Association of Common Variations in the Norepinephrine Transporter Gene With Response to Olanzapine-Fluoxetine Combination Versus Continued-Fluoxetine Treatment in Patients With Treatment-Resistant Depression: A Candidate Gene Analysis

John P. Houston, MD, PhD; Kit Lau, PhD; Virginie Aris, PhD; Wenlei Liu, PhD; Bonnie A. Fijal, PhD; Alexandra N. Heinloth, MD; and Roy H. Perlis, MD, MSc

ABSTRACT

Objective: To determine whether single-nucleotide polymorphisms (SNPs) in candidate genes are associated with response to olanzapine-fluoxetine combination.

Method: A post hoc analysis of a priori–selected SNPs used data from a clinical trial (dates: April 2002–July 2005) of olanzapine-fluoxetine combination, fluoxetine, and olanzapine in patients with major depressive disorder (*DSM-IV* criteria) and with nonresponse to prestudy antidepressant treatment and nonresponse to fluoxetine treatment during the study. Patients received open-label treatment with fluoxetine for 8 weeks (2 weeks, 25 mg/d; then 6 weeks, 50 mg/d), at the end of which nonresponders (< 25% decline in the 17-item Hamilton Depression Rating Scale score) were randomized to receive double-blind, monotherapy treatment with olanzapine-fluoxetine combination (6/50–18/50 mg/d, n = 71), fluoxetine (50 mg/d, n = 78), or olanzapine (6–18 mg/d, n = 56) for 8 weeks. Statistical significance was assessed at $P < .05$. The primary efficacy measure for within-study treatment was improvement on the Montgomery-Asberg Depression Rating Scale (MADRS).

Results: Rs36024, an intronic SNP in the norepinephrine transporter (*SLC6A2*), as well as 3 SNPs in melanocortin 3 receptor (*MC3R*) and 2 SNPs in tryptophan hydroxylase 2 (*TPH2*), were associated with MADRS-defined response to treatment with olanzapine-fluoxetine combination (adjusted Li-Nyholt $P < .05$). Except for 1 SNP in *TPH2*, identified SNPs were not significantly associated with response to continued-fluoxetine or olanzapine treatments.

Conclusions: Our findings further support the hypothesis that the synergistic effect of olanzapine and fluoxetine on prefrontal cortical levels of norepinephrine and dopamine might be an underlying mechanism for the efficacy of olanzapine-fluoxetine combination in the treatment of treatment-resistant depression and, if replicated, may form a basis on which response to olanzapine-fluoxetine combination versus continued fluoxetine can be predicted based on variants in *SLC6A2*.

Trial Registration: Parent study registered at ClinicalTrials.gov identifier: NCT00035321

J Clin Psychiatry 2012;73(6):878–885

Submitted: *November 24, 2010; accepted July 29, 2011.*
Online ahead of print: *March 6, 2012 (doi:10.4088/JCP.10m06744).*
Corresponding author: *John P. Houston, MD, PhD, Medical Fellow I, Lilly USA, LLC, Mail Code 4103, Indianapolis, IN 46285 (Houston_John_P@lilly.com).*

One-tenth of the US population is affected annually by major depressive disorder[1,2]; less than 30% attain remission with monotherapy during antidepressant trials.[3] Olanzapine-fluoxetine combination has been approved by the US Food and Drug Administration for treatment-resistant depression, which is defined by failure to respond to adequate treatment with 2 different antidepressants during the current episode.[4]

Response is variable in patients with major depressive disorder or treatment-resistant depression. Numerous genes have been associated with response in both candidate gene and genome-wide association studies, with few consistently reported associations (1 previously examined treatment-resistant depression).[5] The polymorphisms, identified as associated with treatment response, are pharmacologically diverse.[6–18] Genome-wide association studies have also failed to yield consistent results with respect to antidepressant response.[19–22]

We recently reported associations between single-nucleotide polymorphisms (SNPs) in *DRD3* and *HRH1* genes with olanzapine-fluoxetine combination[11] treatment response in bipolar depression. That analysis did not address whether this association is specific to bipolar depression or olanzapine-fluoxetine combination. Here, we examined genetic associations with olanzapine-fluoxetine combination response in treatment-resistant depression patients nonresponsive to fluoxetine monotherapy in a prospective lead-in phase.[23] Our goal was to identify genetic variants that might predict improvement in patients not initially responsive to fluoxetine after switching to olanzapine-fluoxetine combination versus continued treatment with fluoxetine monotherapy. We were especially interested in variants of the norepinephrine transporter gene because of olanzapine and fluoxetine effects on prefrontal norepinephrine.[24]

METHOD

Patients and Study Design

Data were from self-identified white treatment-resistant depression patients, with recurrent major depressive disorder (*DSM-IV* criteria), who consented to genetic testing in a clinical trial of olanzapine-fluoxetine combination, fluoxetine, and olanzapine. Clinical trial details are described elsewhere.[23] The parent study is registered at ClinicalTrials.gov (identifier: NCT00035321). Patients with a documented clinical history of failure to respond to a non-fluoxetine antidepressant during their current depressive episode entered an 8-week, open-label dose titration lead-in phase with fluoxetine (2 weeks, 25 mg/d; then 6

Clinical Points

- If genetic associations can be replicated, they might allow for clinically useful predictive testing to guide antidepressant response.
- Some patients may be nonresponsive to fluoxetine monotherapy, due to minimal effects on norepinephrine, but responsive to olanzapine augmentation.

weeks, 50 mg/d). Patients who were nonresponders (< 25% decline in score on the 17-item Hamilton Depression Rating Scale [HDRS-17], an HDRS-17 score ≥ 18, or a ≤ 15% decrease in interactive voice response HDRS-17 between week 7 and week 8)[23] were randomly assigned double-blind (1:1:1 ratio) to olanzapine-fluoxetine combination (6/50–18/50 mg/d), continued fluoxetine (50 mg/d), or olanzapine (6–18 mg/d) for 8 weeks.

To investigate associations between genetic polymorphisms and treatment response after lack of fluoxetine response, we included all patients who consented to genetic testing, passed genotyping quality control checks, and had baseline and > 1 postbaseline Montgomery-Asberg Depression Rating Scale (MADRS)[25] total measurement.

Baseline and Treatment Assessments and Candidate Genes

Baseline measures were collected at the beginning of the randomization phase with depressive symptom changes quantified by changes in MADRS total from baseline to each visit. Associations of genetic polymorphisms with changes in depressive symptoms were tested in a 2-tier approach in all groups. For the first-tier analysis in the continued-fluoxetine group, SNPs in the following genes were selected (based on the literature or HapMap CEU data [National Center for Biotechnology Information, build 36]): *BDNF*,[26] *CRHR2*,[10,17] *HTR1A*,[27] *HTR2A*,[28,29] *TPH1*,[27] and *TPH2*.[30,31]

In the olanzapine group, associations between changes in depressive symptoms and SNPs in the following preselected (based on literature reports, ongoing studies) genes were examined in the first tier: *DRD2*, *DRD3*,[32] *BDNF*, *COMT*, *RGS4*, *ADRA1A*, *ABCB1*, *GRIA4*, *HTR6*, *MC2R*,[33] *HTR1A*,[34] *HTR2A*,[35] and *HTR6*.[36]

Associations between changes in depressive symptoms and SNPs that were identified as statistically significantly within either the continued-fluoxetine or olanzapine groups were examined in the olanzapine-fluoxetine combination group. Additionally, SNPs in the following preselected genes (based on literature reports, ongoing studies) were examined in the first tier: *DRD3, HRH1, MC2R, COMT*,[11] and *SLC6A2*.[37]

For all treatments, the second tier included the remainder of the 411 genetic variants in 44 genes (Supplementary eTable 1). Due to the increased likelihood of variants in *SLC6A2* to influence response of treatment-resistant depression to olanzapine-fluoxetine combination, based on the reported synergistic effect of olanzapine on prefrontal cortical levels of norepinephrine,[24] 77 individual SNPs located in this gene were assayed to provide comprehensive coverage of the gene, the surrounding promoter region, and the 3′ untranslated region.

Genotyping and Quality Control

Genotyping was performed by Cogenics (Newton, Massachusetts) and Sequenom (San Diego, California). Genotyping quality control was based on all genotyped SNPs, independent of their use in the current analyses. Individuals with a genotype call rate < 90% for all genetic markers and male patients with a heterozygosity rate > 20% across X chromosome SNPs were excluded from analyses. Call rates for each SNP were calculated across all samples; genetic markers with a call rate < 90% were excluded. Single-nucleotide polymorphisms located on the X chromosome with > 10% heterozygosity in male patients were removed and changed to missing. The SNPs with a Hardy-Weinberg equilibrium of $P < .0001$ within white patients were flagged, but not excluded. The SNPs that had been genotyped more than once and had a discordance rate > 5% were excluded.

Statistical Analyses

Analyses were done on an intent-to-treat basis, which is a widely accepted approach in clinical trial studies because it avoids the bias associated with the nonrandom loss of participants. In the randomization phase, the primary measure for response in patients nonresponsive to lead-in fluoxetine for 8 weeks was MADRS score change from baseline. Mixed-effects repeated-measures (MMRM) analysis of variance was performed for each of the treatments separately (fixed covariates: baseline MADRS total score, country, visit, genotype, and genotype-by-visit interaction, subject as random effect with repeated measures in 9 visits over 8 weeks, and the baseline visit). For the continued-fluoxetine group, the model also included gender (significant, with $P < .05$ in the MMRM model, in this arm before adding genotype terms). The additive genetic model was used; SNPs were entered as 0, 1, or 2, corresponding to the number of minor alleles. Covariance structures used for repeated measures in olanzapine-fluoxetine combination and fluoxetine were Toeplitz; in olanzapine, the autoregressive of order 1. The covariance structures were chosen from the best-fitting models before adding the genotype terms among models with the following covariance structures: unstructured, Toeplitz, autoregressive of order 1, and compound symmetric. Akaike information criterion (AIC)[38] was used to determine the best-fitting models. Analyses were done using SAS PROC MIXED procedure (9.1, SAS Institute Inc, Cary, North Carolina).

For SNPs with $P < .10$, least squares (LS) mean values of MADRS score change were estimated with standard error stratified by genotype at week 8. Week 8 differences in MADRS scores across genotypes were tested with analysis of variance of genotype as the contrast variable in the MMRM (covariates specified above). The MADRS score LS mean change versus time for the entire 8 weeks was plotted.

Response and remission rates for the different genotypes were calculated by Kaplan-Meier estimates. Response was defined as achieving a decrease in MADRS score > 50% of baseline; remission, as a MADRS score of < 10. Subjects were considered to achieve response or remission if they retained that status at the end of the trial (or last visit). Log-rank tests assessed the differences in the response and remission rates across different genotypes.

Multiple test correction adjusted for the effective number of SNPs tested within a gene. The method of Li and Ji,[39] implemented by Nyholt,[40] was used to estimate the effective number of independent tests within a gene. Bonferroni correction was then applied to adjust for the effective number of SNPs in a given gene (usually much smaller than the actual number because of high correlation among SNPs within a gene). The SNPs with Li-Nyholt–adjusted (corrected) 2-sided $P < .05$ were considered to be significantly associated with response in these groups. The SNPs with unadjusted $P < .05$ but adjusted $P > .05$ were considered to be nominally associated, especially if multiple SNPs in high linkage disequilibrium in the same gene achieved this level of significance. While it is more robust to control the family-wise error rate or false discovery rate at the level of .05 for all the SNPs, such a criterion would be too strict for these modest sample sizes. We assessed the false discovery rate[41] for the first-tier SNPs and the second-tier SNPs separately to elucidate highly significant SNPs that could achieve false discovery rate < .05. Due to the relatively small sample sizes in the treatment cohorts, raising greater concern for type II error, SNPs with unadjusted 2-sided $P > .05$ but $P < .10$ were retained for follow-up analyses.

RESULTS

Patients

Two hundred five self-reported white patients (olanzapine-fluoxetine combination, n = 71; continued fluoxetine, n = 78; and olanzapine, n = 56) were included (Supplementary eTables 2 and 3).

Genetic Association With Continued-Fluoxetine Response

Four hundred eleven SNPs met quality control criteria and were included. In the continued-fluoxetine group, 1 first-tier candidate SNP, *HTR1A* rs6295 had an unadjusted $P < .05$ for an association with change in MADRS score, after 8 weeks of treatment ($P = .037$). In the first- and second-tier analyses combined, 33/411 tested genetic variants had an unadjusted $P < .05$ (Supplementary eTable 4) after 8 weeks of treatment. After correction for multiple SNPs comparison, none had an adjusted $P < .05$ for an association with change in MADRS during continued-fluoxetine treatment.

Genetic Association With Olanzapine Response

In the first- and second-tier analyses combined, 67/411 SNPs showed associations with change in depression with olanzapine, with an unadjusted $P < .05$; 3 SNPs, *CYP2D6* rs9623531 and rs16947 and *DRD2* rs1125393, had a $P < .05$ after false discovery rate multiple comparison adjustment for all SNPs (false discovery rate $P = .00012$, $P = .016$, $P = .043$, respectively [Supplementary eTable 5]), while 1 SNP, *TPH2* rs7305115, had a Li-Nyholt adjusted $P = .041$ (Supplementary eTable 6). Because these results are of questionable clinical utility and interpretable only with limitations (limited overall treatment response and the extended half-life of fluoxetine [and norfluoxetine] from lead-in treatment), they receive less attention (Supplementary eTables 5 and 6).

Genetic Association With Olanzapine-Fluoxetine Combination Response

For olanzapine-fluoxetine combination, 2 of the candidate SNPs, *COMT* rs174697 and *CYP2D6* rs9623531, had an unadjusted $P < .05$ when tested for association with change in depression ($P = .014$ and $P = .029$, respectively), with the same direction of effect as previously reported *COMT* rs174697 in duloxetine major depressive disorder treatment.[11] After false discovery rate adjustment for multiple comparisons, neither SNP had a $P < .05$.

In the combined first- and second-tier analyses, 45/411 SNPs had an unadjusted $P < .05$ when tested for associations with depression improvement with olanzapine-fluoxetine combination (Table 1). While none had a false discovery rate < .05 in multiple-test adjustment for all SNPs, several were significant with Li-Nyholt adjusted $P < .05$ when adjusted for the effective number of SNPs tested in the genes. These included *SLC6A2* rs36024 (effective number of independent SNPs [Meff] = 24/77 SNPs), *TPH2* rs7305115 and rs4290270 (Meff = 2/3 SNPs), and *MC3R* rs6014649, rs3746619, and rs3827103 (Meff = 3/5 SNPs; Table 1).

Analysis of LS mean changes of MADRS over time, stratified by *SLC6A2* rs36024 genotype, revealed distinct differences between carriers of 0, 1, or 2 alleles of the polymorphism with olanzapine-fluoxetine combination (Figure 1); no such differences were seen in the continued-fluoxetine group. When comparing olanzapine-fluoxetine combination versus continued-fluoxetine carriers of the *SLC6A2* rs998424 genotype, the groups showed opposite LS mean changes in MADRS score over time (Figure 2).

Linkage Disequilibrium Map Analysis for *SLC6A2*

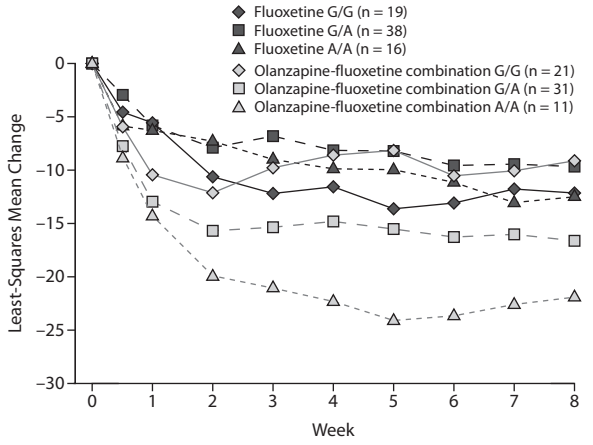
The 77 *SLC6A2* SNPs span a 54.3 kilobase (kb) region that contains 8 haplotype blocks; however, 6 blocks are very small and only 2 main blocks remain (linkage disequilibrium blocks 1 and 2). The first main block spans 16 kb (rs168924 to rs187714) and contains rs36024, which had a Li-Nyholt corrected $P < .05$ in the olanzapine-fluoxetine combination group. Additionally, several SNPs with an uncorrected $P < .05$ in olanzapine-fluoxetine combination reside in the first main linkage disequilibrium block (therefore, these are closely linked with rs36024). For the continued-fluoxetine group, a few SNPs in the first main *SLC6A2* linkage disequilibrium block had an unadjusted $P < .05$. This outcome is contrasted by the second main linkage disequilibrium block, spanning a region of 13 kb (rs12443955 to rs998424), in which several genetic variants had an unadjusted $P < .05$ for continued

Table 1. Single-Nucleotide Polymorphisms (SNPs) Nominally[a] Statistically Significantly Associated With Response to Olanzapine-Fluoxetine Combination

		MADRS Total Score Change						
		AA		AB		BB		
Gene SNP (A/B)[b]	Gene Region[c]	LS Mean (SE)	n	LS Mean (SE)	n	LS Mean (SE)	n	Uncorrected *P* Value[d]
ADRA1A								
rs10503801 (C/A)	Intron 1	−16.1 (1.6)	53	−8.8 (4.1)	7	NA		.011
COMT								
rs174697 (G/A)	Intron 6	−13.7 (1.5)	59	−24.3 (3.0)	10	NA		.014
CRHR2								
rs8192496 (T/C)	Intron 4	−18.9 (2.4)	21	−13.0 (1.8)	41	NA		.049
CYP2D6								
rs9623531 (T/C)	Upstream	−12.2 (2.3)	25	−17.9 (2.1)	31	−21.4 (5.4)	5	.029
DRD3								
rs9817063 (A/G)	Downstream	−14.7 (2.3)	24	−12.5 (2.2)	23	−22.4 (3.2)	13	.043
HTR2A								
rs2296973 (C/A)	Intron 1	−13.2 (2.1)	28	−17.4 (1.9)	33	−20.2 (4.1)	5	.044
HTR2B								
rs17586405 (T/C)	Intron 2	−14.2 (1.5)	58	NA		−24.1 (4.1)	6	.048
HTR6								
rs3790756 (C/T)	Intron 2	−17.3 (1.6)	53	−12.3 (2.5)	15	−4.6 (6.5)	2	.035
MC3R								
rs6014649 (G/A)	**Upstream**	**−14.5 (1.5)**	**52**	**−22.8 (2.9)**	**14**	**NA**		**.0015**
rs3746619 (C/A)	**5′ UTR**	**−14.0 (1.5)**	**53**	**−23.0 (2.8)**	**15**	**NA**		**.0018**
rs3827103 (G/A)	**Exon 1**	**−14.3 (1.5)**	**51**	**−23.0 (2.8)**	**15**	**NA**		**.0031**
SLC6A2								
rs4783899 (T/G)	Upstream	−13.1 (3.0)	14	−12.8 (2.2)	24	−19.6 (2.1)	22	.031
rs168924 (A/G)	Upstream (LD1)	−13.8 (1.6)	48	−22.3 (3.3)	14	−23.4 (9.2)	1	.015
rs36031 (A/G)	Intron 2 (LD1)	−19.5 (2.1)	27	−12.7 (2.3)	22	−11.8 (3.8)	8	.0086
rs36030 (T/C)	Intron 2 (LD1)	−13.8 (1.6)	48	−22.3 (3.3)	14	−23.4 (9.2)	1	.015
rs11076111 (C/G)	Intron 2 (LD1)	−13.6 (2.3)	30	−16.5 (2.4)	21	−23.0 (4.4)	5	.038
rs36029 (A/G)	Intron 2 (LD1)	−19.4 (2.1)	26	−12.1 (2.2)	25	−11.0 (3.5)	10	.014
rs36028 (G/T)	Intron 2 (LD1)	−13.0 (1.6)	46	−22.4 (3.1)	15	NA		.0049
rs40519 (G/C)	Intron 2 (LD1)	−14.1 (1.6)	45	−22.5 (3.3)	13	−22.0 (8.4)	2	.019
rs17307291(A/G)	Intron 2 (LD1)	−13.0 (2.2)	32	−15.8 (2.1)	25	−22.5 (3.8)	6	.025
rs40434 (A/G)	Intron 2 (LD1)	−19.8 (2.0)	28	−13.3 (2.1)	26	−9.2 (4.1)	8	.0095
rs17247999 (A/G)	Intron 2 (LD1)	−13.0 (2.2)	32	−15.8 (2.1)	25	−22.5 (3.8)	6	.025
rs40518 (G/C)	Intron 2 (LD1)	−20.0 (2.0)	28	−13.7 (2.4)	21	−11.7 (3.5)	10	.0090
rs41154 (A/G)	Intron 2 (LD1)	−18.9 (2.0)	27	−11.0 (2.3)	24	−11.0 (3.4)	10	.010
rs13333066 (C/T)	Intron 2 (LD1)	−13.0 (2.2)	31	−15.4 (2.2)	23	−22.7 (3.9)	6	.033
rs933555 (T/A)	Intron 3 (LD1)	−19.8 (2.0)	27	−12.7 (2.3)	23	−11.3 (3.4)	10	.0058
rs36026 (C/A)	Intron 3 (LD1)	−14.0 (1.6)	50	−22.0 (3.6)	12	−23.4 (9.3)	1	.030
rs36025 (C/T)	Intron 3 (LD1)	−13.8 (1.6)	48	−22.3 (3.3)	14	−23.4 (9.2)	1	.015
rs36024 (G/A)	**Intron 4** (LD1)	**−9.2 (2.4)**	**21**	**−16.7 (2.0)**	**31**	**−21.9 (2.8)**	**11**	**.00034**
rs187714 (A/G)	Intron 4 (LD1)	−20.4 (2.0)	27	−12.2 (2.2)	25	−11.2 (3.5)	10	.0079
rs36021 (A/T)	Intron 4	−13.4 (2.7)	16	−15.4 (2.1)	30	−20.1 (2.8)	16	.039
rs36020 (C/T)	Intron 4	−13.1 (1.7)	47	−20.1 (3.1)	14	−30.0 (6.5)	2	.017
rs11862589 (T/C)	Intron 5 (LD2)	−15.5 (2.8)	14	−14.2 (1.9)	36	−24.4 (3.6)	10	.035
rs2397772 (C/G)	Intron 5 (LD2)	−14.9 (2.1)	29	−13.8 (2.2)	25	−21.7 (3.8)	9	.048
rs12920735 (A/T)	Intron 5 (LD2)	−14.7 (2.0)	29	−14.6 (2.2)	24	−21.1 (3.7)	9	.036
rs47958 (C/A)	Intron 6 (LD2)	−18.5 (2.6)	17	−12.9 (2.1)	29	−13.5 (3.0)	13	.046
rs998424 (G/A)	Intron 10 (LD2)	−15.3 (1.9)	33	−15.2 (2.3)	22	−27.5 (4.2)	7	.0099
SLC6A4								
rs8071667 (C/T)[e]	Intron 1	−18.3 (1.8)	42	−9.9 (2.6)	17	−7.5 (6.5)	4	.012
rs2020936 (T/C)[e]	Intron 1	−18.3 (1.8)	42	−9.9 (2.6)	17	−7.5 (6.5)	4	.012
rs6354 (A/C)	Intron 1	−17.9 (1.8)	40	−10.0 (2.6)	17	−7.6 (6.5)	4	.016
rs2020933 (T/A)	Intron 1	−16.7 (1.6)	52	−10.0 (3.3)	9	NA		.030
rs1872924 (T/C)	Intron 2	−17.4 (1.8)	38	−14.6 (2.5)	18	2.0 (8.9)	3	.049
rs140700 (G/A)	Intron 5	−16.9 (1.7)	48	−10.0 (3.2)	13	NA		.032
TPH2								
rs7305115 (G/A)	**Exon 7 end region**	**−20.4 (2.2)**	**22**	**−11.4 (2.1)**	**29**	**−14.0 (2.8)**	**15**	**.0021**
rs4290270 (T/A)	**Exon 9**	**−18.6 (2.1)**	**27**	**−13.0 (2.1)**	**28**	**−13.0 (3.3)**	**12**	**.015**

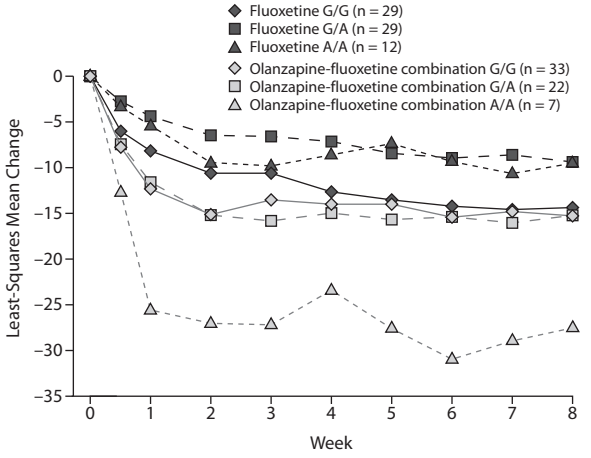
[a]SNPs for which uncorrected *P* value < .05.
[b]The alleles in parentheses following the SNP number represent A and B.
[c]LD1 and LD2 are *SLC6A2* linkage disequilibrium blocks as described in Results.
[d]SNPs with within-gene Li-Nyholt–corrected *P* values < .05 are shown in boldface: *SLC6A2* rs36024, *P* = .0082; *MC3R* rs6014649, *P* = .0045, *MC3R* rs3746619, *P* = .0054; *MC3R* rs3827103, *P* = .0093; *TPH2* rs7305115, *P* = .0042; *TPH2* rs4290270, *P* = .030. All other corrected *P* values were nonsignificant. White allele frequencies in public databases (Perlegen, HGBase, HapMap) for significant SNPs: rs6014649 A.125, G.875; rs3746619 A.145, G.854; rs3827103 A.125, G.875; rs7305115 A.333, G.667; rs4290270 A.3, T.7; rs36024 A.385, G.615.
[e]Li-Nyholt *P* = .074.
Abbreviations: LD = linkage disequilibrium, LS = least squares, MADRS = Montgomery-Asberg Depression Rating Scale, NA = not available, SE = standard error, UTR = untranslated region.

Figure 1. Least-Squares Mean Change by Visit for the MADRS Total Score by *SLC6A2* rs36024 Genotype[a]



[a]The continued fluoxetine–treated group did not differ significantly by genotype (uncorrected $P = .58$); olanzapine-fluoxetine combination–treated group had uncorrected $P < .001$, corrected $P = .008$ by genotype.
Abbreviation: MADRS = Montgomery-Asberg Depression Rating Scale.

Figure 2. Least-Squares Mean Change by Visit for the MADRS Total Score by *SLC6A2* rs998424 Genotype[a]



[a]The continued fluoxetine–treated group had uncorrected $P = .049$ by genotype; olanzapine-fluoxetine combination–treated group had uncorrected $P = .010$ by genotype.
Abbreviation: MADRS = Montgomery-Asberg Depression Rating Scale.

fluoxetine, while a few SNPs in this block had a $P < .05$ for olanzapine-fluoxetine combination (Figure 3).

We also compared the change in MADRS score from baseline for both continued fluoxetine and olanzapine-fluoxetine combination in 8 *SLC6A2* SNPs (Supplementary eTable 7) because of the possible relationship between norepinephrine transport and prefrontal cortical concentration increases in norepinephrine with olanzapine. Consistent with this potential link, we found that the minor allele associated with greater improvement with olanzapine-fluoxetine combination was associated with less improvement with continued fluoxetine for 6/7 SNPs ($P < .10$ for each). This finding was mirrored by changes in MADRS over time when stratified by genotype (rs998424 in Figure 2).

DISCUSSION

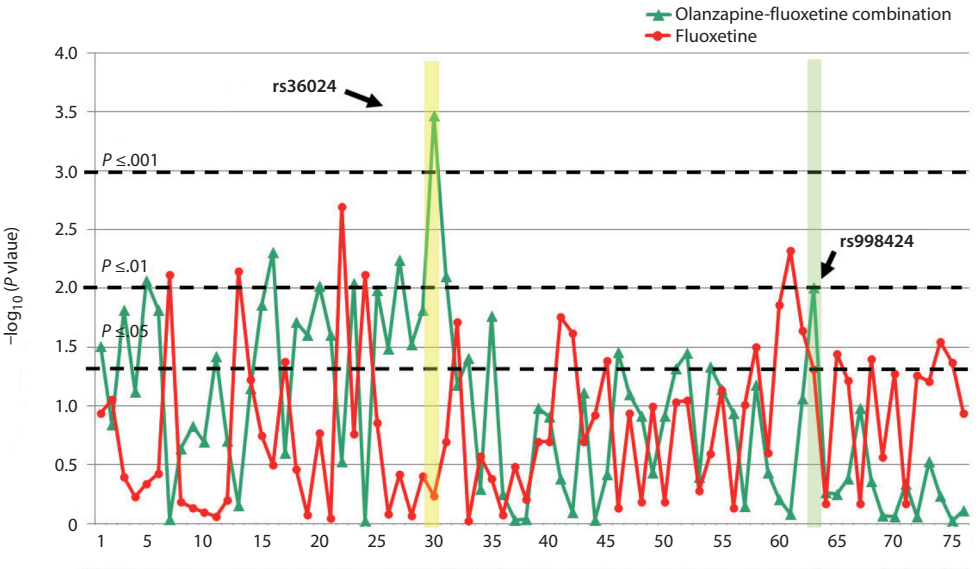
This study demonstrated an association of *SLC6A2* rs36024 with improvement of depressive symptoms in patients with treatment-resistant depression during olanzapine-fluoxetine combination treatment. Therefore, *SLC6A2* rs36024 might be a marker for response to olanzapine-fluoxetine combination in patients with treatment-resistant depression who did not respond to fluoxetine monotherapy. Response and remission rates by genotype for rs36024 suggest that the AA genotype is more strongly associated with both response (90.9%) and remission (63.6%) than the GA genotype (53.2% and 42.7%) or the GG genotype (23.1% and 18.5%) (response [$P = .001$] and remission rates [$P = .037$]). These rates are in agreement with the decreases in MADRS scores observed over time (Figure 1). Residual fluoxetine and norfluoxetine, due to prolonged half-lives, most likely affected response to olanzapine during randomization for the first weeks. Similar patterns of response for these treatments over the first few weeks, with subsequent divergence thereafter, were reported (Figure 2),[23] which limit the value of potential interpretations.

SLC6A2 codes the norepinephrine transporter, and polymorphisms have been previously associated with antidepressant response.[42,43] Zhang and colleagues[24] described synergistic effects of olanzapine and fluoxetine on the prefrontal cortical levels of norepinephrine and dopamine in rodents and proposed that this synergy might be an underlying mechanism for the effectiveness of olanzapine-fluoxetine combination in treatment-resistant depression. Our findings support this hypothesis and demonstrate that carriers of the rs36024 SNP who are nonresponsive to fluoxetine benefit in symptom improvement with added olanzapine.

In our analysis, 26/77 *SLC6A2* SNPs had uncorrected $P < .05$ for an association with depression improvement. Noteworthy is the potential opposite direction of effect for olanzapine and fluoxetine versus continued fluoxetine, which results in potential use of these SNPs as a potent differentiator of response. For example, in the SNP rs998424 (G/A), with an uncorrected $P = .010$ for association with MADRS improvement during olanzapine-fluoxetine combination treatment (versus $P = .049$ during continued fluoxetine), the AA genotype had response rates of 80.0% versus 25.0%; the AG genotype, 50.3% versus 29.4%; and the GG genotype, 48.8% versus 40.5% for olanzapine-fluoxetine combination and continued fluoxetine, respectively. This result requires replication in a separate dataset.

In our analysis, nominal *P* values for association with olanzapine-fluoxetine combination response for SNPs previously reported to be associated with amphetamine response (rs36017, $P = .104$; rs1861647, $P = .072$)[37] and response to milnacipran (rs5569/Val499Ile, $P = .087$; rs224244, $P = .076$),[12] while nonsignificant, may be of interest given our dataset's power.

Figure 3. Linkage Disequilibrium Map and *P* Values for *SLC6A2* Single-Nucleotide Polymorphisms (SNPs) and Change in Depression Severity



SNP in order from left to right

snp 1 to 10	RS4783899	RS2397771	RS168924	RS2242446	RS36031	RS36030	RS17306977	RS9937434	RS17841327	RS17307096
snp 11 to 20	RS11076111	RS12232407	RS12932949	RS3785143	RS36029	RS36028	RS2062723	RS40519	RS17307291	RS40434
snp 21 to 30	RS17247999	RS6499771	RS40518	RS734980	RS41154	RS13333066	RS933555	RS36026	RS36025	**RS36024**
snp 31 to 40	RS187714	RS36023	RS36021	RS3785151	RS36020	RS3785152	RS1814269	RS1814270	RS12443955	RS36017
snp 41 to 50	RS36016	RS10521329	RS1861646	RS40616	RS3785155	RS880711	RS11862589	RS4436775	RS879519	RS11649052
snp 51 to 60	RS879522	RS2397772	RS12920735	RS5564	RS47958	RS1861647	RS2279805	RS3785157	RS12924088	RS1566652
snp 61 to 70	RS12708954	RS8047672	RS5569	**RS998424**	RS36009	RS1800887	RS8049681	RS36007	RS8050050	RS2242447
snp 71 to 77	RS15534	RS42460	RS7194256	RS10521330	RS9930182	RS16955708	RS12929956			

The large number of *SLC6A2* SNPs across the gene indicates promise as predictors of response, given the proposed mechanism of olanzapine-fluoxetine combination in treating treatment-resistant depression. A haplotype analysis might be productive in a larger dataset. Lack of congruence between our individual SNP and previous results[44] suggests that a haplotype analysis would not be of value in haplotype selection in our study. The differences in distribution of *SLC6A2* SNPs that are nominally significant for continued-fluoxetine response in 1 large linkage disequilibrium region versus those for olanzapine-fluoxetine combination response in the other large linkage disequilibrium region of the gene and differences in the direction of effect at given SNPs that are significant for both suggest that patients who are responsive to continued fluoxetine versus olanzapine-fluoxetine combination have differences in norepinephrine transporter activity, where lack of norepinephrine transporter with fluoxetine effects would render those patients nonresponsive to fluoxetine monotherapy, and compensation with olanzapine in olanzapine-fluoxetine combination effects would render the same patients more responsive to olanzapine-fluoxetine combination.

TPH2 codes for tryptophan hydroxylase 2, the rate-limiting enzyme of serotonin biosynthesis, and *TPH2* rs7305115 and rs4290270 were associated with depressive symptom response. An association of these SNPs with depressive symptom response has been described previously.[9,11,45] Additionally, these 2 SNPs have been associated with variations in *TPH2* messenger ribonucleic acid expression in the dorsal and median raphe,[46] the main sources of

serotonin in the forebrain. *TPH2* rs7305115 and rs4290270 in olanzapine-fluoxetine combination and rs7305115 in olanzapine had Li-Nyholt–corrected $P < .05$ each for association with depression improvement. However, the olanzapine group had remaining levels of fluoxetine and norfluoxetine during the initial weeks of treatment, which limit the interpretation of the observed rs7305115 *P* value and explain the relatively low olanzapine *P* value despite very similar changes in MADRS by *TPH2* genotype.

MC3R encodes the melanocortin-3 receptor, and rs6014649, rs3746619, and rs3827103 had a Li-Nyholt–corrected $P < .05$ in olanzapine-fluoxetine combination and an uncorrected $P < .05$ in olanzapine for association with depression improvement. Neither an association of *MC3R* polymorphisms with depressive symptoms nor response to olanzapine-fluoxetine combination or olanzapine has been reported previously. Interestingly, genetic variations in *MC3R* have been associated with obesity[47,48] and, during olanzapine and olanzapine-fluoxetine combination treatment, weight gain has been observed.[4] Possible associations of depression response with *MC3R* variants may be due to effects mediated by the hypothalamic-pituitary-adrenal axis, but future research is needed. Again, the validity of the observed *P* value in the olanzapine group might be impaired by the remaining fluoxetine and norfluoxetie levels in this group.

Nonreplication of previous associations with depression response to olanzapine-fluoxetine combination in depressed patients with bipolar disorder[49] in our analysis involving unipolar patients with treatment-resistant depression may be due to either power limitations or possible differences in unipolar versus bipolar depression.

The interpretation of these results is limited to white patients. Although SNPs had within-gene significance for an association with response after multiple testing corrections, significance after correction among genes is lost. However, many candidate genes would not be expected to have a direct effect on olanzapine augmentation of fluoxetine effects on prefrontal norepinephrine, with the gene with the strongest likelihood for association (*SLC6A2*) being most closely linked to the proposed treatment-resistant depression olanzapine-fluoxetine combination mechanism of action. Limited coverage of some candidate genes precludes the exclusion of associations between other parts of those genes and changes in depressive symptoms. Interpretation of our results is useful for purposes of determining appropriate patients for continued fluoxetine versus olanzapine-fluoxetine combination, rather than for predicting response to initial olanzapine-fluoxetine combination or fluoxetine, since patients in the randomization phase had already failed with fluoxetine. Additionally, an initial power analysis for olanzapine-fluoxetine combination indicated that our sample has a power of > 80% to achieve a nominal significance level of .05 and a significance level of .05 adjusted by ~ 24 SNPs (number of effective SNPs in *SLC6A2*). However, power is limited when adjusting for all 411 SNPs with genetic effects in the olanzapine-fluoxetine combination arm. Analyses testing differential effect of SNPs by comparing treatment arms were not performed due to small sample sizes that would have resulted in very limited power.

In conclusion, we were able to demonstrate an association of *SLC6A2* rs36024 with improvement of depressive symptoms in patients with treatment-resistant depression during olanzapine-fluoxetine combination treatment, which is consistent with a model in which olanzapine added to fluoxetine potentiates prefrontal norepinephrine levels. Correlative relations with response to fluoxetine monotherapy suggest some patients are nonresponsive to fluoxetine due to minimal effects on norepinephrine. This finding may help identify patients with treatment-resistant depression who will benefit most from olanzapine-fluoxetine combination versus fluoxetine treatment. Additional work with larger datasets would be helpful in clarifying, refining, and replicating associations with response.

Drug names: duloxetine (Cymbalta), fluoxetine (Prozac and others), milnacipran (Savella), olanzapine (Zyprexa and others), olanzapine-fluoxetine combination (Symbyax).

Author affiliations: Lilly USA, LLC, and Department of Psychiatry, Indiana University School of Medicine (Dr Houston), and Eli Lilly and Company (Drs Liu and Fijal), Indianapolis, Indiana; PharmaNet/i3, an inVentiv Health Company, Ann Arbor, Michigan (Drs Lau, Aris, and Heinloth); and Department of Psychiatry, Massachusetts General Hospital, Boston (Dr Perlis).

Potential conflicts of interest: **Dr Houston** is a full-time employee of Lilly USA, LLC, and a minor stockholder of Eli Lilly and Company. **Drs Lau, Aris**, and **Heinloth** are full-time employees of PharmaNet/i3, an inVentiv Health Company, which is a subsidiary of UnitedHealth Group. **Dr Liu** was a full-time employee and minor stockholder of Eli Lilly and Company at the time of manuscript completion and is now an employee of Pfizer. **Dr Fijal** is a full-time employee and minor stockholder of Eli Lilly and Company. **Dr Perlis** has received consulting fees from Eli Lilly and Company, Proteus Biomedical, RIDventures, and Genomind Equity; has received grant/research support from Proteus Biomedical; and holds patents with and receives royalties from Concordant Rater Systems.

Funding/support: This study was supported by Eli Lilly and Company and Lilly USA, LLC.

Role of sponsor: Eli Lilly designed and conducted the study; collected, managed, analyzed, and interpreted the data; and prepared, reviewed, and approved the manuscript. Eli Lilly contracted PharmaNet/i3 for statistical and writing support.

Acknowledgments: The authors thank Laura Tyler, BA; Barbara McLean, BA; and Angela Lorio, BA, of PharmaNet/i3, part of the inVentiv Health Company, for editorial assistance. Mss Tyler, McLean, and Lorio have no additional conflicts of interest to report.

Supplementary material: Available at PSYCHIATRIST.COM

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Supplementary Material

Article Title: Association of Common Variations in the Norepinephrine Transporter Gene With Response to Olanzapine-Fluoxetine Combination Versus Continued-Fluoxetine Treatment in Patients With Treatment-Resistant Depression: A Candidate Gene Analysis

Author(s): John P. Houston, MD, PhD; Kit Lau, PhD; Virginie Aris, PhD; Wenlei Liu, PhD; Bonnie A. Fijal, PhD; Alexandra N. Heinloth, MD; and Roy H. Perlis, MD, MSc

Citation: J Clin Psychiatry 2012;73

DOI Number: 10.4088/JCP.10m06744

List of Supplementary Material for the article

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eTable 1. Candidate Genes Used in the Association Analyses.

Gene	Number of genetic variants analyzed
ABCB1	2
ADRA1A	22
ADRA2A	4
ANKK1	6
BDNF	1
COMT	13
CRH	6
CRHR1	15
CRHR2	21
CYP2D6	12
DBH	2
DRD1	5
DRD2	59
DRD3	26
DRD4	1
GNB3	1
GRIA4	1
GRIK1	1
GRM3	6
HFE	1
HRH1	7
HTR1A	5
HTR2A	14
HTR2B	10
HTR2C	10
HTR3A	3
HTR6	9
IL6	1
INS	1
KIAA0999	1
MC2R	10
MC3R	5
MTHFR	2
NR3C1	13
NUDT9P1	1
POMC	5
RGS4	5
SLC2A2	1
SLC6A2	77
SLC6A4	14
TNF	1
TPH1	7
TPH2	3
XKR4	1

eTable 2. Baseline Patient Characteristics.

	Study Period 3 atients (Non-responsive to FLX lead-in)		
Parameter	**OFC-treated patients (N=71)**	**Continued-FLX-treated patients (N=78)**	**OLZ-treated patients (N=56)**
Female gender, n (%)	52 (73.2)	48 (61.5)	36 (64.3)
Country of origin, n (%)			
USA	56 (78.9)	68 (87.2)	48 (85.7)
Canada	15 (21.1)	10 (12.8)	8 (14.3)
Age (years), mean (SD)	43.8 (9.5)	45.0 (10.4)	44.9 (9.2)
BMI (kg/m^2)	31.5 (7.8)	30.4 (8.3)	31.7 (6.3)
MADRS-total score, mean (SD)	30.8 (6.1)	30.8 (6.8)	30.7 (6.8)

Abbreviations: BMI = body mass index; FLX = fluoxetine; MADRS = Montgomery-Åsberg Depression Rating Scale; N = total number of patients in group; n = number of affected patients; OFC = olanzapine/fluoxetine combination; OLZ = olanzapine; SD = standard deviation.

eTable 3. Number of Patients in the OFC, OLZ, and FLX Groups from Visit 7 (Baseline) to Visit 16 (End of Trial).

Visit	OFC	OLZ	FLX
7	71	56	78
8	71	56	78
9	67	55	77
10	64	55	74
11	62	51	74
12	59	47	69
13	57	44	69
14	53	40	66
15	51	37	66
16	52	36	64

Abbreviations: FLX = fluoxetine; OFC = olanzapine/fluoxetine combination; OLZ = olanzapine.

eTable 4. Tier 2 SNPs, Nominally Statistically Significantly Associated with Response to Continued-Fluoxetine During Study Period 3.

		LS mean change (SE) MADRS-TS and n			
Gene SNP	**Gene region[a]**	**AA**	**AB**	**BB**	**Uncorrected p-value**
ADRA1A					
rs526302 (G/T)	Intron-1	-11.8 (1.6), n=40	-10.1 (2.0), n=25	-5.4 (3.5), n=6	.016
rs472865 (C/T)	Intron-1	-9.3 (1.5), n=54	-14.4 (2.1), n=18	-22.9 (8.2), n=1	.020
DRD3					
rs3732783 (A/G)	Exon-2	-10.3 (1.3), n=64	-20.8 (3.9), n=6	NA	.011
GRM3					
rs1989796 (C/T)	Intron-5	-11.4 (2.1), n=21	-8.7 (1.6), n=36	-13.6 (2.3), n=16	.041
HTR2A					
rs912127 (G/A)	Intron-2	-10.2 (1.6), n=34	-11.6 (1.8), n=36	-22.7 (6.8), n=2	.014
rs3742278 (A/G)	Intron-2	-9.6 (1.4), n=49	-14.9 (2.1), n=22	-5.5 (8.1), n=1	.015
HTR1A					
rs10042486 (G/A)	Upstream	-7.7 (2.1), n=21	-11.4 (1.6), n=40	-11.1 (2.6), n=12	.037
rs6295 (G/C)	Upstream	-8.1 (2.0), n=24	-11.4 (1.6), n=41	-11.9 (2.5), n=13	.037
rs878567 (T/C)	Downstream	-7.7 (2.1), n=21	-11.5 (1.6), n=41	-11.1 (2.6), n=12	.037
rs749099 (G/A)	Downstream	-8.2 (2.1), n=23	-12.0 (1.7), n=35	-11.7 (2.6), n=13	.035
MC3R					
rs6127698 (G/T)	Upstream	-11.4 (2.0), n=23	-12.0 (1.5), n=37	-3.3 (2.5), n=14	.013
MTHFR					
rs1801133 (G/A)	Exon-4	-14.1 (1.9), n=28	-9.1 (1.6), n=33	-8.4 (3.0), n=9	.016
rs1801131 (T/G)	Exon-7	-10.0 (1.6), n=43	-10.5 (1.9), n=26	-15.3 (4.1), n=4	.044
POMC					
rs7582597 (C/T)	Upstream	-15.7 (2.3), n=21	-8.6 (1.4), n=51	NA	.032
SLC6A2					
rs17306977 (A/T)	Intron-2 (LD1)	-10.5 (1.3), n=65	-16.7 (3.4), n=7	-17.6 (8.1), n=1	.0077
rs12932949 (G/C)	Intron-2 (LD1)	-10.0 (1.4), n=61	-16.5 (3.5), n=7	-17.7 (8.2), n=1	.0072
rs2062723 (T/C)	Intron-2 (LD1)	-9.8 (1.7), n=27	-10.4 (1.6), n=35	-14.6 (3.0), n=9	.042
rs6499771 (A/G)	Intron-2 (LD1)	-10.2 (1.3), n=63	-18.4 (3.0), n=9	-17.8 (8.0), n=1	.0020
rs734980 (A/G)	Intron-2 (LD1)	-10.5 (1.3), n=65	-16.7 (3.4), n=7	-17.6 (8.1), n=1	.0077
rs36023 (G/A)	Intron-4	-8.1 (1.8), n=29	-11.6 (1.7), n=33	-17.4 (2.9), n=11	.019

rs10521329 (C/A)	Intron-5 (LD2)	-9.0 (1.5),, n=51	-15.0 (1.9), n=21	NA	.017
rs1861646 (G/T)	Intron-5 (LD2)	-8.9 (1.5), n=52	-14.6 (2.0), n=20	NA	.024
rs880711 (G/A)	Intron-5 (LD2)	-8.4 (1.6), n=49	-15.5 (2.2), n=18	NA	.041
rs12924088 (A/G)	Intron-9 (LD2)	-14.4 (1.8), n=30	-9.4 (1.7), n=29	-8.0 (2.4), n=14	.031
rs12708954 (C/A)	Intron-9 (LD2)	-9.1 (1.5), n=52	-15.4 (2.0), n=20	NA	.014
rs8047672 (G/A)	Intron-9 (LD2)	-8.6 (1.5), n=52	-16.3 (2.0), n=19	NA	.005
rs5569 (G/A)	Exon-9 (LD2)	-14.3 (1.8), n=32	-8.8 (1.7), n=31	-8.1 (2.4), n=14	.023
rs998424 (G/A)	Intron-10 (LD2)	-14.4 (1.9), n=29	-9.3 (1.8), n=29	-9.5 (2.6), n=12	.049
rs1800887 (T/C)	Intron-12	-9.0 (1.6), n=44	-13.1 (1.8), n=28	NA	.036
rs8050050 (G/A)	Intron-13	-8.6 (1.4), n=51	-15.8 (2.1), n=17	NA	.040
rs9930182 (T/G)	Downstream	-8.7 (1.6), n=45	-13.5 (1.8), n=27	NA	.028
rs16955708 (T/C)	Downstream	-9.8 (1.5), n=55	-13.4 (2.0), n=18	NA	.043
TNF					
rs1800610 (C/T)	Intron-2	-11.6 (1.3), n=61	-1.7 (2.7), n=12	NA	.0042

Abbreviations: LS mean=least-squared mean; MADRS-TS = Montgomery-Åsberg Depression Rating Scale Total Score; n=number of patients; NA=not available; SE = standard error; SNP = single nucleotide polymorphism

[a] LD1 and LD2 are *SLC6A2* linkage disequilibrium blocks as described in Results.

Note: the alleles in parentheses following the SNP number represent A and B.

eTable 5. Candidate SNPs Statistically Significantly Associated with Response to Olanzapine.

		LS mean change (SE) MADRS-TS and n			
Gene SNP (A/B)	**Gene region**	**AA**	**AB**	**BB**	**Uncorrected p-value**
RGS4 rs6678136 (G/A)					
	Upstream	-7 (2.7), n=11	-13 (1.8), n=28	-13 (2.2), n=14	.008
HTR6 rs6693503 (G/A) rs9659997 (T/C)					
	Upstream	-15 (1.9), n=24	-11 (1.8), n=28	-8 (5.0), n=4	.021
	Intron-2	-15 (1.9), n=24	-11 (1.8), n=28	-8 (5.0), n=4	.021
COMT rs174696 (T/C)					
	Intron-6	-10 (1.8), n=31	-13 (2.2), n=18	-19 (4.3), n=3	.023

Abbreviations: LS mean=least-squared mean; MADRS-TS = Montgomery-Åsberg Depression Rating Scale Total Score; n=number of patients;SE = standard error; SNP = single nucleotide polymorphism.

Note: the alleles in parentheses following the SNP number represent A and B.

eTable 6. Tier 2 SNPs, Nominally Statistically Significantly Associated with Response to Olanzapine.

		LS mean change (SE) MADRS-TS and n			
Gene SNP (A/B)	**Gene region**	**AA**	**AB**	**BB**	**Uncorrected p-value**
ADRA1A					
rs2291775 (A/G)	Intron-2	-10.3 (1.6), n=46	-20.3 (4.1), n=4	NA	.032
rs10503799 (G/A)	Downstream	-10.2 (1.5), n=48	-20.4 (4.1), n=4	NA	.024
ANKK1					
rs2734848 (A/G)	Exon-8	-14.2 (1.8), n=29	-10.8 (1.9), n=22	-7.1 (3.9), n=5	.0059
rs1800497 (G/A)	Exon-8	-9.3 (1.7), n=37	-15.6 (1.9), n=16	NA	.0061
rs11214601 (C/T)	Downstream	-10.0 (1.6), n=43	-16.0 (2.1), n=13	NA	.0030
COMT					
rs174696 (T/C)	Intron-6	-9.8 (1.8), n=31	-13.2 (2.2), n=18	-19.5 (4.3), n=3	.023
rs165774 (G/A)	Intron-6	-12.2 (1.8), n=24	-12.2 (1.9), n=25	-6.4 (5.2), n=4	.049
CRHR1					
rs242924 (C/A)	Intron-3	-17.0 (2.5), n=18	-11.3 (1.7), n=25	-7.2 (2.7), n=10	.011
rs242940 (T/C)	Intron-3	-16.1 (2.7), n=16	-12.1 (1.8), n=21	-7.7 (2.4), n=14	.037
rs171440 (C/T)	Intron-3	-16.1 (2.7), n=16	-12.1 (1.8), n=22	-8.3 (2.3), n=15	.049
CRHR2					
rs255121 (C/T)	Upstream	-13.2 (1.3), n=44	-7.3 (3.5), n=8	NA	.0058
rs255099 (A/G)	Upstream	-14.0 (2.0), n=16	-12.0 (2.1), n=24	-7.4 (3.5), n=10	.011
rs255100 (T/A)	Upstream	-13.5 (1.9), n=17	-12.0 (2.0), n=26	-7.1 (3.4), n=10	.014
rs2267716 (T/C)	Intron-2	-13.4 (1.7), n=26	-11.7 (2.2), n=17	-8.1 (4.6), n=7	.026
rs975537 (A/T)	Intron-8	-13.4 (1.5), n=34	-7.8 (2.3), n=19	NA	.031
CYP2D6					
rs9623531 (T/C)[a]	**Upstream**	**-15.2 (1.8), n=24**	**-9.1 (1.8), n=24**	**1.3 (3.9), n=4**	**<.00001**
rs2267448 (A/G)	Exon-4	-6.7 (2.2), n=17	-11.7 (2.4), n=23	-15.4 (2.5), n=12)	.012
rs16947 (G/A)[b]	**Exon-6**	**-15.2 (1.8), n=25**	**-9.7 (2.2), n=20**	**-3.2 (3.7), n=5**	**.00008**
rs1135840 (C/G)	Exon-9	-10.1 (2.0), n=20	-10.6 (2.5), n=19	-15.9 (2.5), n=13	.0158
rs5758589 (G/A)	Downstream	-6.4 (2.3), n=14	-11.2 (2.3), n=24	-15.7 (2.5), n=13	.0014
rs6002626 (G/C)	Downstream	-6.6 (2.2), n=15	-11.1 (2.3), n=24	-15.6 (2.5), n=13	.0017
DRD2					
rs1125393 (G/A)[c]	**Intron-1**	**-9.7 (1.6), n=41**	**-15.8 (2.1), n=12**	**NA**	**.0062**

rs1079596 (G/A)	Intron-1	-9.7 (1.6), n=41	-15.8 (2.1), n=12	NA	.0062
rs1125394 (A/G)	Intron-1	-9.9 (1.7), n=39	-16.3 (2.4), n=10	NA	.0075
rs4648319 (C/T)	Intron-1	-9.8 (1.6), n=40	-15.8 (2.1), n=13	NA	.0093
rs7103679 (G/A)	Intron-1	-9.8 (1.7), n=41	-15.6 (2.5), n=9	NA	.036
rs2734836 (G/A)	Intron-2	-9.5 (1.7), n=41	-15.7 (2.5), n=10	NA	.0020
rs2075654 (G/A)	Intron-2	-9.7 (1.6), n=41	-15.8 (2.1), n=12	NA	.0062
rs1076560 (G/T)	Intron-6	-9.5 (1.7), n=41	-15.2 (2.4), n=11	NA	.0021
rs1124491 (G/A)	Intron-7	-9.4 (1.8), n=38	-16.9 (2.7), n=9	NA	.00032
rs1079594 (T/G)	Intron-7	-9.5 (1.7), n=40	-15.2 (2.4), n=11	NA	.0026
rs6278 (G/T)	3Prime_UTR	-10.0 (1.6), n=43	-16.0 (2.1), n=13	NA	.0030
rs2242591 (G/A)	Downstream	-9.7 (1.6), n=41	-15.8 (2.1), n=12	NA	.0062
rs2242593 (A/G)	Downstream	10.0 (1.6), n=43	-16.0 (2.1), n=13	NA	.0030
rs2234689 (G/C)	Downstream	-14.2 (1.8), n=28	-10.3 (1.9), n=20	-7.3 (3.9), n=5	.0067
HRH1					
rs346070 (C/T)	3Prime_UTR	-14.1 (1.6), n=39	-9.4 (2.3), n=14	-5.4 (4.8), n=3	.029
HTR2A					
rs3125 (G/C)	3Prime_UTR	-10.8 (1.6), n=41	-12.8 (2.7), n=11	NA	.0079
HTR2C					
rs518147 (G/C)	5Prime_UTR	-15.1 (1.8), n=27	-9.7 (2.0), n=19	-7.9 (3.3), n=8	.010
rs569959 (T/C)	Intron-2	-14.5 (1.8), n=28	-10.0 (2.1), n=17	-8.0 (3.3), n=8	.010
rs2192372 (G/A)	Intron-3	-14.2 (1.7), n=29	-10.2 (2.2), n=16	-8.0 (3.3), n=8	.013
rs6318 (G/C)	Exon-5	-12.7 (1.4), n=45	-8.6 (7.4), n=3	-5.5 (5.1), n=4	.049
HTR6					
rs6693503 (G/A)	Upstream	-14.6 (1.9), n=24	-10.6 (1.8), n=28	-8.2 (5.0), n=4	.021
rs4912138 (G/A)	Intron-2	-14.4 (1.6), n=36	-9.7 (2.1), n=19	NA	.0031
rs9659997 (T/C)	Intron-2	-14.6 (1.9), n=24	-10.6 (1.8), n=28	-8.2 (5.0), n=4	.021
LLY12 (T/C)	NA	-15 (2.05), n=21	-12 (1.92), n=23	-6 (2.94), n=9	.019
MC3R					
rs6014649 (G/A)	Upstream	-12.3 (1.4), n=46	-11.1 (4.2), n=6	NA	.047
rs3746619 (C/A)	5Prime_UTR	-12.0 (1.4), n=47	-10.8 (4.2), n=6	NA	.041
rs3827103 (G/A)	Exon-1	-12.0 (1.4), n=46	-10.6 (4.2), n=6	NA	.042
NR3C1					
rs13186836 (T/C)	Upstream	-11.7 (1.5), n=37	-13.6 (2.7), n=13	-10.9 (4.3), n=3	.048
rs852983 (G/A)	Intron-2	-15.2 (2.4), n=13	-10.7 (1.5), n=39	NA	.0073
rs33388 (A/T)	Intron-2	-15.1 (2.5), n=13	-12.1 (2.2), n=20	-8.3 (2.4), n=13	.020

rs852979 (T/C)	Intron-3	-10.4 (1.7), n=27	-11.2 (2.4), n=15	-18.5 (3.2), n=8	.017
rs860457 (T/C)	Intron-4	-10.5 (1.7), n=28	-11.7 (2.2), n=17	-18.5 (3.2), n=8	.011
rs852977 (A/G)	Intron-4	-10.5 (1.7), n=28	-11.5 (2.3), n=16	-18.6 (3.2), n=8	.014
rs258750 (T/C)	Intron-8	-10.5 (1.7), n=28	-11.7 (2.2), n=17	-18.5 (3.2), n=8	.011
rs6191 (T/G)	3Prime_UTR	-15.0 (2.4), n=15	-13.7 (1.9), n=26	-7.5 (2.2), n=15	.020
POMC					
rs934778 (T/C)	Intron-1	-10.3 (2.1), n=19	-12.6 (1.8), n=28	-15.7 (3.2), n=6	.0014
rs1009388 (C/G)	Intron-1	-10.2 (1.8), n=27	-11.7 (2.3), n=17	-18.4 (5.1), n=2	.018
rs1042571 (C/T)	3Prime_UTR	-11.5 (1.7), n=30	-11.2 (2.2), n=19	-12.4 (5.2), n=3	.033
RGS4					
rs6678136 (G/A)	Upstream	-7.4 (2.7), n=11	-13.4 (1.8), n=28	-12.8 (2.2), n=14	.0075
rs6427711 (G/A)	Upstream	-10.5 (1.8), n=28	-15.0 (2.4), n=17	-15.2 (3.8), n=5	.032
SLC6A4					
rs1872924 (T/C)	Intron-2	-9.8 (1.8), n=33	-14.0 (2.5), n=13	-15.9 (4.5), n=3	.047
rs3794808 (G/A)	Intron-12	-11.2 (2.4), n=16	-12.6 (1.9), n=27	-6.3 (3.1), n=9	.034
SLC6A2					
rs36021 (A/T)	Intron-4	-12.8 (1.9), n=22	-11.2 (2.5), n=20	-6.3 (3.0), n=10	.035
rs42460 (A/G)	3Prime_UTR	-12.0 (1.6), n=45	-5.6 (3.7), n=6	-2.5 (7.4), n=1	.010
TNF					
rs1800610 (C/T)	Intron-2	-13.9 (1.4), n=41	-6.5 (2.8), n=11	NA	.0071
TPH2					
rs7305115 (G/A)[d]	**Exon-7 end region**	**-12.2 (2.4), n=19**	**-12.0 (1.8), n=25**	**-11.9 (2.9), n=8**	**.020**

Abbreviations: continued-FLX = continued-fluoxetine; LS mean=least-squared mean; MADRS-TS = Montgomery-Åsberg Depression Rating Scale Total Score; NA = not available; SE = standard error; SNP = single nucleotide polymorphism; UTR = untranslated region.
Bolded entries indicate adjusted p<.05.
[a] FDR p=.000123
[b] FDR p=.016
[c] FDR p=.043
[d] Li/Nyholt p=.04
Note: the alleles in parentheses following the SNP number represent A and B.

eTable 7. Comparison of change of MADRS total score from baseline by genotype for OFC versus continued-FLX for selected[a] *SLC6A2* SNPs.

		MMRM (SE) MADRS total score change from baseline and n by genotype			
SNP (A/B)[b] region	**Treatment**	AA	AB	BB	**p-value[c]**
rs3785143 (C/T) Intron-2	OFC	-14.2 (1.7), n=48	-20.9 (3.3), n=11	N/A, n=0	.071
	FLX	-11.5 (1.4), n=57	-7.0 (2.5), n=14	N/A, n=0	.059
rs36024 (G/A) Intron-4	OFC	-9.2 (2.4), n=21	-16.7 (2.0), n=31	-21.9 (2.8), n=11	.00034
	FLX	-12.2 (2.2), n=19	-9.7 (1.6), n=38	-12.5 (2.4), n=16	.582
rs36023 (G/A) Intron-4	OFC	-12.7 (2.2), n=24	-16.5 (2.2), n=27	-22.5 (3.5), n=10	.067
	FLX	-8.1 (1.8), n=29	-11.6 (1.7), n=33	-17.4 (2.9), n=11	.019
rs2397772 (C/G) Intron-5	OFC	-14.9 (2.1), n=29	-13.8 (2.2), n=25	-21.7 (3.8), n=9	.048
	FLX	-14.2 (2.0), n=27	-10.0 (1.7), n=32	-8.1 (2.5), n=14	.092
rs12920735 (A/T) Intron-5	OFC	-14.7 (2.0), n=29	-14.6 (2.2), n=24	-21.1 (3.7), n=9	.036
	FLX	-14.4 (2.0), n=26	-9.6 (1.7), n=32	-8.1 (2.5), n=14	.089
rs12924088 (A/G) Intron-9	OFC	-14.8 (1.9), n=33	-14.5 (2.4), n=22	-21.7 (3.9), n=8	.066
	FLX	-14.4 (1.8), n=30	-9.4 (1.7), n=29	-8.0 (2.4), n=14	.031
rs5569 (G/A) Exon-9	OFC	-14.8 (1.9), n=35	-14.8 (2.1), n=28	-21.7 (3.8), n=8	.087
	FLX	-14.3 (1.8), n=32	-8.8 (1.7), n=31	-8.1 (2.4), n=14	.023
rs998424 (G/A) Intron-10	OFC	-15.3 (1.9), n=33	-15.2 (2.3), n=22	-27.5 (4.2), n=7	.0099
	FLX	-14.4 (1.9), n=29	-9.3 (1.8), n=29	-9.5 (2.6), n=12	.049

[a]SNPs had additive model p-values less than 0.1 for MMRM MADRS total score change from baseline for both OFC and continued-FLX treatment arms and rs36024.

[b]Note: the alleles in parentheses following the SNP number represent A and B.

[c]Uncorrected p-value for MMRM change in MADRS total score from baseline using additive model of genotypes.

Abbreviations: FLX = fluoxetine; MADRS = Montgomery-Åsberg Depression Rating Scale; MMRM = mixed model repeated measure; n = number of patients; OFC = olanzapine/fluoxetine combination; SE = standard error; SNP = single nucleotide polymorphism.