



## **Genetic variation in HTR2A influences serotonin transporter binding potential as measured using PET and [C-11]DASB**

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# Genetic variation in HTR2A influences serotonin transporter binding potential as measured using PET and [<sup>11</sup>C]DASB

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## Abstract

In a previous study we showed that genetic variation in HTR2A, which encodes the serotonin 2A receptor, influenced outcome of citalopram treatment in patients with major depressive disorder. Since chronic administration of citalopram, which selectively and potently inhibits the serotonin transporter (5-HTT), putatively enhances serotonergic transmission, it is conceivable that genetic variation within HTR2A also influences pretreatment 5-HTT function or serotonergic transmission. The present study used positron emission tomography (PET) and the selective 5-HTT ligand, [<sup>11</sup>C]DASB, to investigate whether the HTR2A marker alleles that predict treatment outcome also predict differences in 5-HTT binding. Brain levels of 5-HTT were assessed *in vivo* using PET measures of the non-displaceable component of the [<sup>11</sup>C]DASB binding potential (BP<sub>ND</sub>). DNA from 43 patients and healthy volunteers, all unmedicated, was genotyped with 14 single nucleotide polymorphisms located within or around HTR2A. Allelic association with BP<sub>ND</sub> was assessed in eight brain regions, with covariates to control for race and ethnicity. We detected allelic association between [<sup>11</sup>C]DASB BP<sub>ND</sub> in thalamus and three markers in a region spanning the 3' untranslated region and second intron of HTR2A (rs7333412,  $p=0.000045$ ; rs7997012,  $p=0.000086$ ; rs977003,  $p=0.000069$ ). The association signal at rs7333412 remained significant ( $p<0.05$ ) after applying corrections for multiple testing via permutation. Genetic variation in HTR2A that was previously associated with citalopram treatment outcome was also associated with thalamic 5-HTT binding. While further work is needed to identify the actual functional genetic variants involved, these results suggest that a relationship exists between genetic variation in HTR2A and either 5-HTT expression or central serotonergic transmission that influences the therapeutic response to 5-HTT inhibition in major depression.

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**Key words:** genetic association, HTR2A, positron emission tomography, serotonin transporter, [<sup>11</sup>C]DASB.

## Introduction

Several studies have investigated the use of genetic markers to predict antidepressant treatment outcome in major depressive disorder (MDD) (Laje & McMahon, 2007). However, despite many interesting

findings no consensus exists. A genetic association study of 68 candidate genes in the STAR\*D cohort identified a significant and reproducible association between citalopram treatment outcome and genetic variation occurring near or within the gene encoding the serotonin 2A receptor (HTR2A) (McMahon *et al.* 2006). However, the mechanism by which variation in HTR2A influences outcome of citalopram treatment remains unclear.

Citalopram inhibits the reuptake of serotonin (5-HT) into 5-HT neurons by selectively and potently binding to serotonin transporters (5-HTT). The 5-HTT

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has been the focus of many studies of normal and pathological emotional behaviour. In post-mortem human brain tissue, the 5-HTT density varies by region, with highest values in the raphe nuclei, thalamus, and amygdala, and lowest values in the cerebellar cortex (Cortes *et al.* 1988; Kish *et al.* 2005; Laruelle *et al.* 1988; Varnas *et al.* 2004). The 5-HTT binding potential (a parameter that reflects the product of density and affinity) can be quantified *in vivo* using positron emission tomography (PET) and the radioligand, [<sup>11</sup>C]-3-amino-4-(2-dimethylamino-methyl-phenylsulfanyl)-benzonitrile ([<sup>11</sup>C]DASB). This radioligand has shown relatively greater specific:non-specific binding ratios *in vivo* than previously available 5-HTT radioligands (Ginovart *et al.* 2001; Houle *et al.* 2000; Huang *et al.* 2002; Wilson *et al.* 2002). The non-displaceable component of the 5-HTT binding potential (BP<sub>ND</sub>) obtained using [<sup>11</sup>C]DASB closely corresponds with 5-HTT densities measured post mortem in the human brain (Ginovart *et al.* 2001; Houle *et al.* 2000). Cannon *et al.* (2007) reported differences in [<sup>11</sup>C]DASB BP<sub>ND</sub> between healthy controls and unmedicated subjects with a major depressive episode (MDE).

Since SSRIs bind the 5-HTT, genes important in response to SSRIs might also be important in regulating the 5-HTT, or its expression. DNA samples acquired from some of the subjects that participated in the Cannon *et al.* (2007) study were genotyped using a panel of single nucleotide polymorphisms (SNPs) that were previously tested in the much larger sample from the multicentre, STAR\*D clinical trial (McMahon *et al.* 2006). This SNP profile included 14 markers in and around HTR2A, of which one showed an association with improvement after citalopram treatment. Using these data, we enquired whether genetic variation in HTR2A is associated with 5-HTT BP<sub>ND</sub>, as one of the potential mechanisms by which variation in HTR2A modulates antidepressant response.

## Materials and methods

### *Subject sample, PET data collection and analysis*

A detailed description of the subject entrance criteria, region-of-interest (ROI) definition, and PET data analysis appears in Cannon *et al.* (2006, 2007). Briefly, participants included unmedicated outpatients and healthy volunteers aged 18–55 yr who met DSM-IV criteria for a current MDE, manifested a clinical course consistent with either MDD ( $n=16$ ) or bipolar disorder (BD) ( $n=12$ ), and had experienced mood disorder onset prior to age 40 yr. Healthy controls ( $n=15$ ) who had never met criteria for a major psychiatric

**Table 1.** Basic demographic characteristics of participants by diagnostic group including gender, ethnicity and age at time of PET scan

|                  | MDD<br>( $n=16$ ) | BD<br>( $n=12$ ) | HC<br>( $n=15$ ) |
|------------------|-------------------|------------------|------------------|
| Gender (female)  | 11                | 10               | 9                |
| Ethnicity        |                   |                  |                  |
| Caucasian        | 4                 | 7                | 9                |
| African-American | 8                 | 5                | 5                |
| Other            | 4                 |                  | 1                |
| Mean age (s.d.)  | 36.5 (8.9)        | 29.75 (6.7)      | 35 (6.9)         |

MDD, Major depressive disorder; BD, bipolar disorder; HC, healthy control; s.d., standard deviation.

disorder and denied having a first-degree relative with a major psychiatric disorder also participated (Table 1). All participants provided written informed consent and the study was approved by the NIMH Institutional Review Board. Exclusion criteria included exposure to psychotropic drugs or to any medications or herbal preparations with reported effects on serotonergic function within the 3 wk before scanning (8 wk for fluoxetine), pregnancy, lifetime history of substance dependence, or substance abuse within the previous 1 yr. The severity of depressive symptoms was rated using the Montgomery–Asberg Depression Rating Scale (MADRS; Montgomery & Asberg, 1979) and the Inventory of Depressive Symptoms – Clinician Version (IDS-C; Trivedi *et al.* 2004). Anxiety symptoms were rated using the Hamilton Anxiety Rating Scale (HAMA; Hamilton, 1959), and manic symptoms using the Young Mania Rating Scale (YMRS; Young *et al.* 1978). Only a subset of the total sample reported in Cannon *et al.* (2006, 2007) was available for DNA testing ( $n=43$ ). DNA was extracted from whole blood using GenePure chemistry (Qiagen, USA) at the NIH laboratories.

PET images were acquired using a GE Advance scanner in 3D mode [reconstructed 3D spatial resolution = 6 mm full-width at half maximum (FWHM)]. A 120-min dynamic emission scan was acquired as 33 frames of increasing length. High specific activity [<sup>11</sup>C]DASB was synthesized as described previously (Wilson *et al.* 2000). To provide an anatomical framework for analysis of the PET data, MRI scans were acquired using a GE 1.5 T or 3.0 T scanner and a T<sub>1</sub>-weighted pulse sequence (voxel size 0.86 × 0.86 × 1.2 mm). The co-registered PET and MRI images were spatially normalized to a common stereotaxic array using SPM2 and resampled into 2 mm<sup>3</sup> voxels.

The 5-HTT binding parameter estimates were computed voxel-by-voxel using the MRTM2 method (Ichise *et al.* 2003) as described in Cannon *et al.* (2006). The reference region was defined in cerebellar cortex with the ROI boundaries situated more than one FWHM ventral to the occipital and temporal cortices, lateral to the vermis, and anterior to the posterior surface. Regional templates were defined on a mean MRI image generated by summing the spatially normalized MRI images from all subjects. MRTM2 allows voxel-wise estimation (parametric imaging) of  $BP_{ND} = f_2 B_{max} / K_d$ , which is proportional to the transporter density ( $B_{max}$ ), where  $K_d$  and  $f_2$  are the dissociation constant and free tissue fraction, respectively. The  $BP_{ND}$  values thus were independent of blood flow (K1) (Cannon *et al.* 2007; Ichise *et al.* 2003). The weighted mean tracer clearance time (fixed  $k_2$ ) obtained from the thalamus, midbrain and striatum did not differ ( $F=1.15$ ,  $p=0.323$ ) across the control ( $0.056 \pm 0.011 \text{ min}^{-1}$ ), MDD ( $0.053 \pm 0.009 \text{ min}^{-1}$ ) or BD ( $0.058 \pm 0.009 \text{ min}^{-1}$ ) samples.

The [<sup>11</sup>C]DASB  $BP_{ND}$  values were assessed in eight ROIs where differences were obtained between control and mood-disordered groups in Cannon *et al.* (2007): thalamus (Th), striatum (Str), insula (Ins), midbrain (Midbr), pregenual anterior cingulate cortex (pgACC), dorsal cingulate cortex (DCC) and posterior cingulate cortex (PCC). Additionally, the subgenual anterior cingulate cortex (sgACC) was included due to previous reports of association with depression treatment outcome (Drevets *et al.* 2008; Mayberg *et al.* 1997). Anatomical boundaries for these ROIs appear in Cannon *et al.* (2006). The  $BP_{ND}$  value for each region was computed as the average of the  $BP_{ND}$  values for all voxels within the ROI.

To identify the areas where genotype correlated most significantly with  $BP_{ND}$ , a voxel-wise analysis was conducted *post hoc* using SPM2 (smoothing kernel  $8 \text{ mm}^3$ ). We used two-sample, independent  $t$  tests to compare parametric  $BP_{ND}$  images between genotype-based groups. This analysis was performed under a dominant model in order to generate comparably sized groups for comparison. The significance threshold for the voxel-wise analysis was set at  $p_{corr} < 0.05$ , after applying corrections for multiple comparisons using the cluster test (Poline *et al.* 1995) or the false discovery rate (Genovese *et al.* 2002).

### Selection of SNP markers

Fourteen SNPs were selected to sample common variation in HTR2A, as detailed in (McMahon *et al.* 2006). The program 'LDSelect' (Carlson *et al.* 2004)

was used to choose an optimally uncorrelated set of available SNPs to genotype (marker-marker  $r^2 < 0.8$ ), excluding SNPs with a low minor allele frequency, which lack power to demonstrate association. Due to their potential functional significance, we also included three SNPs reported to have an impact on HTR2A transcription (Erdmann *et al.* 1996; Ozaki *et al.* 1997).

### Genotyping methods

Samples were shipped to Illumina Inc., USA, where they were genotyped on a highly accurate assay (Gunderson *et al.* 2004). Of all SNPs selected, 99.73% could be genotyped, and 99.90% of all possible genotypes were returned, including 35 052 blind duplicate genotypes, all of which matched exactly.

### Genetic association analysis

The  $BP_{ND}$  for each ROI was extracted for each subject and used as a quantitative trait in the association analysis. To improve power depressed patients and healthy volunteers were grouped together for this analysis, since there was no *a-priori* reason to assume that the genetic association between marker and imaging trait would differ between diagnostic subgroups. Association with the genotyped SNPs was assessed via score tests derived from a linear model (Schaid *et al.* 2002; Seaman & Muller-Myhsok, 2005).

We controlled the experiment-wise error rate for the genetic association analysis by permutation. We developed a novel permutation method that accounted for the 14 SNPs and eight ROIs tested. This method breaks any connection between markers and phenotypes, reflecting a global null hypothesis, while maintaining the correlation structure among the markers and, separately, among the phenotypes. We considered the imaging phenotypes from each individual as a unit and randomly permuted entire phenotype units between individuals to form permutation datasets. All the marker association tests were performed on each permutation dataset and the minimum  $p$  value noted. Thus we were able to estimate the permutation distribution of the minimum  $p$  value under the global null hypothesis. Comparing our original raw  $p$  values with the permutation distribution allowed us to estimate  $p$  values that are appropriately adjusted for the number of tests performed. To control for effects of race and ethnicity, the sample was divided into three strata (see below) and permutations were performed within each stratum separately. Additional details of our testing procedure can be found in the Supplementary Appendix (available online).

**Table 2.** Genetic association results. Regions of interest (ROI) were defined bilaterally (to reduce the number of comparisons) in the thalamus (Th), striatum (Str), insula (Ins), and midbrain (Midbr), and in the subgenual anterior (sgACC), pregenual anterior (pgACC), dorsal (DCC) and posterior (PCC) portions of the cingulate cortex

| Marker    | ROI     |         |         |       |         |         |         |         |
|-----------|---------|---------|---------|-------|---------|---------|---------|---------|
|           | Th      | Str     | Ins     | sgACC | pgACC   | DCC     | PCC     | Midbr   |
| rs7333412 | 0.00005 | 0.03415 | 0.00387 | n.s.  | 0.06698 | 0.07041 | 0.07775 | n.s.    |
| rs3125    | n.s.    | n.s.    | n.s.    | n.s.  | n.s.    | n.s.    | n.s.    | n.s.    |
| rs6314    | n.s.    | n.s.    | n.s.    | n.s.  | n.s.    | n.s.    | n.s.    | n.s.    |
| rs7997012 | 0.00009 | n.s.    | 0.00627 | n.s.  | n.s.    | 0.04558 | 0.07356 | 0.07439 |
| rs977003  | 0.00007 | n.s.    | 0.02151 | n.s.  | n.s.    | n.s.    | n.s.    | n.s.    |
| rs1923884 | n.s.    | n.s.    | n.s.    | n.s.  | n.s.    | n.s.    | n.s.    | n.s.    |
| rs1745837 | n.s.    | n.s.    | n.s.    | n.s.  | n.s.    | n.s.    | n.s.    | n.s.    |
| rs2770296 | n.s.    | n.s.    | n.s.    | n.s.  | n.s.    | n.s.    | n.s.    | n.s.    |
| rs1328677 | n.s.    | n.s.    | n.s.    | n.s.  | n.s.    | n.s.    | n.s.    | n.s.    |
| rs1928040 | n.s.    | n.s.    | n.s.    | n.s.  | n.s.    | n.s.    | n.s.    | n.s.    |
| rs731779  | n.s.    | n.s.    | n.s.    | n.s.  | n.s.    | n.s.    | n.s.    | n.s.    |
| rs985933  | 0.02799 | n.s.    | n.s.    | n.s.  | n.s.    | n.s.    | n.s.    | n.s.    |
| rs594242  | 0.01553 | n.s.    | n.s.    | n.s.  | n.s.    | n.s.    | n.s.    | n.s.    |
| rs6312    | n.s.    | n.s.    | n.s.    | n.s.  | n.s.    | n.s.    | n.s.    | n.s.    |

n.s., Not significant.

$p$  values represent the linear regression of allele on 5-HTT binding potential in each ROI, uncorrected for multiple comparisons. Only values  $<0.1$  are shown.

### Population structure

To reduce the risk that population stratification might inflate the results of the genetic associations, we used genotypes of 1764 unlinked SNPs (generated separately on this sample) to estimate posterior probabilities of subpopulation membership for each individual. Based on this analysis, each individual's most likely subpopulation membership was imputed and used as a stratifying variable in the analysis. Using STRUCTURE (Pritchard *et al.* 2000), we evaluated the scenario of three cryptic populations within the sample by running 20 000 burn-in steps followed by 20 000 replications.

Potential confounders were assessed in *post-hoc* tests. The original [ $^{11}\text{C}$ ]DASB PET studies (Cannon *et al.* 2006, 2007) found that  $\text{BP}_{\text{ND}}$  in the thalamus was significantly influenced by age, diagnosis, and symptom severity. Thus, we included these variables as covariates (along with rs7333412 genotype and STRUCTURE posterior probabilities) in a generalized linear model, with thalamic 5-HTT  $\text{BP}_{\text{ND}}$  as the dependent variable. Previous studies have also suggested that smoking status (Staley *et al.* 2001) and season when the scan was obtained (Praschak-Rieder *et al.* 2008) can also affect  $\text{BP}_{\text{ND}}$ , so these were also evaluated in separate covariate analyses.

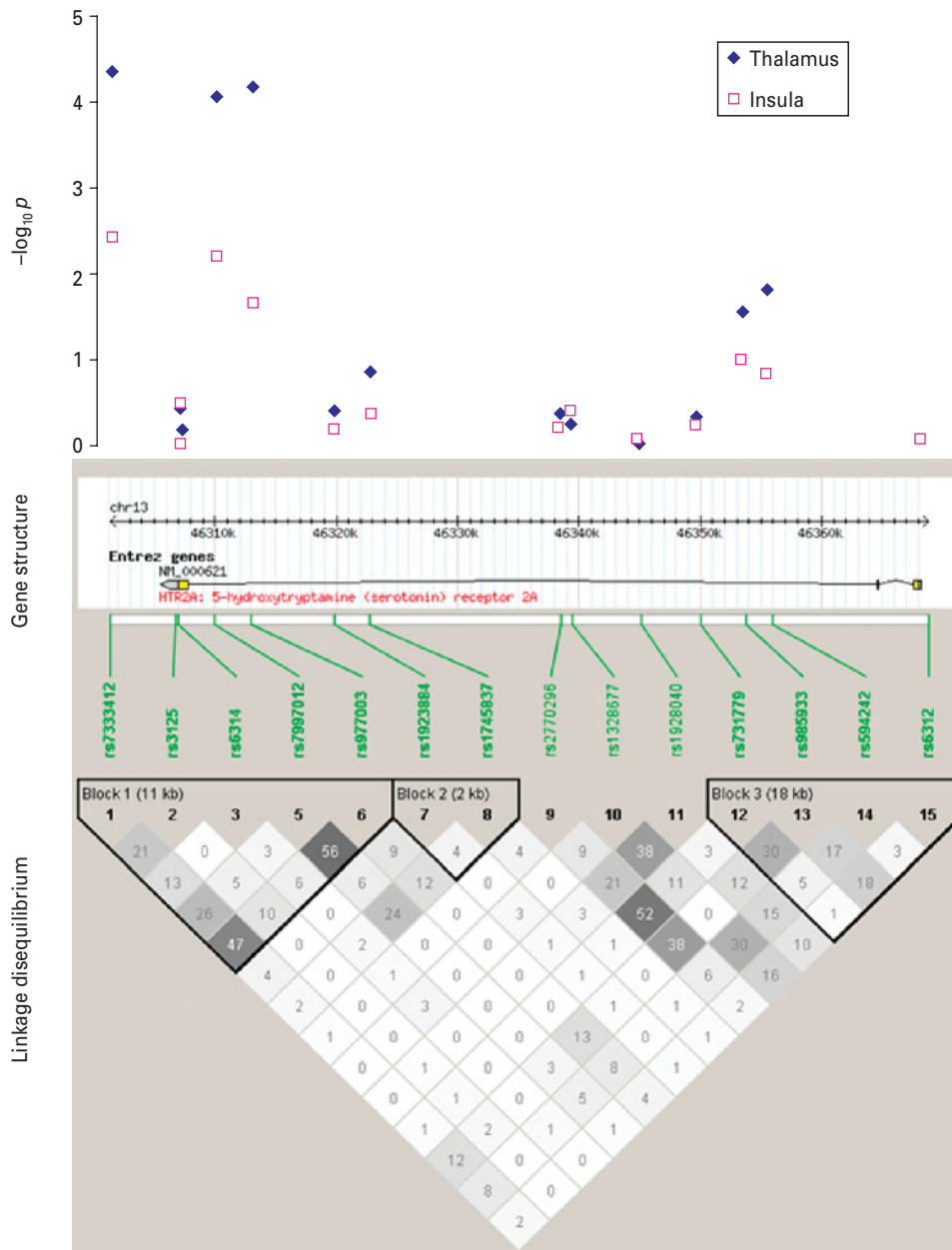
### Results

#### Association analyses

Association results for the 14 SNPs in the eight ROIs appear in Table 2. The markers rs7333412, rs7997012 and rs977003 were associated with 5-HTT  $\text{BP}_{\text{ND}}$  in thalamus and insula at  $p_{\text{uncorr}} < 0.05$ . Only rs7333412 in thalamus remained significant after permutation correction ( $p_{\text{corr}} < 0.05$ ). This marker explained 28% of the variance in thalamic 5-HTT  $\text{BP}_{\text{ND}}$  in this sample (Fig. 1). The lowest  $\text{BP}_{\text{ND}}$  values were observed in AA homozygotes.

The positions of the genotyped markers relative to HTR2A, their linkage disequilibrium relationships, and the allelic association results in thalamus and insula are shown in Fig. 2. All of the associated markers lay in the same haplotype block spanning the 3' untranslated region (UTR) and second intron of HTR2A. Marker rs7333412 represents a G > A substitution in a non-conserved region 4 kb distal to the 3'-UTR of HTR2A. This is the same gene region that was associated with citalopram outcome in our previous study (McMahon *et al.* 2006).

The *post-hoc* voxel-wise analysis of the relationship between rs7333412 and  $\text{BP}_{\text{ND}}$  supported the ROI results. In the thalamus, subjects with the AG or GG genotype had higher 5-HTT binding than those with

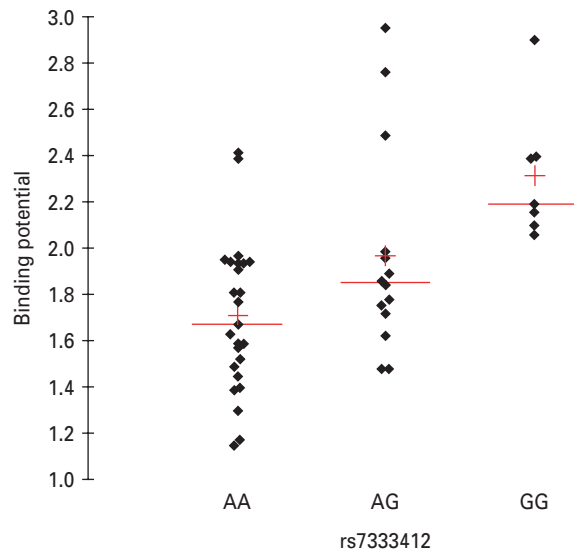


**Fig. 1.** Association results in thalamus and insula, *HTR2A* gene structure, physical positions and linkage disequilibrium relationships of the 14 studied markers (Draft Human Genome Sequence Build 34). Haploview was used to calculate linkage disequilibrium that is shown using  $r^2$  values and colouring (darker shades indicate higher LD). Haplotype blocks were defined using the Four Gamete Test.

the AA genotype ( $T=3.37$ ,  $p=0.03$ ; Fig. 3). AA carriers did not have greater 5-HTT BP<sub>ND</sub> in other brain areas, consistent with the ROI results (data not shown). The genotype-based groups were well-balanced for the proportion of mood disorder cases and healthy controls (AA 53%, AG+GG 43%) and for mean age (AA  $36.7 \pm 7.4$  yr, AG+GG  $32.4 \pm 8.7$  yr).

In *post-hoc* tests of potential confounders, diagnosis and age were found to be significant covariates ( $p=0.01$ ,  $p=0.01$ , respectively). However, the association between 5-HTT BP<sub>ND</sub> and rs7333412 remained significant after inclusion of these covariates in the regression model [ $F(5, 40)=6.14$ ,  $p=0.0003$ ]. Current symptoms of depression, mania, and anxiety,





**Fig. 2.** Serotonin transporter binding potential in thalamus by rs7333412 genotype. All data points are shown, along with mean (–) and median (+).

measured by MADRS, IDS-C, YMRS, and HAMA, showed no significant effect on the relationship between rs7333412 and thalamic 5-HTT BP<sub>ND</sub> (Table 3). Smoking status and season of scan also had no significant effect on the observed association (data not shown). Thus the association observed between rs7333412 and thalamic 5-HTT BP<sub>ND</sub> remained significant despite adjustment for potentially confounding differences between the AA homozygotes and G carriers in age, diagnosis, symptom severity, season assessed, and smoking status.

None of the putatively functional markers we studied showed any evidence of association with 5-HTT BP<sub>ND</sub> in this sample.

## Discussion

This is the first study to detect an association between 5-HTT BP<sub>ND</sub> and genetic variation in HTR2A. These data suggest that either a direct or an indirect functional relationship exists between the HTR2A and 5-HTT systems. Genetic variation in the 3'-UTR and second intron of HTR2A was previously associated with the antidepressant outcome of citalopram treatment in patients with MDD (McMahon *et al.* 2006). Although these data have not localized the specific functional genetic variant within HTR2A, taken together they hold the potential to shed light on the mechanism of antidepressant action of SSRIs.

We found that genetic variation spanning the 3'-UTR and second intron of HTR2A is associated with 5-HTT BP<sub>ND</sub>. This genetic variation lay within the same haplotype block that was implicated in our study of citalopram treatment outcome (McMahon *et al.* 2006). Based on these results, we speculate that HTR2A might exert a *trans*-regulatory effect on 5-HTT expression (SLC6A4), 5-HTT trafficking, or both. The final result may be a relative difference in 5-HTT concentration at the synapse that could impact SSRI efficacy and tolerability.

One variant in this same haplotype block, rs6314, encodes a His to Tyr substitution (His<sup>452</sup>Tyr) and may regulate Ca<sup>2+</sup> mobilization peaks (Ozaki *et al.* 1997). However, both the STAR\*D and the present sample were underpowered to detect association with this very uncommon variant. It is possible that the same functional variant(s) in this region of HTR2A account for both the citalopram response and the 5-HTT BP<sub>ND</sub> association findings, but we cannot definitively implicate a single functional variant based upon the available data.

Differences in [<sup>11</sup>C]DASB BP<sub>ND</sub> probably reflect changes in 5-HTT density or affinity between genotypic groups. The method we applied to obtain 5-HTT BP<sub>ND</sub> used a reference tissue model to obviate the need for arterial blood sampling. Using the cerebellar cortex as the reference tissue (i.e. to correct for the proportions of radiotracer distribution attributable to free or non-specific binding), this model provides valid and reliable assessments of the 5-HTT BP<sub>ND</sub> parameter in [<sup>11</sup>C]DASB images, and yields occupancy measures that compare closely with those determined using an arterial input function (Ichise *et al.* 2003; Parsey *et al.* 2006). Moreover, while the cerebellum also contains very low 5-HTT concentrations (Szabo *et al.* 2002), Parsey *et al.* (2006) used a combination of post-mortem and *in-vivo* neuroimaging assessments to demonstrate that the cerebellar cortex is the optimal reference region for modelling the BP<sub>ND</sub> parameter in [<sup>11</sup>C]DASB images.

A limitation of the BP<sub>ND</sub> parameter is that it reflects the product of density and affinity ( $B_{max}/K_D$ ), so the effects of genetic variation in HTR2A on 5-HTT binding cannot be more specifically attributed to density *vs.* affinity. For example, although physiological changes in the intrasynaptic 5-HT concentration are unlikely to alter [<sup>11</sup>C]DASB binding directly (Ginovart *et al.* 2003; Praschak-Rieder *et al.* 2005; Talbot *et al.* 2005), the trafficking of 5-HTT protein between the cytosol and the cell membrane is affected directly and rapidly by 5-HT levels (Blakely *et al.* 1998). Many radiotracers show a lower affinity for protein binding

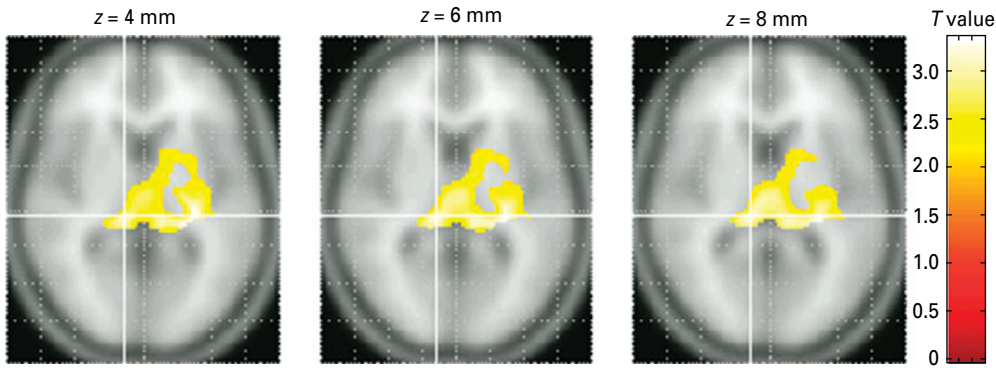


Fig. 3. Voxel-based analysis showing increased thalamic 5-HTT binding potential in subjects with the AG or GG genotypes compared to those with the AA genotype ( $t=3.37$ ,  $p=0.03$ ).

sites situated within the cytosol as opposed to on the cell membrane (Laruelle *et al.* 1988). Although the extent to which [<sup>11</sup>C]DASB affinity for cytosolic 5-HTT protein differs from that for membrane-bound 5-HTT protein has not been characterized, it remains possible that if variation in HTR2A influences 5-HT transmission, this effect may influence 5-HTT BP<sub>ND</sub> in the same direction (i.e. an increase in 5-HT transmission would result in increased 5-HTT trafficking from the cytosol to the membrane). Such an effect would hold major implications regarding the nature of the differential treatment responsiveness to genetic variation in HTR2A. In particular, the observation that individuals homozygous for the A allele at rs7333412 show greater responsiveness to citalopram treatment (McMahon *et al.* 2006) and lower 5-HTT BP<sub>ND</sub> suggests the hypothesis that AA homozygotes benefit from enhancing 5-HT transmission via SSRI pharmacotherapy because these individuals have lower basal 5-HT transmission.

The ROIs chosen for this study were based on known relevance to affective disorders and measurable concentration of 5-HTT. The thalamus, where we find the most robust evidence of association between BP<sub>ND</sub> and rs7333412, has long been thought to be involved in the depression circuitry, due to its connections with subcortical limbic structures such as the amygdala and prefrontal and cingulate cortices. However, our results do not exclude association with other ROIs. Indeed, the uncorrected association results are consistent with signals in insula and striatum. Although these  $p$  values did not remain significant after permutation correction, it is possible that this constitutes a type II error due to limited sample size.

In the present study we performed multiple tests of association between genetic markers in or near the HTR2A gene and the 5-HTT BP<sub>ND</sub> phenotype in

Table 3. General Linear Model results including thalamus binding potential (BP<sub>ND</sub>) by rs7333412 genotype and scores for current symptoms of depression, mania, and anxiety

| Source             | d.f. | Mean square | F value | Pr > F |
|--------------------|------|-------------|---------|--------|
| IDS-C              | 1    | 0.45        | 3.03    | 0.0904 |
| MADRS              | 1    | 0.19        | 1.26    | 0.2693 |
| YMRS               | 1    | 0.00        | 0.00    | 0.9698 |
| HAMA               | 1    | 0.01        | 0.04    | 0.8520 |
| rs7333412 genotype | 2    | 0.95        | 6.44    | 0.0042 |

IDS-C, Inventory for Depressive Symptoms – Clinician rated; MADRS, Montgomery–Asberg Depression Rating Scale; YMRS, Young Mania Rating Scale; HAMA, Hamilton Anxiety Scale.

several brain regions. Multiple testing can lead to spurious findings. To address this issue we used a novel permutation procedure that took into account both the linkage disequilibrium between nearby SNPs and the correlation in BP<sub>ND</sub> across brain ROIs. Where such correlations exist, alternative procedures such as Bonferroni correction would be overly conservative, and might also depend on asymptotic properties that did not hold in the current dataset (Cheng & Lin, 2005; Seaman & Muller-Myhsok, 2005). The permutation procedure we used had neither of these limitations and thus was well-suited to handle multiple correlated phenotypes. Nevertheless, this conservative approach increased the risk for type II error, and conceivably the association between genetic variation at rs7333412 and 5-HTT BP<sub>ND</sub> may have become significant in other regions (e.g. insula) as well in a larger sample size or a more targeted analysis. The regional specificity of our findings thus awaits future studies designed to address this issue more specifically.



Other limitations of our study design also merit comment. The original imaging experiment was not designed primarily to test association between HTR2A and 5-HTT binding. Furthermore, the 14 SNPs tested in HTR2A were extracted from a larger set that included a total of 768 SNPs in 68 genes (the same set reported in McMahon *et al.* 2006), selected to interrogate common variation. Finally, our results do not illuminate whether HTR2A variation affects 5-HTT binding directly, or indirectly through some mediating molecule(s) or system(s). For example, if variation in HTR2A influences serotonergic transmission generally, the 5-HTT BP<sub>ND</sub> values may be influenced indirectly via associated changes in 5-HTT expression or trafficking, as described above (Blakely *et al.* 1998). Arterial samples were not drawn during scanning and BP<sub>ND</sub> therefore equals the product of BP<sub>F</sub> (the ratio of specific binding to free radioligand at equilibrium) and the free fraction of ligand in the non-displaceable tissue compartment ( $f_{ND}$ ). Our results thus constitute preliminary observations until the functional alleles are identified, replication is shown in an independent sample, and the mechanism underlying the relationship between genetic variation in HTR2A and 5-HTT binding is more fully characterized.

In summary, genetic variation in a region of HTR2A that is associated with antidepressant outcome was also associated with 5-HTT binding in the thalamus. These results suggest a novel functional connection between HTR2A and 5-HTT that may shed light on the neurobiological basis underlying individual differences in the responsiveness to SSRI pharmacotherapy.

## Note

Supplementary material accompanies this paper on the Journal's website (<http://journals.cambridge.org/pnp>).

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## Statement of Interest

Dr Laje and Dr McMahon are listed as co-inventors on a patent application the NIH has filed based in part on some of the markers described in this paper. The authors are not involved in the negotiation and execution of this licence, but under federal law the NIH is required to pay them a portion of any royalties the NIH receives under the licence. The authors do not endorse any commercial use of the patent.

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