Converging Evidence for the Association of Functional Genetic Variation in the Serotonin Receptor 2a Gene With Prefrontal Function and Olanzapine Treatment

Giuseppe Blasi, MD, PhD; Caterina De Virgilio, PhD; Apostolos Papazacharias, MD; Paolo Taurisano, PhD; Barbara Gelao, PhD; Leonardo Fazio, PhD, MD; Gianluca Ursini, MD; Lorenzo Sinibaldi, MD, PhD; Ileana Andriola, MD; Rita Masellis, PhD; Raffaella Romano, PhD; Antonio Rampino, MD; Annabella Di Giorgio, MD, PhD; Luciana Lo Bianco, PhD; Grazia Caforio, MD, PhD; Francesco Piva, Eng, PhD; Teresa Popolizio, MD; Cesario Bellantuono, MD; Orlando Todarello, MD; Joel E. Kleinman, MD, PhD; Gemma Gadaleta, PhD; Daniel R. Weinberger, MD; Alessandro Bertolino, MD, PhD

**IMPORTANCE** Serotonin (5-hydroxytryptamine) receptor 2a (5-HT2AR) signaling is important for modulation of corticostriatal pathways and prefrontal activity during cognition. Furthermore, newer antipsychotic drugs target 5-HT2AR. A single-nucleotide polymorphism in the 5-HT2AR gene (HTR2A rs6314, C>T; OMIM 182135) has been weakly associated with differential 5-HT2AR signaling and with physiologic as well as behavioral effects.

**OBJECTIVE** To use a hierarchical approach to determine the functional effects of this single-nucleotide polymorphism on 5-HT2AR messenger RNA and protein expression, on prefrontal phenotypes linked with genetic risk for schizophrenia, and on treatment with olanzapine.

**DESIGN** In silico predictions, in vitro, and case-control investigations.

**SETTING** Academic and clinical facilities.

**PARTICIPANTS** The postmortem study included 112 brains from healthy individuals; the in vivo investigation included a total sample of 371 healthy individuals and patients with schizophrenia.

**EXPOSURES** Patients received olanzapine monotherapy for 8 weeks.

**MAIN OUTCOMES AND MEASURES** In silico predictions, messenger RNA, and protein expression in postmortem human prefrontal cortex and HeLa cells, functional magnetic resonance imaging prefrontal activity and behavior during working memory and attention in healthy individuals, and response to an 8-week trial of olanzapine treatment in patients with schizophrenia.

**RESULTS** Bioinformatic analysis predicted that rs6314 alters patterns of splicing, with possible effects on HTR2A expression. Moreover, the T allele was associated with reduced prefrontal messenger RNA expression in postmortem prefrontal cortex, with reduced protein expression in vitro, inefficient prefrontal blood oxygen level-dependent functional magnetic resonance imaging response during working memory and attentional control processing, and impaired working memory and attention behavior, as well as with attenuated improvement in negative symptoms after olanzapine treatment.

**CONCLUSIONS AND RELEVANCE** Our results suggest that HTR2A rs6314 affects 5-HT2AR expression and functionally contributes to genetic modulation of known endophenotypes of schizophrenia-like higher-level cognitive behaviors and related prefrontal activity, as well as response to treatment with olanzapine.
Many studies have demonstrated that serotonin projections from the dorsal raphe to the prefrontal cortex are implicated in several aspects of higher-order cognitive behavior. A crucial factor of serotonergic signaling is the phospholipase C and D activating G protein-coupled serotonin (5-hydroxytryptamine) receptor 2a (5-HT2AR). This receptor is abundantly expressed on pyramidal cells and interneurons in prefrontal cortex, where it is well positioned to regulate the balance between excitatory and inhibitory responses. Consistently, pharmacologic studies in animal models have demonstrated that infusion of 5-HT2AR agonists improves prefrontal cognition. Moreover, single-cell recording studies in nonhuman primates performing a working memory task have indicated that stimulation of 5-HT2AR contributes to greater spatial tuning of prefrontal pyramidal neurons as reflected by increased activity for preferred target locations and/or lower activity for nonpreferred locations.

Prefrontal cognitive deficits related to working memory, attention, and executive function are core symptoms of schizophrenia. Furthermore, abnormalities of 5-HT2AR may be important for this brain disorder. Several postmortem studies have revealed reduced prefrontal cortical 5-HT2AR and 5-HT2CR binding in patients with schizophrenia. Despite this evidence, another study has demonstrated prefrontal up-regulation of 5-HT2AR density in patients with untreated schizophrenia along with reduction of receptor density and expression associated with administration of atypical antipsychotics in patients and in mice. These discrepancies may be related to the typical limitations and confounds of postmortem studies of schizophrenia. However, a recent in vivo study with positron emission tomography using a 5-HT2AR-specific ligand in antipsychotic-naive patients has demonstrated reduction of 5-HT2AR binding that was also correlated with positive psychotic symptoms. Together, these results suggest that schizophrenia may be associated with dysregulation of 5-HT2AR brain expression, which may in turn be modulated by antipsychotic treatment. Relevance of 5-HT2AR for schizophrenia is also implicated by pharmacologic evidence in humans and animal models indicating that psychotogenic drugs such as lysergic acid diethylamide act as agonists also on postsynaptic cortical 5-HT2ARs. On the contrary, drugs that antagonize both dopamine D2 receptors and 5-HT2ARs are well-known effective antipsychotics.

Evidence of an association between a diagnosis of schizophrenia and the gene coding for 5-HT2AR (HTR2A, 13q14-21; OMIM 182135) has been weak. More recently, a study using data on gene-level integration of genome-wide association with other genetic and gene expression studies in humans and animal models indicated an association of HTR2A with a diagnosis of schizophrenia. Two of the most studied single-nucleotide polymorphisms (SNPs) within HTRA2 include the promoter variant rs6311 and the synonymous SNP rs6313, which are in strong linkage disequilibrium. These 2 SNPs have been inconsistently associated with a diagnosis of schizophrenia and with response to treatment with antipsychotics. Conflicting results also have been reported on the functional significance of rs6311/rs6313 including the association with postmortem expression. In particular, a postmortem study controlling for a series of experimental variables indicated that these SNPs are not associated with messenger RNA (mRNA) expression in several brain regions. Together, these results suggest that functional effects, if any, of rs6311/rs6313 are weak.

The HTRA2 gene also contains a SNP at exon 3 (rs6314, C>T, minor allele frequency in white individuals: 0.075) implying a missense substitution at amino acid 452 in the C-terminal region of the receptor (histidine > tyrosine). In vitro studies using platelets or fibroblasts have provided some suggestion that rs6314 has an effect on calcium signaling and mobilization as well as on reduced activation of phospholipases C and D, although this evidence is not necessarily related to neural molecular mechanisms and it is weak. However, this polymorphism has been associated with hippocampal volume and activity, and with episodic memory performance, as well as with response to treatment with clozapine in patients with schizophrenia. Moreover, association between rs6314 and diagnosis of schizophrenia has been described, even though it has not been supported by genome-wide association studies. Together, these results suggest that rs6314 may be a potentially important functional candidate, although its relevance for genetic risk for schizophrenia is poorly understood.

Given the key role of 5-HT2AR in molecular mechanisms related to serotonergic neurotransmission in the prefrontal cortex, we hypothesized that rs6314 would be associated in humans with higher-level cognitive behaviors implicated in prefrontal activity and contribute genetically to variation in these functions. Because genetic variation does not directly cause behavioral phenotypes but rather affects neuronal features that influence processing at the level of neural networks, we used a hierarchical stepwise translational genetic approach to investigate the contribution of rs6314 to prefrontal behavior and activity, endophenotypes known to be related to genetic risk for schizophrenia. In particular, our first objective was to determine the functional effects of this SNP on mRNA and protein expression in silico, postmortem, and in vitro methods. Furthermore, we tested the association of this SNP with prefrontal activity and behavior during higher-order cognition. Moreover, based on the hypothesis that antipsychotic efficacy is also obtained with 5-HT2AR antagonism, we evaluated the association of rs6314 with response to an 8-week trial of treatment with olanzapine in patients with schizophrenia. Of note, based on our hypothesis and making use of a hierarchical translational genetic approach, we investigated only one SNP in our subjects: rs6314.

Methods

The present experimental protocol (Supplement eFigure) was approved by the local institutional review board, located at the Policlinico Hospital, Bari, Italy. After complete description of the study to the participants, written informed consent was obtained (see also the Supplement eMethods).

Bioinformatic Modeling

We used bioinformatic tools to predict the association of this SNP with splicing processes, mRNA, and protein expression.
In particular, SpliceAid\(^{40}\) was used to predict the position of splicing motifs in HTR2A wild type and polymorphic regulatory sequences. This software uses a database collecting all experimentally assessed target RNA sequences in humans, thus minimizing issues related to false-positive predictions.\(^{41}\) Furthermore, NNSPLICE\(^{42}\) was used to detect 5′ and 3′ splice sites, Human Transcriptome Map\(^{43}\) and ArrayExpress\(^{44}\) were used to retrieve mRNA expression data, and protein expression data were extracted from the Human Protein Atlas\(^{45}\) and the Human Protein Reference Database.\(^{46}\) Finally, to determine whether HTR2A rs6314 affects protein function, we used SIFT\(^{47}\) and PolyPhen.\(^{48}\) Secondary structure prediction has been performed with Jpred\(^{49}\) and PSIpred.\(^{50}\)

**HTR2A mRNA Expression in Postmortem Prefrontal Cortex of Healthy Humans**

Data from 112 brains of healthy white individuals (32 females; mean [SD] age, 29.0 [21.4] years; pH, 6.5 [0.3]; postmortem interval, 26.2 [15.2]) were used for this study. These data were obtained from a publicly available database\(^{51}\) (Supplement [eMethods]) and included 93 CC and 19 CT individuals. Postmortem prefrontal mRNA expression values were used as the dependent variable in an analysis of covariance (ANCOVA), with rs6314 as the independent variable and age at death, postmortem interval, pH, and sex as covariates of no interest. Analysis of covariance with rs6311 and rs6313 as independent factors was used to replicate earlier studies,\(^{29}\) suggesting that these SNPs are not associated with 5-HT2AR expression in the prefrontal cortex (Supplement [eMethods]).

**HTR2A mRNA and Protein Expression in HeLa Cells**

HTR2A rs6314 C and T complementary DNA synthesis and cloning in expression vectors, as well as their transient overexpression in HeLa cells, are described in the Supplement. Quantitative real-time reverse transcriptase–polymerase chain reaction was used to measure HTR2A mRNA levels in these cells (Supplement [eMethods]). All samples were run in triplicate. The HTR2A mRNA levels were assessed using the comparative cycle threshold method, with β-actin as a reference gene (2\(^{-}\Delta\Delta CT)\(^{52}\) Furthermore, Western blotting was used for protein quantification (Supplement [eMethods]). Optical density values were normalized to β-actin for variation in loading and transfer. Resulting measures are reported as percentages of expression relative to those of the rs6314 ancestral (C) allele. Thus, rs6314 genotype was used as the predictor in analyses of variance (ANOVA) with either mRNA or protein expression as the dependent variables.

**Prefrontal Function, Prefrontal Behavior, and Response to Olanzapine in Humans**

We collected data from 310 healthy individuals and 61 patients with schizophrenia and evaluated the participants with the Structured Clinical Interview for DSM-IV\(^{53}\) to exclude any psychiatric disorder or to confirm the diagnosis of schizophrenia. All individuals were white and were from the region of Puglia, Italy. Exclusion criteria were a history of significant drug or alcohol abuse, active drug use in the past year, head trauma with loss of consciousness, and any significant medical condition. All participants underwent 1 or more of the procedures described here and were genotyped for HTR2A rs6314.

**Genotyping**

The DNA was extracted from whole blood using standard procedures. Determination of HTR2A rs6314 genotype was conducted using the 5′ exonuclease TaqMan assay (Supplement [eMethods]). Genotype groups in healthy individuals and patients with schizophrenia displayed Hardy-Weinberg equilibrium (P > .20). Minor allele frequency was 0.09 in healthy individuals and 0.11 in patients. Given the low number of TT participants, we collapsed homozygous (when present) and heterozygous individuals within one group for further analyses in the procedures described below.

**Prefrontal Function During Cognition in Healthy Individuals**

One hundred ninety-seven healthy individuals (166 CC and 31 T carriers [29 CT and 2 TT]) underwent functional magnetic resonance imaging (fMRI) while performing the N-back working memory task. Furthermore, 92 of these participants (77 CC and 15 CT) were scanned while performing the variable attentional control (VAC) task, a paradigm eliciting attentional control processing\(^{54-56}\) (Table 1). Genotype groups were matched in terms of age, sex, IQ, and handedness (all P > .11).

**fMRI Tasks**

Working memory was assessed with the N-back task as described in earlier reports.\(^{57}\) Briefly, N-back refers to how far back in the sequence of stimuli the participant was able to recall (Supplement [eMethods]). For the fMRI setting, we used a simple block design in which each block consisted of 8 alternating 0-back and rest (participants were instructed to fixate on the diamond on the screen) conditions (each lasting 30
Table 2. Demographics of Patients With Schizophrenia

<table>
<thead>
<tr>
<th>No. of Patients</th>
<th>No. of Females</th>
<th>Age, y</th>
<th>Premorbid IQ WRAT</th>
<th>Length of Illness, mo</th>
<th>Mean Olanzapine Dose, mg/d</th>
<th>PANSS Scores at Baseline</th>
<th>Drug-Free Period, mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>61 (21 Drug naive, 30 drug free)*</td>
<td>15</td>
<td>28.5 (7.7)</td>
<td>102.1 (7.2)</td>
<td>65.3 (52.3)</td>
<td>21.1 (7.9)</td>
<td>Total: 103.3 (21.2) Positive: 25.5 (21.2) Negative: 25.8 (10.1) General psychopathology: 52.0 (12.1)</td>
<td>11.8 (19.5)</td>
</tr>
</tbody>
</table>

Abbreviations: PANSS, Positive and Negative Syndrome Scale; WRAT, Wide Range Achievement Test.

*Drug-free period is relative to the 30 patients with schizophrenia who were previously treated with antipsychotic drugs.

seconds). Similar blocks were used for the 1-back or 2-back alternating with the 0-back condition. Each task combination was obtained in 4 minutes and 8 seconds. Presentation of the runs was counterbalanced.

The VAC task was used to elicit increasing demands of attentional control. This event-related design task was identical to that published in previous studies54-56 and allows investigation of brain activity during 3 levels of attentional control (low, intermediate, and high), which were obtained manipulating both the relative directions of arrows with different sizes and the related cue words (Supplement [eMethods]).

For both tasks, stimuli were presented via a back-projection system. Responses were recorded through a fiber optic response box allowing measurement of accuracy and reaction time. Analysis of variance was used as appropriate to investigate genotype effects on behavioral data.

fMRI Data Acquisition and Analysis | The fMRI data were acquired with a 3.0-T scanner (GE Healthcare) (Supplement [eMethods]), and image analysis was completed using Statistical Parametric Mapping 5 (SPM5) software.58 After pre-processing (Supplement [eMethods]), the individual contrast images relative to the N-back (0-back vs rest, 1- or 2- vs 0-back) and the VAC (low, intermediate, and high levels of attentional control vs baseline) tasks were used in second-level random-effects models to determine task-specific regional responses at the group level. In particular, ANCOVA was performed on these contrasts, with genotype as a predictor and working memory or attentional control loads as repeated-measures factors. Residual movement was modeled as a regressor of no interest. These analyses were constrained by a mask obtained by combining group activation maps of both genotype groups of participants. We used a statistical threshold of $P < .05$, minimum cluster size ($k$) = 5, and familywise error corrected within volumes of interest, ie, the WFU_PickAtlas59 Brodmann areas (BAs), in which significant clusters were located (BA 46 for the N-back task, BA 9 and BA 46 for the VAC task).

Cognitive Behavioral Performance in Healthy Participants

To investigate the association between HTR2A rs6314 and behavioral correlates of prefrontal cognition, 254 healthy individuals (211 CC and 43 T carriers [39 CT and 4 TTT]) performed the N-back task, 187 (154 CC and 33 T carriers [29 CT and 4 TTT]) performed the Trail Making Test,60 and 212 (178 CC and 34 T carriers [30 CT and 4 TTT]) performed the Wisconsin Card Sorting Test (WCST) (Table 1). All genotype groups were matched in terms of age, sex, IQ, and handedness (all $P > .10$).

Cognitive Tasks | In the present study, we used the 0-, 1-, and 2-back conditions of the N-back working memory task to investigate the interaction between HTR2A rs6314 genotype and working memory load. Performance data were recorded as the number of correct responses (accuracy) and reaction time. The Trail Making Test consists of 2 versions: Trail Making Test Parts A and B (Supplement [eMethods]). Both versions reflect top-down modulation of attention, visual scanning, visuospatial memory, and speed of processing. However, the Trail Making Test Part B is associated with greater cognitive demand. Trail Making Test Parts A and B scores are reported as the time (in seconds) required to complete the task. Therefore, faster processing is an index of better performance. Trail Making Test Part B − A scores have been proposed as specific indicators of executive control function.60 The WCST evaluates abstract thinking, planning, and ability to modify mental set as circumstances require (Supplement [eMethods]). Performance data were recorded as the numbers of completed categories and of perseverative errors.

Statistical Analysis | The ANOVA with rs6314 as the predictor and the chi-squared test were used to compare demographics; ANOVA was also used to investigate the association of rs6314 with behavioral performance.

Response to Treatment With Olanzapine in Patients With Schizophrenia

Subjects and Experimental Design | Sixty-one white patients with schizophrenia (48 CC and 13 CT), matched in age, sex, and premorbid IQ ($P > .10$), were studied (Table 2). Fifty-five patients had paranoid schizophrenia; 4, disorganized schizophrenia; and 2, undifferentiated schizophrenia. As per protocol, all patients had received no psychotropic medication, including benzodiazepines, antidepressants, and mood stabilizers, for at least 1 week or 1 month if they were receiving depot medication. Thirty-one individuals were drug naïve; 15 previously received first-generation antipsychotics; 5, second-generation antipsychotics; and 10, drugs from both classes. According to available clinical his-
tory information, none of the patients had previously received antidepressants. Other patients’ characteristics are specified in Table 2. All patients received olanzapine monotherapy for 8 weeks. Titration was allowed for the first 4 weeks, and the dose was then kept constant until week 8. Symptoms were assessed at study entry (day 0) and at day 56 (8 weeks) with the Positive and Negative Syndrome Scale (PANSS) by only one trained psychiatrist (G.C.), who was blind to genotype.

Statistical Analysis | The ANOVA and χ² tests were used to compare demographics and mean dose of olanzapine as a function of rs6314 genotype. For a qualitative analysis, PANSS scores (total, positive, negative, and general psychopathology symptoms scores) were entered into separate χ² analyses with rs6314 genotype as predictor; 30% improvement from baseline at 8 weeks was used as a cutoff to determine treatment response and nonresponse. Furthermore, the difference between PANSS scores at 8 weeks and baseline was entered into separate ANCOVs with rs6314 genotype as predictor. Because the genotype groups differed for olanzapine mean dose and because previous evidence has suggested that estrogens may be associated with response to treatment and with psychotic symptoms, this analysis was covaried for olanzapine mean dose and sex.

Results

Bioinformatic Modeling of rs6314 Variation and 5-HT2AR Expression

Our analyses predicted that HTR2A rs6314 variation destroys the binding site for SRp40, a protein expressed in neuronal tissues that binds exonic splicing enhancer cis-acting elements and promotes the efficient and/or accurate splicing of pre-mRNA. Because function and the presence of exonic splicing enhancers is partially redundant, destruction of one exonic splicing enhancer is not generally associated with severe effects. However, we also found competition between SRp40 and hnRNP A1, a strong exonic splicing silencer expressed in neuronal tissues. In particular, the steric hindrance associated with SRp40 binding could reduce or even prevent binding of hnRNP A1. Therefore, destruction of the SRp40 binding site could increase, or even allow, hnRNP A1 binding. This effect on SRp40 binding can produce splicing isoforms containing in-frame stop codons. These defective isoforms trigger mechanisms of RNA surveillance, allowing degradation of defective transcripts. As a result, these mechanisms potentially lead to a decrease in the amount of total mRNA. For further results, see the Supplement (eResults).

Association of HTR2A rs6314 With Postmortem and In Vitro 5-HT2AR Expression

Postmortem Study

Analysis of covariance indicated a trend for an effect of rs6314 genotype on mRNA expression in postmortem prefrontal cortex (F₁,₁₀₆ = 3.8; P = .06), with T-carrier individuals having reduced mean expression values relative to CC (Figure 1). Rs6311 and rs6313 were not associated with prefrontal mRNA expression (Supplement [eResults]).

5-HT2AR Overexpression in HeLa Cells

Analysis of variance revealed an effect of rs6314 on 5-HT2AR mRNA (F = 34.6; P = .004) and protein (F = 114.5; P < .001) expression in HeLa cells. Again, the T allele is associated with reduced expression values relative to the C allele for both variables (Figure 2).

Association Between HTR2A rs6314 and Prefrontal Function During Cognition in Healthy Individuals

There was no effect of genotype on N-back and VAC behavioral performance, thus allowing us to investigate the asso-

Figure 1. Serotonin (5-Hydroxytryptamine) Receptor 2a (5-HT2AR) Messenger RNA (mRNA) Prefrontal Expression

Figure 2. Serotonin (5-Hydroxytryptamine) Receptor 2a Expression in HeLa Cells
Association of rs6314 with brain responses during working memory and attentional control processing regardless of differences in behavior. Consistent with previous studies, ANOVA of N-back data with the SPM5 software package indicated a main effect of load in bilateral regions of the dorsolateral, ventrolateral, and medial prefrontal cortex. Furthermore, there was a main effect of genotype in a cluster in the left dorsolateral prefrontal cortex (BA 46: x = –51, y = 38, z = 12; k = 6; z = 3.1), where T carriers have greater activity relative to CC individuals (Figure 3A). No interaction between HTR2A rs6314 genotype and working memory load was found.

The VAC imaging data revealed a main effect of load in several regions of prefrontal cortex, including dorsolateral, ventrolateral, and medial prefrontal areas, as well as in cingulate cortex, consistent with previous studies using this task. Furthermore, there was an effect of HTR2A rs6314 genotype on a right dorsolateral prefrontal cluster, which peaked in BA 9 extending to BA 46 (x = 45, y = 26, z = 26; k = 67; z = 4.3). Again, CT individuals have greater activity relative to CC individuals (Figure 3B). No interaction between load and genotype was found.

Association between rs6314 and higher-order cognitive behavior in healthy individuals

N-Back
Repeated-measures ANOVA of N-back accuracy data indicated a main effect of load (F_{2.504} = 97.9; P < .001), no effect of HTR2A rs6314 genotype (F_{1.252} = 1.5; P > .20), and an interaction between load and genotype (F_{2.504} = 3.1; P = .04). Fisher test post hoc analysis revealed reduced accuracy at 2-back for T carriers relative to CC genotype individuals (P = .007) (Figure 4). No significant genotype effects or interactions were found on reaction time data (all P > .11).

Figure 3. rs6314 and Prefrontal Activity During Cognition

Figure 4. rs6314 and Accuracy During Working Memory

Reduced accuracy in T carriers compared with CC-genotype individuals at the 2-back working memory task (P = .007). Error bars indicate confidence intervals.
Functional Genetic Variation in HTR2A

Original Investigation  Research

Figure 5. rs6314 and Performance on Trail Making Test

![Figure 5](image)

Statistically significantly reduced speed of processing (longer time in seconds to perform the task) in healthy individuals with the CT genotype relative to those with the CC genotype in Trail Making Test Part A and Trail Making Test Parts B − A (B) (P = .04). Data are given as mean (bullets) and 95% CI (error bars).

Trail Making Test
Analysis of variance revealed no main effect of HTR2A rs6314 genotype on Trail Making Test Part A performance (F₁,185 = 0.5; P > .50). On the other hand, T carriers had reduced performance at the Trail Making Test Part B (longer time to complete the task, F₁,185 = 4.2; P = .04) and B − A scores (F₁,185 = 4.1; P = .04) (Figure 5).

Wisconsin Card Sorting Test
Analysis of variance was also performed on performance of WCST. Results did not show any effect of HTR2A rs6314 genotype on WCST number of completed categories (F₁,210 = 0.1; P > .72) or perseverative errors (F₁,210 = 0.3; P > .55).

Association Between HTR2A rs6314 and Response to Treatment With Olanzapine in Patients With Schizophrenia

There was no significant difference between genotype groups in terms of PANSS total, positive, negative, and general psychopathology scores at day 0 (all P > .05). On the other hand, CT patients received a greater mean dose of olanzapine during the 2 months of treatment (F₁,159 = 7.5; P = .008). A χ² test on the proportion of patients who qualified as responders based on at least 30% improvement of PANSS negative symptoms scores from baseline after 8 weeks of treatment revealed a qualitative effect of HTR2A rs6314 genotype, with a greater proportion of CC participants responding (CC: 25 of 48; CT: 2 of 13; χ² = 6.2; P = .01) (Figure 6). This analysis did not demonstrate any other genotype effect on total PANSS scores or on other subscales (all χ² < 1.1; all P > .32). Furthermore, ANCOVA (covarying for mean olanzapine dose and sex) on the difference between PANSS scores at 8 weeks and baseline indicated an effect of genotype on negative symptoms scores (F₁,57 = 5.8; P = .02), with greater improvement for CC individuals. This analysis did not demonstrate any other significant effect on total PANSS scores or other subscales (all F < 1.5; all P > .20). The effect size of genotype as measured with Cohen d was 0.6. See the Supplement for an analysis of potential outliers.

Discussion

The results of the present study converge in suggesting an association of HTR2A rs6314 genotype with molecular, brain imaging, and behavioral phenotypes linked with genetic risk for schizophrenia. In particular, the possible molecular effects of HTR2A rs6314 were predicted by computational modeling, which suggest effects of this SNP on splicing and HTR2A expression. Confirmatory investigation of bioinformatic predictions revealed that presence of the T allele of rs6314 predicts a trend for reduced expression of postmortem prefrontal mRNA. Consistent with these results, presence of the T allele is also associated with reduced 5-HT2AR mRNA and protein expression in HeLa cells. Functional brain imaging and behavioral results also indicate that healthy individuals carrying the T allele have exaggerated prefrontal responses during working memory and attentional control tasks as well as impaired cognitive behavioral performance on tests tapping into prefrontal cognition, which represent endophenotypes known to be related to genetic risk for schizophrenia.69 Finally, patients with this brain disorder who carry the T allele have relatively reduced improvement of negative symptoms scores after 8 weeks of olanzapine treatment. Of course, these results do not explain the whole of variation in the phenotypes investigated here, and they should be interpreted with consideration of the complexity of genetic and environmental loads acting on the function of the brain.

The present data are consistent with previous studies implicating 5-HT2AR signaling during working memory7 and in the mechanism of action of newer antipsychotic medications.14,70 In particular, our findings indicating an association of T allele presence with lower 5-HT2AR mRNA and protein expression in HeLa cells and in postmortem prefrontal cortex suggest that rs6314 contributes to genetic variation of prefrontal 5-HT2AR signaling.

These molecular changes may affect phenotypes at greater biological distance from gene effects. In fact, earlier studies
had indicated that presence of the T allele is linked with reduced temporal lobe volume,35 hippocampal activity,34 and reduced behavioral performance24,35 during episodic memory. In our fMRI samples, healthy participants carrying the T allele had greater lateral prefrontal activity during the N-back and VAC tasks despite no significant difference in behavioral performance. These effects were also present in a subsample of individuals after covariation for prefrontal gray matter volume. As in previous studies,55,71,72 a plausible interpretation of these results is that T-allele carriers need more prefrontal resources to perform these tasks as well as CC individuals. This “inefficient” prefrontal response is a well-known endophenotype linked with genetic risk for schizophrenia and has been repeatedly associated with the effect of risk alleles in healthy people55 and in patients with schizophrenia.57 Another more speculative interpretation is that differential expression of 5-HT2AR as a function of rs6314 modifies mechanisms of synaptic plasticity associated with 5HT2AR signaling73,74 and its interaction with the glutamate system,75 which is then reflected in differential functional responses during cognition.

The behavioral results of the present study are internally consistent with the imaging associations. We found that healthy individuals carrying the T allele had reduced accuracy on the 2-back task and reduced speed of processing on the Trail Making Test Part B and Part B − A tests. Both of these tests tap into prefrontal cognitive domains associated with genetic risk for schizophrenia,69 including working memory, executive function, and executive control of attention.60,76 Previous studies77 in nonhuman primates and healthy volunteers have suggested that pharmacologic stimulation or antagonism of 5-HT2AR impairs spatial working memory and deteriorates sustained attention, respectively. In the present study, we found that having the C allele is associated with greater expression of 5-HT2AR and is also linked to better behavioral performance. Therefore, our results are consistent and may add further evidence to the literature suggesting that greater 5-HT2AR signaling may be beneficial for cognitive processing. On the other hand, we did not find an association of HTR2A rs6314 with WCST performance, suggesting that this test is associated with lower sensitivity to the subtle genetic effects investigated herein. In particular, a possible interpretation of this result is that this task does not provide a robust enough cognitive load on prefrontal function such that in the context of serotonin signaling, subtle genetic effects are not captured by the simple WCST indices used in this study. This possibility is also supported by the N-back results, which demonstrated a load × genotype interaction and indicated that only greater cognitive load elicits genotype effects.

Consistent with the direction of the effects of the T allele on the phenotypes investigated in the present study, we also found that the rs6314 genotype is associated with both qualitative and quantitative indices of response to antipsychotic treatment in patients with schizophrenia. In particular, our results indicate that individuals with schizophrenia who are carrying the T allele have attenuated improvement of PANSS negative symptoms scores after 8 weeks of olanzapine treatment. These data appear to be consistent with previous findings55,24,36 showing that presence of the T allele of rs6314 is associated with poorer response to treatment with clozapine. The specific effect we found on negative symptoms scores is consistent with the hypothesis that treatment with second-generation antipsychotics may provide some benefit on negative symptoms in patients with schizophrenia via 5-HT2AR signaling.78

Some limitations of these pharmacogenetic results must be acknowledged. First, the sample size is certainly not large. However, this was a 2-month longitudinal study in acutely ill patients; the primary objective of the study was to investigate the association of genetic variation with response to antipsychotic treatment and not with drug effectiveness per se. Furthermore, patients were recruited, treated, and evaluated in a single center, thus limiting potential confounding effects associated with intrinsic variability in enrollment and evaluation of multicenter studies. Finally, the effect size of genotype as measured with the Cohen d is 0.6, which indicates a medium effect size. Another limitation is that almost half of the patients with schizophrenia included in this study had been treated in the past with either first- or second-generation antipsychotics, or with both (n = 30), before entering the study. Previous evidence has indicated paradoxical downregulation of 5-HT2AR associated with previous treatment with both typical and atypical antipsychotics.11,79 This effect correlates positively with an affinity of antipsychotics for 5-HT2AR,80 even if not all studies agree.81 However, we believe it is important that half of our present sample had been drug-naïve and the other participants who had been treated had received no treatment for an average of 11.8 months. Therefore, we believe that there was modest, if any, potential for previous antipsychotic treatment to represent a confounding factor on the present results. Finally, the olanzapine dose in the pharmacogenetic study was different between genotype groups, with a greater mean dose in CT participants. These individuals had attenuated improvement in negative symptoms relative to CC patients with schizophrenia. On the other hand, previous studies82 have suggested that atypical antipsychotics actually improve negative symptoms, if anything. Furthermore, to our knowledge, there have been no studies suggesting that larger doses of second-generation antipsychotics are associated with greater levels of negative symptoms in patients with schizophrenia. Finally, our ANCOVA for olanzapine dose indicating a significant effect of rs6314 on negative symptoms temper this limitation.

In conclusion, our data suggest the HTR2A rs6314 genotype as being functionally implicated in phenotypes relevant to schizophrenia and located at different points along the pathway going from molecular to behavioral and symptomatology, genetic effects.
Functional Genetic Variation in HTR2A

Biochemistry and Molecular Biology Ernesto Quagliariello, Aldo Moro University, Bari, Italy (De Virgilio, Gadaleta); Istituto di Neurocigna and Cura a Carattere Scientifico “Casa Sollievo della Sofferenza,” San Giovanni Rotondo, Italy (Sibalinbi, Di Giorgio, Popolizio); Psychiatric Unit, Department of Mental Health, United Hospitals of Ancona, Polytechnic University of Marche, Italy (Lo Bianco, Bellantuono); Department of Biochemistry, Biology and Genetics, Polytechnic University of Marche, Ancona, Italy (Piva); Department of Neuroscience and Sense Organs, Aldo Moro University, Bari, Italy (Todarello); Clinical Brain Disorders Branch, Genes, Cognition and Psychosis Program, National Institute of Mental Health, National Institutes of Health, Bethesda, Maryland (Kleinman); Lieber Institute for Brain Development, Johns Hopkins Medical Campus, Baltimore, Maryland (Weinberger)

Author Contributions: All authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Blasi, Taurisano, Ursini, Sibalinbi, Romano, Rampino, Caforio, Todarello, Todarello, Gadaleta, Weinberger, Bertolino.

Acquisition of data: De Virgilio, Papazacharias, Taurisano, Gelao, Fazio, Ursini, Andriola, Maselli, Romano, Rampino, Di Giorgio, Lo Bianco, Caforio, Popolizio, Bellantuono, Kleinman.

Analysis and interpretation of data: Blasi, De Virgilio, Papazacharias, Taurisano, Gelao, Fazio, Ursini, Maselli, Romano, Rampino, Di Giorgio, Caforio, Piva, Weinberger, Bertolino.

Drafting of the manuscript: Blasi, Papazacharias, Gelao, Fazio, Ursini, Piva, Weinberger, Bertolino.

Critical revision of the manuscript for important intellectual content: Blasi, De Virgilio, Taurisano, Ursini, Sibalinbi, Andriola, Maselli, Romano, Rampino, Di Giorgio, Lo Bianco, Caforio, Piva, Popolizio, Bellantuono, Todarello, Kleinman, Gadaleta, Weinberger.


Administrative, technical, and material support: Papazacharias, Gelao, Fazio, Ursini, Sibalinbi, Andriola, Maselli, Romano, Rampino, Caforio, Kleinman, Weinberger, Bertolino.

Study supervision: Blasi, Ursini, Rampino, Caforio, Popolizio, Bellantuono, Todarello, Kleinman, Gadaleta, Weinberger.

Conflict of Interest Disclosures: None reported.

Funding/Support: This study was supported in part by a National Alliance for Research on Schizophrenia and Depression (now Brain and Behavior Research Foundation) Young Investigator Award to Dr Blasi.

Additional Contributions: Riccarda Lomuscio, BA, assisted in data acquisition. We thank all people who participated in this study.

REFERENCES


Functional Genetic Variation in HTR2A


