

Association of Serotonin 5-HT_{2A} Receptor Binding and the T102C Polymorphism in Depressed and Healthy Caucasian Subjects

Vadim D Khait^{1,2}, Yung-yu Huang^{1,2}, Gil Zalsman^{1,2}, Maria A Oquendo^{1,2}, David A Brent³, Jill M Harkavy-Friedman^{1,2} and J John Mann^{*,1,2}

¹Department of Neuroscience, New York State Psychiatric Institute, New York, NY, USA; ²Department of Psychiatry, Columbia University, New York, NY, USA; ³Division of Child and Adolescent Psychiatry, Western Psychiatric Institute and Clinic, Pittsburgh, PA, USA

Serotonin 5-HT_{2A} receptor (5-HT_{2A}) binding is reported to be altered in individuals with suicidal behavior, mood disorders, and aggressive-impulsive traits. Genetic association with major depression, suicidal behavior, and aggressive-impulsive traits has not been established. This study examines the possible association of the 5-HT_{2A} gene C102T polymorphism with the receptor binding kinetics, and clinical overt phenotypes. The study population included 63 healthy volunteers and 152 subjects with mood disorders, 56 of whom had a history of suicide attempts. All were Caucasian. Platelet 5-HT_{2A} binding kinetics (B_{max} and K_D) were assayed and adjusted for seasonal variation. All subjects were genotyped for the T102C polymorphism. Clinical phenotype was determined by structured clinical interview. The TT genotype was associated with higher B_{max} in all subjects ($F = 3.53$, $df = 2,211$; $p = 0.03$), controlling for diagnosis. Bonferroni-adjusted *post hoc* testing showed higher binding in the TT compared with TC genotype in the control group ($F = 7.56$, $df = 2,60$, $p = 0.001$), but not in the mood-disordered subjects. No difference was found in genotype and allele distribution between the mood-disordered subjects, with and without suicide attempt history, and controls. B_{max} was not related to a diagnosis of mood disorders. The TT genotype appears associated with higher platelet 5-HT_{2A} B_{max} in the healthy population, but this genotypic effect appears absent in mood disorders and unrelated to psychopathology.

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INTRODUCTION

The human serotonin 5-HT_{2A} receptor (5-HT_{2A}) binding kinetics are altered in major depression, suicidal and aggressive behaviors, and schizophrenia. Greater 5-HT_{2A} binding has been reported in post-mortem brain tissue from individuals with major depression and suicide victims by some but not all studies (Stanley and Mann 1983; Mann *et al*, 1986; Hrdina and Du 2001; Kato *et al*, 1996; Ono *et al*, 2001; Hrdina *et al*, 1993; Arora and Meltzer 1989; Yates *et al*, 1990). Pandey *et al* (1995) found that higher prefrontal cortical 5-HT_{2A} binding was associated with greater gene expression in youth suicide.

The platelet is a readily accessible source of 5-HT_{2A} receptors with characteristics similar to the brain receptor (Cook Jr *et al*, 1994; Elliott and Kent, 1989). Greater platelet 5-HT_{2A} binding has been reported in association with major depression and a history of suicidal acts (Hrdina *et al*, 1995; Hrdina *et al*, 1997; Pandey *et al*, 1995; Sheline *et al*, 1995). Our own study found higher platelet 5-HT_{2A} binding to be related to suicidal behavior and aggression history, but not to a diagnosis of mood disorder (McBride *et al*, 1994).

The 5-HT_{2A} 20-kb gene consists of three exons and two introns (Chen *et al*, 1992) and is located on chromosome 13q14–q21 (Sparkes *et al*, 1991). A significant association between T102C and schizophrenia is reported (Williams *et al*, 1996; Williams *et al*, 1997). The C allele was associated with diagnosis of schizophrenia with an odds ratio of 1.3 ($p = 0.008$). These findings are confirmed by a meta-analysis of previous studies (Williams *et al*, 1997). A more recent meta-analysis demonstrated a stronger association with schizophrenia in Caucasians compared with other ethnic groups (Abdolmaleky *et al*, 2004). No association of

*Correspondence: Dr Jj Mann, Department of Neuroscience, New York State Psychiatric Institute, 1051 Riverside Drive, Box 42, New York, NY 10032, USA, Tel: +1 212 543 5571, Fax: +1 212 543 6017, E-mail: jjm@columbia.edu

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genotype or allele frequencies is found with suicide or mood disorders (Arranz *et al*, 1997; Bondy *et al*, 2000; Crawford *et al*, 2000; Mahieu *et al*, 1997; Minov *et al*, 2001; Tsai *et al*, 1999; Ono *et al*, 2001; Correa *et al*, 2002; Geijer *et al*, 2000). Turecki *et al* (2003) found that higher 5-HT_{2A} binding in the brain is associated with the T102C polymorphism in the 5-HT_{2A} gene independently of suicide, although others disagree (Hrdina and Du, 2001). The T and C alleles may show differential expression (Poleskaya and Sokolov 2002; Bray *et al*, 2004).

To clarify the relationship of the T and C alleles to 5-HT_{2A} receptor number and to mood disorders, we investigated the association of the T102C polymorphism with platelet 5-HT_{2A} receptor binding indices and with clinical phenotype in subjects with mood disorders, with and without a history of suicide attempts, and a control group of healthy volunteers.

PATIENTS AND METHODS

Subjects

Patients ($N=152$) were recruited from the subjects presenting to the research clinics of a university-affiliated psychiatric hospital for evaluation and treatment of a mood disorders. All subjects met DSM-IV criteria for a current major depressive episode ($N=90$, 59.2% of the patients), or bipolar disorder with depressed mood ($N=25$, 16.4%), or dysthymia ($N=2$, 1.3%), or remitted major depression ($N=35$, 23.0%). Healthy controls ($N=63$) were recruited through advertisements. Only Caucasian subjects of European origin were included to reduce ethnic variation and risk of genetic stratification (Gelernter *et al*, 1993). Subjects were free from drugs known to affect the serotonin system for at least 14 days (with a median of 30 days). The drug-free interval was longer for drugs with a longer half-life (6 weeks for fluoxetine and 4 weeks for an oral antipsychotic). The duration of the drug-free period of the patients was established by a combination of drug screen, observation in the hospital, and a history obtained from the subject's family and the referring physician. Written informed consent was obtained as approved by the Institutional Review Board. Demographic and clinical data from the two groups are presented in Table 1.

Clinical Phenotype Assessment

DSM-IV (APA 1994) Axis I and II diagnoses were made using the Structured Clinical Interview (SCID-I and -II) (Spitzer *et al*, 1989). Lifetime aggression was rated using the Brown–Goodwin Aggression Inventory (BG) (Brown and Goodwin 1986), lifetime hostility, using the Buss–Durkee Hostility Inventory (BDHI) (Buss and Durkee 1957), and impulsivity, using the Barratt Impulsiveness Scale (BIS) (Barratt 1994). Depression was evaluated by the Beck Depression Inventory (BDI) (Beck *et al*, 1961) and the 17-item Hamilton Depression Rating Scale (HAM-17) (Hamilton 1960). Hopelessness was measured by the Beck Hopelessness Scale (BHS) (Beck *et al*, 1985). For healthy volunteers, any Axis I diagnosis by the SCID-NP (non-patient version) and any history of first-degree relative with a mood or psychotic disorder were ruled out. Subjects and healthy volunteers had a physical examination and routine laboratory screening tests to detect neurological disease and any active physical disease that could affect their mental status or serotonin function.

DNA Isolation and Extraction

Venous blood samples were collected in EDTA tubes. The samples were first centrifuged (150g) for 15 min at room temperature to obtain platelet-rich plasma (PRP). After removal of PRP, the remaining blood fraction was centrifuged at 750g for 15 min to obtain the buffy coat, which was further purified by layering on top of a 4-ml Ficoll[®]/hpaque (Pharmacia) gradient. The tubes were centrifuged at 750g for 15 min in a Sorvall GLC-2B centrifuge. The white cell interface layer was transferred into new plastic tubes and 3 ml of PBS buffer added. Then, the fraction was further centrifuged at 11 000g in a Sorvall RC-5B centrifuge for 5 min at 4°C. A measure of 3 ml of PBS buffer were used to wash the white cell pellet, which was then centrifuged at 11 000g for 5 min. The supernatant was discarded, and the pellet stored at –20°C pending DNA extraction.

DNA extraction from lymphocytes pellets was performed as described by Higuchi (1992). Thawed pellets were resuspended in 3 ml PBS buffer. The suspension was centrifuged at 11 000g for 5 min at 4°C, and the supernatant discarded. Pellets were resuspended in a 500 µl of polymerase chain reaction (PCR) buffer (50 mM KCl, 10 mM

Table 1 Demographic and Clinical Description of Mood-Disordered Patients and Healthy Controls ($N=215$)

Group	Age±SD	Sex (M/F)	HDRS	BDI	BIS	BG
<i>Mood disorders</i>						
Nonattempters, $N=100$	40.92±15.5	40/58	11.4±8.80	17.7±12.3	49.38±17.98	16.8±5.02
Attempters, $N=52$	37.42±12.75	12/42	16.16±7.31	28.6±11.3	57.48±20.08	18.5±5.8
All, $N=152$	39±14.68	52/100	13.12±8.58	21.6±13.08	52.31±19.10	17.4±5.36
Controls, $N=63$	40±17 ^a	30/33 ^a	1.77±3.2 ^b	2.89±3.17 ^b	34.64±3.47 ^b	14.5±3.78 ^b

^aDifference between the two groups is not significant.

^bDifference between the two groups is significant $p<0.001$. HDRS = Hamilton Depression Scale; BDI = Beck Depression Inventory; BIS = Barratt Impulsivity Scale; BG = Brown Goodwin Life History of Aggression Scale.

Tris-HCl (pH 8.3), 2.5 mM MgCl₂, 0.1 mg/ml gelatin, 0.45% NP40, 0.45% Tween 20) containing 12 µg Proteinase K. After incubation at 55–60°C for 1 h, the proteinase K was inactivated by heating at 95°C for 10 min. The samples were diluted with 10 mM Tris-EDTA buffer at 1:5 dilution and pipetted (2 µl) for PCR. Genomic DNA fractions were stored at –20°C.

Polymerase Chain Reaction

Genotyping of the 102T/C polymorphism of the 5-HT_{2A} receptor gene (a synonymous allele, dbSNP:6313) was carried out by PCR and *MspI* restriction enzyme digestion, as described previously (Higuchi 1992) with modifications. Briefly, the oligonucleotide primers sense Mann7 (5'-CAAGGTGAATGGTGAGCAGAAA-3') and antisense Mann8 (5'-TGGCAAGTGACATCAGGAAATAGT-3') were used to amplify a PCR fragment of 425 bp length. PCR was carried out in a 25 µl volume, containing 100 ng DNA, 1 pmol of each primer, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 2 mM MgCl₂, 0.01% gelatin, 200 µM of each dNTP, and 1 U of Red Tag DNA Polymerase (Sigma, St Louis, MO). Samples were processed in a GeneAmp PCR System 2400 (Perkin-Elmer). In all, 30 temperature cycles were carried out, consisting of 30 s at 94°C, 30 s at 55°C, and 40 s at 72°C, followed by a final extension step at 72°C for 4 min. After the amplification, each aliquot of 10 µl PCR product was digested with *MspI* restriction enzyme (Gibco BRL, Rockville, MD) in a volume of 15 µl at 37°C overnight. The digested PCR products were separated on a 1.2% agarose gel. The 102C allele resulted in DNA fragments of 176 and 249 bp, whereas the 102T allele PCR fragments remained uncut.

5-HT_{2A} Receptor Binding Assay

The assay was performed as described previously (Khait *et al*, 1999). Briefly, platelet membranes were prepared as follows. PRP obtained as described above was centrifuged at 16 000g for 10 min at 4°C. The resultant pellet was resuspended in 5 ml of normal saline and spun again. We have shown (Khait *et al*, 1999) that a prolonged storage of platelet pellets affects binding. Therefore, in the present study storage time was limited to less than a month. At the time of the binding assay, the pellet was lysed by suspension in hypotonic solution (Tris-EDTA buffer: 5 mM Tris-HCl, 0.1% EDTA, pH 7.5), and the suspension was homogenized using a Brinkmann homogenizer (setting 6) for 10 s. The mixture was centrifuged twice at 16 000g for 10 min at 4°C, once with hypotonic buffer, and finally with the incubation buffer (Tris-HCl 50 mM (pH 7.4) containing 120 mM NaCl, 5 mM KCl, and 2 mM MgCl). The pellet was then resuspended in the incubation buffer to form the final membrane suspension for binding studies. LSD binding assay was performed as described by Geaney *et al* (1984). Four-tenths (0.4) ml of the membrane suspension (containing 20–150 µg protein) was incubated with [³H]LSD (NEN Life Science Products, Boston, MA) in a final volume of 0.5 ml for 4 h at 37°C. After incubation, the reaction was terminated by the addition of 4 ml ice-cold Tris buffer (50 mM of Tris-HCl, pH 7.7) and rapidly filtered through Whatman GF/B filters using a Brandel 24-channel cell harvester. The filters were prewashed with buffer before

filtration and washed three times with 4 ml of buffer after filtration. All filters had been soaked in 2.5% polyethylenimine (Fluka Chemie) and dried prior to filtration, before being counted in a Packard Liquid Scintillation Analyzer. Filters were placed in scintillation vials with scintillation cocktail for 48 h. Saturation binding isotherms were performed using a range of ligand concentrations (0.1–2.0 nM). The specific binding of [³H]LSD was defined as the difference between total and nonspecific binding determined by the addition of 300 nM spiperone. Binding indices, B_{max} and K_D , were calculated by the EDBA/LIGAND program (Biosoft, UK). Total bound did not exceed 5% of the total radioactivity. Protein content was determined by Folin-reagent procedure.

Analyses Controlling for Seasonal Effects and Relationship of Binding to Genotype

Seasonal variation of platelet 5-HTR_{2A} density or B_{max} is an additional source of variance that has been reported in healthy volunteers (Spigset and Mjorndal, 1997; Spigset *et al*, 1998), and by us in subjects with a major depressive episode (Khait *et al*, 1999; Khait *et al*, 2002). In order to control for this variance, an adjusted B_{max} was calculated for each subject by the following procedure. For each subject group, a monthly average $\bar{B}_{max}(m)$, where $m = 1, 2, \dots, 12$ months was calculated and a monthly adjustment coefficient $A(m) = \bar{B}_{max}(m)/B_0$, where B_0 is the group average, was assigned to the month m . Seasonally adjusted B_{max} for each subject was calculated by dividing raw B_{max} by the coefficient $A(m)$ corresponding to the month m of the sample blood drawing. With this correction, the monthly averages for seasonally adjusted B_{max} ($\bar{B}_{max}(m)$) are all the same and equal to the whole group average.

RESULTS

Descriptive

Demographic and clinical data from mood-disordered subjects and normal controls are presented in Table 1. There were no significant differences in age distribution between the mood-disordered group and controls ($t = 0.32$, $df = 213$, $p = 0.748$). There was a trend for more females in mood-disordered subjects compared with controls ($\chi^2 = 3.39$, $df = 1$, $p = 0.065$). Therefore, we controlled for sex in analyses where appropriate. As a group, the patients were moderately depressed and, as expected, scored significantly higher in all rating scales compared with controls ($p < 0.0001$; see Table 1). The observed genotype distributions for patients and controls were not significantly different from Hardy-Weinberg equilibrium (data not shown).

Relationship Between Clinical Parameters and 102T/C Genotype

We found no significant difference in either genotype distribution or allele frequencies between the mood-disordered group and the control group (Table 2). Within the mood-disorder group, suicide attempters had significantly more heterozygotes compared with nonattempters

Table 2 102T/C Genotype and Allele Frequencies and 5-HT_{2A} Receptor Indices in Mood-Disordered Patients and Healthy Controls

Group	102T/C genotype and allele frequencies					χ^2 (for alleles)	<i>p</i>	<i>B</i> _{max} corrected (fmol/mg) mean (SD) [<i>K</i> _D nM (SD)]
	C/C	T/C	T/T	C allele	T allele			
<i>Mood disorders</i>								
Nonattempters, <i>N</i> = 100	39	29	30	107	89	7.64 (0.41)	0.02* (0.51)	82.76 (29.67) [0.33 (0.49)]
Attempters, <i>N</i> = 52	13	28	13	54	54			87.86 (34.23) [0.35 (0.48)]
All, <i>N</i> = 152	52	57	43	161	143	3.07 (1.82)	21 (0.17)	84.58 (31.35) [0.34 (0.48)]
<i>Healthy controls, N</i> = 63								
	14	29	20	57	69			78.51 (22.57) [0.38 (0.55)]

**p* < 0.05.

($\chi^2 = 7.64$, *df* = 2, *p* = 0.020). There was no significant difference in severity of depression, lifetime aggression, hostility or impulsiveness across genotype in the depressed subject group or the controls, and in each of the subgroups, that is, suicide attempters and nonattempters. The same analysis for females only did not change the results (data not shown).

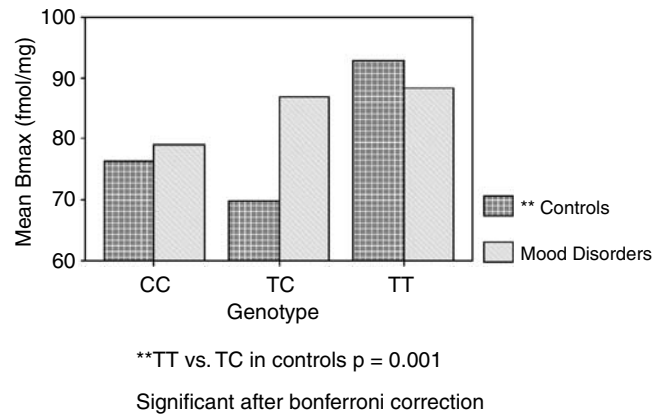
5-HT_{2A} Receptor Binding Indices and Clinical Parameters

There were no significant effects for age (*F* = 0.027, *df* = 213,1; *p* = 0.871) or gender (*t* = -0.582, *df* = 213; *p* = 0.561) on the season-adjusted 5-HT_{2A} *B*_{max}. Therefore, we combined binding data for both genders. Table 2 presents the platelet 5-HT_{2A} *B*_{max} and *K*_D for each group and subgroups of suicide attempters and nonattempters. No group differences were observed.

In this sample, *B*_{max} did not correlate significantly with lifetime aggression score as measured by BG (*r* = 0.011, *p* = 0.89), hostility on the BDHI (*r* = 0.102, *p* = 0.229), HDRS (*r* = 0.125, *p* = 0.126), or impulsivity on the BIS (0.084, *p* = 0.328).

5-HT_{2A} *B*_{max} and 102C/T Genotype

A linear regression model, constructed with season-adjusted *B*_{max} as the dependent variable, and genotype and diagnosis (mood disorder or healthy control) as independent variables, was significant for the whole model (*F* = 2.56, *df* = 5, 209; *p* = 0.028). The association of genotype with *B*_{max} was significant (*F* = 3.53, *df* = 2,213; *p* = 0.031), and the interaction between diagnosis and genotype approached significance (*p* = 0.089). Diagnosis alone was not significant (*p* = 0.251). The reason that the interaction term approaches significance is that the genotype effect on binding was significant for the control group (*F* = 7.56, *df* = 2,60; *p* = 0.001) and not significant for the mood-disordered group (*F* = 1.276, *df* = 2,149; *p* = 0.282) or subgroups of mood disorders (bipolar, major depressive disorder). Adding sex to the model did not change the results, and there was no effect of sex on *B*_{max} (*F* = 0.001, *df* = 1,214, *p* = 0.98). As shown in Figure 1, control subjects with the TT genotype had higher *B*_{max} than subjects with the other genotypes (92.7 vs 69.7 fmol/mg in the TC genotype and 76.3 in the CC genotype). Mood-disordered subjects with the TT

**Figure 1** Platelet 5-HT_{2A} binding density and C102T genotype in mood-disordered subjects and healthy volunteers.

genotype had a *B*_{max} (88.2 fmol/mg) closer to that of the TC genotype (86.9 fmol/mg) and the CC genotype (79.0 fmol/mg) (*F* = 1.26, *df* = 2,149; *p* = 0.282), although the rank order was the same as for controls. A *post hoc* test for multiple comparisons confirmed the difference in the control group between the TT and the TC (23.0 fmol/mg) as significant after Bonferroni correction (*p* = 0.001), whereas the difference between TT and CC (16.4 fmol/mg) approached significance (*p* = 0.074), while the difference between CC and TC (6.55 fmol/mg) was negligible (*p* = 0.99) (see Figure 1). No effect of genotype was found on *K*_D.

DISCUSSION

Our results confirm previous reports that genotype frequencies of 102T/C 5-HT_{2A} receptor gene polymorphism in mood disorders do not differ from healthy volunteers (Arranz *et al*, 1997; Bondy *et al*, 2000; Crawford *et al*, 2000; Mahieu *et al*, 1997; Minov *et al*, 2001; Tsai *et al*, 1999; Ono *et al*, 2001; Correa *et al*, 2002; Geijer *et al*, 2000). Nevertheless, we found an excess of the TC genotype in suicide attempters compared with nonattempters. This is an exploratory finding requiring replication. We found a significant association between platelet 5-HT_{2A} receptor density (*B*_{max}) and the TT genotype of this polymorphism.

This effect appears stronger or is perhaps confined to normal volunteers compared with depressed subjects. There was no sex effect on the findings. The apparent absence or much weaker nature of this relationship in depressed subjects is not explained by a difference in statistical power because we had more depressed subjects ($N = 63$ controls vs $N = 152$ mood-disordered subjects). The finding that affinity (K_D) did not differ significantly between healthy controls and mood-disordered patients indicates that the genetic effect involves B_{max} or receptor number. The TT genotype is associated with more 5-HT_{2A} receptors in healthy volunteers but apparently not in the mood disorders group even though there was the same rank order of binding by genotype (TT > TC > CC). This finding is consistent with Poleskaya and Sokolov (2002), who studied the relationship of the C and T alleles to the expression of the *HTR_{2A}* gene in post-mortem brain tissue and found the expression level with the T allele was higher compared with the C allele in schizophrenia and a control group. They did not include a sample with mood disorders.

Turecki *et al* (2003) studied post-mortem brain samples of suicide victims and comparison subjects and found higher 5-HT_{2A} B_{max} in suicide victims with the C allele than in controls with the C allele, excluding the polymorphism as an explanation of greater binding in suicide, although Turecki studied a population of completers rather than attempters and post-mortem brains instead of platelets. Our data, in combination with those two reports, showed that having the T allele might be associated with higher B_{max} in platelets and brain. Our group demonstrated in the past that higher platelet 5-HT_{2A} binding is related to suicidal behavior and aggression history, but not to a diagnosis of mood disorder (McBride *et al*, 1994). Since the number of suicide attempters in this study was relatively small ($N = 52$), the lack of association of suicidality with higher B_{max} in this sample may be a type II error due to size effect. However, not finding more 5-HT_{2A} binding in the mood-disordered subjects is consistent with our previous report (McBride *et al*, 1994). Another explanation for lack of association with suicidality in the current study may be a complex mode of inheritance in the *HTR_{2A}* gene, rather than simple Mendelian transmission that makes each subject different in expression of its genetic traits. For example, Kato *et al* (1996) found a genomic imprinting mechanism in the *HTR_{2A}* gene, namely, only maternal alleles will be expressed. A trait of imprinting and anticipation was described in the inheritance of mood disorders in Caucasian and Japanese populations (Ohara *et al*, 1998). Given the possibility of imprinting at the C102T locus, the lack of parental data in our study is a limitation.

If this genetic effect occurs in the brain, namely an association between the TT genotype and more 5-HT_{2A} receptors, there is the possibility of greater signal transduction via phosphoinositide turnover and second messengers inositol triphosphate and diacylglycerol. This may enhance 5-HT_{2A}-mediated effects on brain function and effects of antidepressant and atypical antipsychotics. Electroconvulsive shock upregulates 5-HT_{2A} receptors as does stress, perhaps mediating antidepressant and homeostatic effects. The *HTR_{2A}* 102T/C polymorphism or another polymorphism in linkage disequilibrium with it, including the 1438A/G

promoter polymorphism, may affect gene regulation in ways that have not yet been elucidated.

Limitations

As in all case-control studies in psychiatric genetics, we must be aware of false-positive and false-negative findings due to sample size and ethnic stratification. We included only Caucasian subjects to minimize the stratification error. Notably, the association with schizophrenia seems stronger in Caucasians (Abdolmaleky *et al*, 2004). Another limitation is that the peripheral 5-HT_{2A} platelet binding is not a direct measure of the brain 5-HT_{2A} binding, and different transcription factors may regulate the gene in neurons expressing *HTR_{2A}* gene centrally compared with megakaryocytes (Mann *et al*, 1992; Pandey *et al*, 1995). The nonassociation of this genotype with binding in the depressed population may reflect other regulatory effects, associated with diagnosis, that need to be identified. This study applies the same correction for seasonal effect to both groups, whereas there might be a different pattern of seasonal variability. This could potentially distort the comparisons between the two groups. Given the known imprinting at the C102T locus (Kato *et al*, 1996), the lack of parental data in our study limits interpretation of the results since we could not assess such an effect. *HTR_{2A}* gene transcription is regulated in the megakaryocyte, and the half-life of platelets is 9 days. A 14-day drug-free period represents $1\frac{1}{2}$ lives and might not be long enough for drug effects to wear off. Most of our subjects were drug free for 4 weeks, reducing the likelihood of a drug effect. There are other variants in the *HTR_{2A}* gene that were not studied here, for example, the 1438A/G promoter polymorphism with which the C102T is in perfect linkage disequilibrium. The other variants might be more directly related to receptor binding. We studied major depression and bipolar disorder and, although we did not detect an effect of these subgroups, a larger study is needed for adequate statistical power.

In conclusion, this study replicates the lack of association of the T102C with suicidality and mood disorders, but demonstrates an association of the TT genotype with a higher number of 5-HT_{2A} receptors in the platelets of healthy but apparently not in mood-disordered subjects. This difference in the gene to receptor number association between healthy and mood-disordered subjects may reflect pathological mechanisms of importance that warrant further study.

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