive-aggressive dog, as measured with 123I-5-I-R91150 SPECT. *Eur J Nucl Med Mol Imaging* 2005; **32**: 708–716.
42 Weisstaub NV, Zhou M, Lira A, Lambe E, Gonzalez-Maeso J, Hornung JP *et al*. Cortical 5-HT2A receptor signaling modulates anxiety-like behaviors in mice. *Science* 2006; **313**: 536–540.