Association Study of Genotype by Depressive Response during Acute Tryptophan Depletion in Subjects Recovered from Major Depression

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Key Words

Neurotransmitter depletion · Depression endophenotype · Serotonin candidate genes · *SLC6A4* · 5-HTTLPR · *STin2* · *HTR1A* · *HTR2A* · TPH2

Abstract

Purpose: To study the brief and reversible mood response to acute tryptophan depletion (ATD) as a trait marker in subjects considered at risk for major depressive disorder (MDD).

Procedures: ATD was administered to 64 subjects (54 European-Americans and 10 from other races) with a personal and family history of MDD. They were in remission and had been medication-free for at least 3 months. Subjects were randomly assignment to an active or sham condition in a double-blind crossover design. They were genotyped for serotonin-related candidate genes, and mood response was quantified with the Hamilton Depression Rating Scale (HDRS). Data were analyzed using Poisson regression with repeated measures and latent trajectory models. **Results:** Compared to the sham controls, active ATD caused modest depressive changes showing significant main effects of test condition ($\chi^2 = 5.14$, d.f. = 1, $p = 0.023$) and time ($\chi^2 = 12.22$,

d.f. = 3, $p = 0.007$), but no significant interaction of time and test condition. Latent trajectory analysis revealed two groups, identified as depletion responders and non-responders. Those with the *HTR2A* rs6313 CC genotype had significantly higher HDRS scores during ATD ($\chi^2 = 11.72$, d.f. = 1, $p = 0.0006$). **Conclusions:** ATD may help identifying the biological subtypes of MDD. These data are consistent with imaging reports implicating 5-HT_{2A} receptor function in ATD phenotypes.

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Introduction

The acute tryptophan depletion (ATD) test has been widely used to test hypotheses about the function of the brain serotonin (5-HT) system. The ATD test is based on the fact that tryptophan (Trp) is an essential amino acid,

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i.e. a necessary component of most proteins, that mammals cannot synthesize [1]. It is the foundational precursor for 5-HT synthesis, a pathway whose rate-limiting enzyme is usually not saturated, setting the stage for a rapid reduction in 5-HT synthesis as Trp availability diminishes [2]. When mammals ingest a bolus of amino acids, hepatic protein synthesis ensues; however, if the bolus is missing any essential amino acid, plasma stores of the missing amino acid are tapped, causing acute depletion. Ingestion of a 50–100 g solution of 15 amino acids without Trp induces a 4–5-hour-long, 60–80% depletion of plasma Trp in humans [3–6]. When such a solution is used in the ATD test, it is most often administered using a double-blind, sham-controlled, randomized crossover design, with each subject ingesting a Trp-free amino acid solution (true test ‘active’) and an identical but Trp-supplemented solution (‘sham’) on distinct test days separated by an interval of several days.

Human studies have largely confirmed that ATD acutely causes small to modest reductions in 5-HT neurotransmission. For example, positron emission tomography (PET) with a putative marker for 5-HT synthesis rate showed an 80% decrease after ATD [7], and three separate PET studies using radioligands for 5-HT₂ receptors showed that ATD causes a reduction in 5-HT₂ receptor binding in specific brain regions [8–10]. Measurement of the 5-HT metabolite 5-hydroxyindoleacetic acid (5-HIAA) in the cerebrospinal fluid of healthy subjects tested with ATD showed a 25% decrease when measured with either continuous cerebrospinal fluid sampling [11] or a single lumbar puncture 9 h after the Trp-free drink [12]. Although it has been suggested that non-5-HT-related processes may contribute to the human behavioral responses to ATD [13], it is generally accepted that the effects of ATD on 5-HT more fully explain the behavioral and biochemical effects [14].

The effects of ATD on behavior and cognition in healthy subjects have generally been consistent with the known functions of 5-HT. For example, ATD can induce depressive mood in some healthy subjects [15], modify pain sensitivity [16], impair learning [17], reduce punishment-induced inhibition [18], decrease long-term memory [19–21] and hinder executive function [22, 23]. However, the effects on mood and cognition have been small and have not always been replicated [24–26].

In clinical populations, the effects of ATD are more pronounced, the most dramatic effect being the ability of ATD to cause a brief return of depressive symptoms in patients with major depressive disorder (MDD) who had improved with and were continuing to take 5-HT selec-

tive serotonin reuptake inhibitor (SSRI) antidepressants during the ATD test. Similar responses are seen in MDD patients with a seasonal pattern who have responded to light therapy [27, 28]. In contrast, the lack of ATD mood response in healthy subjects dosed for 4 weeks with fluvoxamine as well as never-depressed SSRI-treated patients with obsessive-compulsive disorder [29, 30], panic disorder [31] or generalized anxiety disorder [32] suggests that the mood response to ATD is not simply due to an adverse interaction with the antidepressant or a non-specific effect. ATD also showed no significant behavioral effects in symptomatic, medication-free patients with MDD [5, 33] or obsessive-compulsive disorder [34].

Given the extensive body of data supporting involvement of the 5-HT system in the pathophysiology of MDD and the depressive responses to ATD in some healthy subjects and MDD patients, several groups have sought to determine whether a particularly intense mood response to ATD could be the phenotype of a set of individuals predisposed to future MDD. In this context, ATD induced mild to moderate depressive symptoms [35–37] or cognitive changes [37, 38] to a much greater extent in subjects with a multigenerational family history of affective disorder but no personal history of MDD in comparison to subjects with no personal or family history of affective disorder. Most [6, 39], but not all [40] studies have shown that compared to sham control, ATD causes mild depressive symptoms in untreated euthymic subjects with a past history of MDD compared with personal and family history-negative controls, although depressive symptoms are seen more often in healthy women compared to healthy men, irrespective of whether there is a history of MDD [41–43].

Given that MDD is a phenotypically heterogeneous, and likely biologically heterogeneous disorder, some of the variability in mood response to ATD may be due to genomic differences between subjects. For example, MDD patients homozygous for the long ‘l’ vs. short ‘s’ allele of the 5-HT transporter protein gene (*SLC6A4*) 5-HTTLPR polymorphism [44] were found to be more likely to have a depressive response to ATD [45–47], but the results have not always been replicated [48], and one study found that the ATD response in healthy subjects was greater in those who were homozygous for the ‘s’ allele of *SLC6A4* [49]. These data suggest that the presence of genetic factors that modify 5-HT neurotransmission may contribute to a greater likelihood of having a mood response to ATD, a finding supported by a retrospective study showing that subjects with an increased number of proposed risk genotypes for MDD have a greater risk of

Table 1. Schedule for ATD and sham condition test sessions

Day 1	8:00 a.m.	arrive at the research clinic (fasting after midnight) clinician and self-ratings, plasma Trp (10 cm ³), vital signs
	8:30 a.m.	administration of amino acid drink
	1:30 p.m.	clinician and self-ratings, plasma Trp (10 cm ³), vital signs
	3:30 p.m.	clinician and self-ratings, plasma Trp (10 cm ³), vital signs
	4:00 p.m.	return to unrestricted food intake
Day 2	8:00 a.m.	clinician and self-ratings, plasma Trp (10 cm ³)
Day 3	8:00 a.m.	clinician and self-ratings

having the disorder [50]. Other studies in healthy subjects [8] and SSRI-treated MDD patients [9] have shown that mood response to ATD was associated with a failure to show a decrease in PET measures of 5-HT₂ receptor binding during the procedure.

The present study was designed to test the hypothesis that the depressive response to ATD is associated with certain proposed risk genotypes in subsets of people with MDD, possibly identifying a subset of MDD patients with more homogeneous underlying causal mechanisms. Since ATD has minimal acute effects in symptomatic patients with MDD and the effects of ATD on MDD patients taking antidepressants are highly associated with the pharmacology of the antidepressant [51], the present study focused on medication-free non-depressed subjects who reported both a past personal and family history of MDD.

Subjects and Methods

Subjects

After review and approval of the protocol for this study by the Investigational Review Boards of the University of Arizona, Case Western Reserve University and the University of Texas Health Sciences Center at San Antonio, 64 psychotropic medication-free subjects aged 18–80 years (mean age 53 years, 66% women, 84% European-American [EA]) were recruited by advertisement and provided written informed consent to participate. Interested persons underwent initial screening with a brief, focused review of the inclusion and exclusion criteria as well as medical and psychiatric history. Prospective subjects then met with an investigator for informed consent and/or further assessment and received complete physical and neurological exams as well as pertinent laboratory studies (complete blood count, liver and thyroid function tests, urinalysis, urine drug screen and urine pregnancy test for females). Additional laboratory studies were obtained when indicated.

Subjects were selected for inclusion if, after a structured interview with the Diagnostic Interview for Genetic Studies (DIGS) and

the Family Interview for Genetic Studies (FIGS) [52], they met Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) [53] criteria for at least one prior but not for a current major depressive episode, had a family history of MDD in at least one first-degree relative, and had been free of psychotropic medication for at least 3 months but less than 2 years at the time of enrollment. Subjects had to have a total Hamilton Depression Rating Scale (HDRS) score <10 on the 25-item version [54].

Subjects were excluded for (1) lifetime history of any DSM-IV axis I disorder except MDD, (2) borderline or antisocial personality disorder, (3) history or manifest signs of serious medical or neurological illness on examination or as a result of laboratory studies that might induce depressive symptoms or increase the risk of adverse events during testing, (4) current or prior psychosis, (5) need for any psychotropic medication, including the use of medication for hypnotic purposes (including antihistamines) or use of benzodiazepines for the treatment of anxiety, (6) ongoing suicidal ideation or assessed by one of the investigators to pose a risk of suicide based on clinical presentation and past history, or (7) not using an acceptable method of contraception if they were female and capable of childbearing. Neither sex nor ethnicity/race were exclusion criteria.

About 70% of the subjects were recruited and tested at the Arizona Health Sciences Center, 10% at Case Western Reserve University (Cleveland, Ohio, USA) and 20% at the University of Texas Health Sciences Center San Antonio.

Candidate Gene Selection

The following candidate genes were selected prospectively based on their mechanistic involvement in the brain synthesis (Trp hydroxylase-2), reuptake (5-HT transporter) and principal receptor activity of 5-HT (5-HT_{1A} and 5-HT_{2A}) as well as their suggestive evidence of risk for mood disorders based on evidence available at the time of study.

ATD Procedures

ATD tests were performed in a double blind, sham-controlled fashion and took place between 2003 and 2009. All subjects received two ATD procedures 1 week apart. Each ATD session involved three consecutive days: an amino acid drink day and two follow-up days (see table 1 for schedule of testing). One of the tests involved consumption of a 102.5-g Trp-free amino acid drink (active ATD), and the sham condition involved an identical mixture

Table 2. Amino acid content (100% strength)

L-Alanine	5.5 g
Glycine	3.2 g
L-Histidine	3.2 g
L-Isoleucine	8.0 g
L-Leucine	13.5 g
L-Lysine hydrochloride	11.0 g
L-Phenylalanine	5.7 g
L-Proline	12.2 g
L-Serine	6.9 g
L-Threonine	6.9 g
L-Tyrosine	6.9 g
L-Valine	8.9 g
L-Methionine	3.0 g
L-Arginine	4.9 g
L-Cysteine	2.7 g
<i>With or without</i>	
L-Trp	2.3 g
75% strength used in women.	

of amino acids but containing 2.3 g L-Trp (see table 2 for amino acid drink composition). Subjects were randomly assigned to receive either active ATD or sham condition on the first week and then crossed over to receive the alternative condition the week later. The order of conditions was unknown to the subjects, the supervising physicians and the study raters. Because of sex differences in tolerability of this amino acid drink, women received a 75% strength drink. Given that Trp depletion had been reported to exacerbate premenstrual dysphoric disorder symptomatology [55], the dates of depletion were assigned to coincide with the follicular phase of menstruating women to minimize confounding behavioral effects. The phase of the menstrual cycle was calculated with support from a daily temperature log.

For each test session, subjects arrived at the test site after an overnight fast on day 1 of the study. Depression ratings and blood sampling for Trp levels and vital signs were performed, followed by ingestion of the amino acid drink. Subjects remained in the testing room for the next 7 h but were free to walk about the room, use the toilet and drink water. All subjects remained fasted throughout the day until the test was completed (approximately 4:30 p.m.), when they returned to regular unrestricted food intake. At that time, they were provided a Trp-rich meal for immediate ingestion and an additional Trp-rich meal to take home for dinner. Research subjects were continuously monitored during the day of the amino acid drink by an experienced nurse clinician and one of the investigators was available at all times.

On the day of testing, the amino acid drink was prepared by mixing the blinded amino acid powder with enough water for a final volume of 350 ml and flavored to taste with Hershey's chocolate syrup. The three least palatable amino acids, cysteine, methionine and arginine, were encapsulated and given separately from the drink.

Compensation of USD 100 was offered for participation in each of the blinded procedures (ATD and sham condition.)

Depression Measurements

Depression ratings were obtained at specified intervals during each testing period (see table 1 for schedule of testing). Clinician-rated instruments included the HDRS [54, 56, 57]. The HDRS was used in this study because it provides the best comparison to earlier investigations utilizing neurotransmitter depletion paradigms in clinical populations. Experienced clinicians with extensive experience using the scale performed all ratings. The raters were blinded to the sequence of testing conditions.

Biochemical Measurements

Plasma for free Trp was obtained immediately prior to ingestion of the test drink and 5 h later. Free plasma Trp is assayed by obtaining an ultrafiltrate through a commercially available membrane system and subjecting the ultrafiltrate to high-performance liquid chromatography with fluorometric detection [58].

Genetic Analysis

DNA was extracted by standard techniques from whole blood. Blood from subjects at all sites was frozen and shipped to the Arizona Health Sciences Center for genotyping. Assuming selectivity of ATD for serotonin neurotransmission, the following candidate genes were selected a priori based on their role in serotonin synthesis, transport, and recognition: *SLC6A4* gene including the promoter region polymorphism (5-HTTLPR) [44], its triallelic *L_{A-G}* polymorphism (rs25531) [59], and the intron-2 variable number tandem repeat of the 5-HT transporter (STin2) [60, 61], the serotonin receptor-1A (rs6295) (*HTR1A*) [62], the serotonin receptor-2A (rs6313) (*HTR2A*) [63] and the Trp hydroxylase-2 gene (rs1386494) (*TPH-2*) [64].

Adverse Events

No significant adverse events were observed in any of the subjects during testing. One patient died the week after sham testing for reasons unrelated to this study.

Statistical Analysis

The effect of ATD on Trp levels was analyzed using repeated measures ANOVA with measures at baseline and 5 h. The effect of ATD on mood was assessed using a generalized linear regression model with generalized estimating equations (SAS GENMOD) specifying an overdispersed Poisson distribution (non-normal distribution of the error). Fixed design effects were test condition (depletion vs. sham), time (5, 7, 24 and 48 h after ingestion) and the condition by time interaction. Baseline measures were used as covariates in each test condition. Supplemental analyses explored the effects of participants' sex on HDRS by adding main and interaction effects to the model. Two subjects who completed only the active depletion session were included in these analyses. Genetic data were analyzed by adding genotype as an additional classification factor to these models. Preliminary models were run including order of testing (depletion vs. sham first) as an additional fixed design effect. Neither main nor interaction effects of note were observed, so it was dropped from the final models. A simple confirmatory analysis used a paired-samples *t* test to test the difference between the mean of each post-baseline measure in depletion vs. sham. We also conducted a latent trajectory analysis on the data from the active depletion condition using SAS PROC TRAJ [65]. Rather than characterizing average trends as a function of depletion condition, latent trajectory analysis attempts to identify sub-

groups that have homogeneous trends over time. We fit multiple models specifying increasing numbers of latent groups, and selected a 'best' model based on Schwarz's Bayesian information criterion [66]. The Akaike information criterion and the Bayesian information criterion are well-known statistics used to quantify goodness of fit in regression models that use likelihood-based estimation, and are very widely used to evaluate and choose among models of differing complexity. Given the inclusion of a mixed racial/ethnic subject sample, the genetic analyses were also done excluding non-EA subjects (10/64) to control for possible population stratification.

Results

Trp Levels

The Trp-free test caused an 80.81% decrease from baseline in free plasma levels of Trp (from 8.17 ± 4.01 to $1.57 \pm 1.8 \mu\text{M/l}$), and the Trp-supplemented sham test caused free Trp to increase from baseline by 81.77% (from 8.41 ± 4.7 to $15.29 \pm 7.67 \mu\text{M/l}$). ANOVA with repeated measures showed a highly significant test by time interaction ($F = 110.46$, d.f. = 1,120, $p < 0.001$).

Depressive Response

Generalized estimating equation analysis of HDRS showed a significant main effect of test condition ($\chi^2 = 5.14$, d.f. = 1, $p = 0.023$). The result is depicted in figure 1, which displays both the actual means and the model estimates (the slight differences between line and triangle alignment reflect effects of missing data). Mean HDRS was almost identical at baseline in both conditions and higher across time in the ATD than in the sham condition. The main effect of time was also significant ($\chi^2 = 12.22$, d.f. = 3, $p = 0.007$), but the interaction of condition and time was not ($\chi^2 = 0.89$, d.f. = 3, $p = 0.829$). Supplemental analyses exploring differences between men ($n = 22$) and women ($n = 42$) revealed no significant effects (sex main effect: $\chi^2 = 0.95$, d.f. = 1, $p = 0.33$; sex by task: $\chi^2 = 0.07$, d.f. = 1, $p = 0.79$; sex by time: $\chi^2 = 0.71$, d.f. = 3, $p = 0.87$; sex by task by time: $\chi^2 = 2.99$, d.f. = 3, $p = 0.39$).

Latent Trajectory Modeling of Mood

The final latent trajectory model had two latent classes. The results from PROC TRAJ are presented in table 3. Group 1 was defined by an intercept-only trajectory, with no significant trend component ($p = 0.22$), meaning no change over time. Group 2 began with a higher baseline score (intercept) than group 1 and was defined by a significant ($p = 0.02$) inverted quadratic function, i.e. scores that went up and then down, with a peak at roughly 24 h

Fig. 1. Depressive response during ATD and sham condition ($n = 64$). The figure displays both the actual mean HDRS scores during ATD (squares) and sham condition (triangles) and the model estimates utilizing the generalized linear regression model with generalized estimating equations during ATD (solid line) and sham condition (dotted line).

Table 3. Latent trajectory analysis of HDRS over time

Group	Parameter	Estimate	SE	T for H0	p value
1	intercept	0.293	0.153	1.914	0.056
	linear	-0.007	0.006	-1.229	0.220
2	intercept	1.579	0.065	24.391	<0.000
	linear	0.020	0.008	2.444	0.015
	quadratic	<-0.000	<0.000	-2.345	0.020
	alpha	-2.170	0.280	-7.741	<0.000
<i>Group membership</i>					
1	%	45.692	6.630	6.892	<0.000
2	%	54.308	6.630	8.192	<0.000

Group 1 represents individuals considered non-responders; group 2 represents individuals considered responders.

Likelihood criteria: Bayesian information criterion = -726.47 ($n = 314$); Bayesian information criterion = -720.91 ($n = 64$); Akaike information criterion = -713.35; likelihood criterion = -706.35.

SE = Standard error; T for H0 = test for null hypothesis (parameter = 0).

Fig. 2. Depressive response during ATD in latent trajectory groups ($n = 64$). The figure displays both the actual mean HDRS scores for ATD responders (squares) and non-responders (triangles) at each time point and the model estimates for responders (solid line) and non-responders (dotted line) utilizing generalized linear regression model with generalized estimating equations.

post baseline. Group 1 comprised 46% of the cases and group 2 comprised 54%. We interpret the inverted U response in group 2 as ‘depletion responders’ and the flat trajectory of group 1 as ‘non-responders’. Mean PEAK D (the highest value of HDRS from 5–48 h post depletion in group 2 was 8.4 ± 4.0 (median = 7, range = 2–21), while in group 1 it was 1.9 ± 1.1 (median = 2, range = 0–4). Figure 2 is a graphic of this analysis with actual sample means and model-based trajectories.

Genotype and Depletion Response

Primary analyses of genotypes were done using data only from EA subjects (54/64) to decrease the stratification risk. Six SNPs were analyzed, so the Bonferroni-adjusted p value was $0.05/6 = 0.008$. Only the rs6313 SNP in *HTR2A* yielded a statistically significant association with depression response ($\chi^2 = 11.85$, d.f. = 2, $p = 0.003$). The depletion response (mean difference between depletion and sham conditions) was highly significant only for those with the CC genotype (32.1%, $z = 4.2$, $p < 0.0001$), and non-significant for those with the TC (52.8%, $z = 1.06$, $p = 0.29$) or TT genotypes (15.1%, $z = 0.14$, $p = 0.89$). When the TC and TT groups were pooled and compared with the CC group, the genotype by task interaction yielded $\chi^2 = 11.72$, d.f. = 1, $p = 0.0006$. Furthermore, the *HTR2A*

genotype groups were associated with the latent trajectory classes as expected. Individuals with the CC genotype were more likely to be classified as ATD responders, with an odds ratio of 6.5, a positive predictive value of 82%, a negative predictive value of 58% and a kappa of 0.34 ($\chi^2 = 7.71$, d.f. = 1, $p = 0.006$). These results were weaker when the analysis was done using data from the entire racially heterogeneous sample, but *HTR2A* (CC vs. TC/TT) still had a nominally significant genotype by test condition interaction ($\chi^2 = 5.65$, d.f. = 1, $p = 0.017$) for the HDRS, and individuals with the CC genotype were more likely to be considered ATD responders, with an odds ratio of 3.9, a positive predictive value of 76%, a negative predictive value of 55% and a kappa of 0.265 ($\chi^2 = 5.43$, d.f. = 1, $p = 0.02$). This result is depicted in figure 3, which suggests that the depletion response in depressive mood occurs primarily in those with the CC genotype. For the rest of the genes, the genotype frequencies are 5-HTTLPR ll 32.8%, ls 50%, ss 17.2%; L_{A-G} triallelic sa/sa 15.5% + sa/lg 3.4% = 18.9%, sa/la 46.5% + la/lg 6.9%, and la/la 27.6%; STin2 ss 17.2%, sl 46.9%, ll 35.9%; *HTR1A* cc 14%, cg 26%, gg 22%; TPH2 intron-5 tt 1.6%, tc 22.6%, cc 75.8%. None of these SNPs achieved statistical significance for an association with depressive response during ATD.

Discussion

Active but not sham Trp depletion caused depressive mood reactions in this sample of euthymic, psychotropic medication-free subjects with both a personal and family history of MDD. As a group, our subjects showed a smaller depressive response during active depletion than we had anticipated, and our latent trajectory analysis showed that a modest mood response occurred in a subgroup of about one-half of the subjects. One variable that may help explain this is the selection of subjects who were in sustained remission from depression from 3 to 24 months. Recently remitted individuals may be more likely to experience a depressive response during active depletion. We tested women with a 75% strength drink, which may have led to less dramatic brain serotonin depletion than the 100-g drink previously utilized. Additionally, in women of reproductive age we conducted our testing only during the first phase of their menstrual cycle. It has been suspected that women undergoing ATD during the late luteal phase are more likely to experience negative mood changes [55].

Genetic analyses showed that in a mixed racial sample, subjects with the CC genotype of the rs6313 SNP in the

Fig. 3. Depressive response during ATD (squares) and sham condition (triangles) by *HTR2A* (rs6313) genotype. The figure displays differences in HDRS scores between risk genotype (CC) and non-risk genotype (TT/TC) for *HTR2A* rs6313 for both the racially heterogeneous sample (n = 64, **a**) and the European descent sample (n = 54, **b**). CC = Homozygous C allele; TT = homozygous T allele; TC = heterozygous.

HTR2A gene accounted for a proportion of the mood responses; the result was only nominally significant. When controlling for the effect of race by testing EA subjects only (84% of sample), the association of CC genotype and mood response to ATD was stronger and met corrected significant levels. The present study – while small – is still the largest prospective study to date in which an association between genotype and the depressive response to ATD was analyzed in subjects in remission from MDD.

The *HTR2A**C allele is associated with lower quantity of mRNA and protein made [67] and higher 5-HT_{2A} receptor density in the cerebral cortex [68], and it has been considered a risk allele for various psychiatric conditions including schizophrenia, alcohol dependence and mood disorders by some investigators. Recent PET study findings suggest that the status of 5-HT₂ receptor availability correlates with the depressive response during ATD in SSRI-treated MDD patients and healthy volunteers [8, 9]. The fact that subjects who showed greater decreases in 5-HT₂ binding during ATD were less likely to experience depressive responses is consistent with observations in treated animal models and patients who are in remission from MDD after treatment [69]. Antagonists of the 5-HT_{2A} receptor are effective as single or combined clinical treatments for MDD, and various other antidepressants with different receptor or transporter affinity profiles, including electroconvulsive therapy, reportedly lead to downregulation of postsynaptic 5-HT₂ receptors, a phenomenon believed to be relevant to their mechanism

of action [70]. Yatham et al. [9] hypothesized that the decrease in 5-HT_{2A} binding observed during ATD is a compensatory mechanism that prevents induction of depression. This phenomenon may be similar to the increase in brain-derived neurotrophic factor plasma levels observed during ATD in healthy volunteers, which is believed to be a compensatory mood-stabilizing temporary adaptation [71].

Although theoretically *HTR2A* 'CC', which results in less mRNA and protein, may explain mechanistically a greater decrease in 5-HT_{2A} receptor availability during ATD, the dynamic adaptability of 5-HT_{2A} appears to explain more broadly changes in expression, and response to both agonists and antagonists of 5-HT_{2A} leads to downregulation of 5-HT_{2A} receptor [69]. Should future efforts utilizing ATD or other methods be able to identify specific depression subgroups, the potential for advanced understanding of the mechanism of illness may facilitate the identification of specific risk factors and the development of tailored therapeutic interventions.

This study did not confirm prior reports [45, 46] of increased depressive response to ATD in subjects homozygous to the 'T' allele of *SLC6A4*. According to findings from EA female-female twin pairs over the course of illness, the onset of depressive episodes may become increasingly autonomous, suggesting a sensitization or 'kindling' to the depressive state [72]. If this is the case, subjects without vulnerability genes may be equally likely to express depressive episodes after experiencing recur-

rent lifetime episodes. Note that we included subjects who had experienced several depressive episodes.

Population stratification is a well-known source of artifact in genetic association studies. In the present study, when we considered only self-identified EAs, the result was statistically significant when taking into account the testing of six SNPs. When we considered the entire heterogeneous sample, the results were only nominally significant. It is impossible to draw specific conclusions because of the limited sample size, but the results are consistent with this variant having an effect on Trp depletion response only in EA subjects. Larger groups of non-EA subjects would need to be evaluated to draw a more definitive conclusion.

In summary, this study replicated consistent reports of robust and safe depletion of plasma Trp and the induc-

tion of depressive symptoms in a subgroup of patients recovered from depression. Among the group experiencing depressive changes, the *HTR2A* CC genotype predicted greater symptom induction.

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Disclosure Statement

All authors declare the absence of any competing financial interests in relation to the work described.

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