Altered Effect of Dopamine Transporter 3’UTR VNTR Genotype on Prefrontal and Striatal Function in Schizophrenia

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Context: The dopamine transporter plays a key role in the regulation of central dopaminergic transmission, which modulates cognitive processing. Disrupted dopamine function and impaired executive processing are robust features of schizophrenia.

Objective: To examine the effect of a polymorphism in the dopamine transporter gene (the variable number of tandem repeats in the 3’ untranslated region) on brain function during executive processing in healthy volunteers and patients with schizophrenia. We hypothesized that this variation would have a different effect on prefrontal and striatal activation in schizophrenia, reflecting altered dopamine function.

Design: Case-control study.

Setting: Psychiatric research center.

Participants: Eighty-five subjects, comprising 44 healthy volunteers (18 who were 9-repeat carriers and 26 who were 10-repeat homozygotes) and 41 patients with DSM-IV schizophrenia (18 who were 9-repeat carriers and 23 who were 10-repeat homozygotes).

Main Outcome Measures: Regional brain activation during word generation relative to repetition in an overt verbal fluency task measured by functional magnetic resonance imaging. Main effects of genotype and diagnosis on activation and their interaction were estimated with analysis of variance in SPM5.

Results: Irrespective of diagnosis, the 10-repeat allele was associated with greater activation than the 9-repeat allele in the left anterior insula and right caudate nucleus. Trends for the same effect in the right insula and for greater deactivation in the rostral anterior cingulate cortex were also detected. There were diagnosis × genotype interactions in the left middle frontal gyrus and left nucleus accumbens, where the 9-repeat allele was associated with greater activation than the 10-repeat allele in patients but not controls.

Conclusions: Insular, cingulate, and striatal function during an executive task is normally modulated by variation in the dopamine transporter gene. Its effect on activation in the dorsolateral prefrontal cortex and ventral striatum is altered in patients with schizophrenia. This may reflect altered dopamine function in these regions in schizophrenia.

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The dopamine transporter (DAT; SLC6A3 [GenBank DQ307031]) plays a key role in the regulation of central dopaminergic transmission by mediating dopamine reuptake from the synaptic cleft into the presynaptic terminal. In the mammalian brain, DAT messenger RNA is localized in cell bodies of dopaminergic neurons. Its expression is highest in synapses in the striatum, substantia nigra, and ventral tegmentum, although it is also abundant in the thalamus and in the insular, motor, posterior parietal, and posterior cingulate cortices. It is expressed in lower levels in the prefrontal, anterior cingulate, primary sensory, and occipital cortices, especially within intrasynaptic as opposed to extrasynaptic extracellular space, and the rate of dopamine uptake in these areas is relatively slow. In these brain regions with low levels of DAT, intracellular degradation by catechol O-methyltransferase (COMT) and uptake by nonspecific transporters such as the norepinephrine transporter may play a relatively greater role in the regulation of local dopamine availability. In these regions, DAT is mainly extrasynaptic and may primarily regulate dopamine volume transmission, the spillover of dopamine into the extrasynaptic space.

The human DAT gene has a polymorphic 40–base pair (bp) variable number of tandem repeats (VNTR) in the 3’ untranslated region (DAT 3’UTR VNTR). This yields several alleles ranging from 3 to 11...
copies of the 40-bp repeats, with 9 and 10 being the most common.13 Although this polymorphism does not affect protein structure,13 it may influence transcription. Four independent studies15-18 have found the 10-repeat allele to be associated with higher levels of DAT expression, although there is 1 report of lower expression19 and 1 of no association.20 This DAT 3′UTR VNTR has been previously associated with Parkinson disease,21 alcoholism,22 attention-deficit/hyperactivity disorder,23,24 and Tourette syndrome.25,26

Previous functional neuroimaging studies of memory paradigms in healthy subjects have reported an effect of DAT 3′UTR VNTR on prefrontal activation, and an additive interaction between this effect and that of a functional polymorphism for COMT (Val158Met) in prefrontal cortex.11,27-29 Nonlinear interactions between the effects of the DAT and COMT polymorphisms on hippocampal28 and striatal29 activation have also been reported in the context of reward and memory tasks, respectively.

Schizophrenia is associated with alterations in the dopaminergic input to the cerebral cortex30-34 and the striatum.35-38 The same variation in DAT activity may thus have different effects on brain function in patients with schizophrenia and healthy volunteers. The aims of the present study were to examine the influence of DAT genotype on regional brain function during a verbal fluency task and to assess the extent to which this is altered in schizophrenia. We used functional magnetic resonance imaging to study samples of healthy volunteers and patients large enough to yield subgroups of sufficient size to detect effects of the DAT 3′UTR VNTR genotype on activation. Subjects underwent imaging while they performed a phonologic verbal fluency task, which normally engages the prefrontal, insular, and cingulate cortex; the striatum; and the thalamus39-42 and is associated with impaired performance43,44 and altered prefrontal activation45,47,50-53 in patients with schizophrenia.44,53 Because they express high levels of DAT4-7 and are also engaged during verbal fluency tasks,45,46 we predicted that variation in DAT 3′UTR VNTR would modulate activation in the striatum, thalamus, and insula. Our second hypothesis was that variation in the DAT 3′UTR VNTR genotype would have a different effect in patients compared with controls in 2 areas where there is good evidence that dopamine function is perturbed in schizophrenia: the striatum35-38 and the dorsolateral prefrontal cortex.30-34 Although the prefrontal cortex does not express high levels of DAT, it is connected to the striatum via the corticothalamosstriatal loop.11,27 and variation in the DAT 3′UTR VNTR genotype influences activation in the prefrontal cortex as well as the striatum.27,28 In addition, the prefrontal cortex is a robust site of altered activation in schizophrenia during verbal fluency and other cognitive tasks.30-34

## METHODS

### SUBJECTS

A total of 85 subjects participated. All were native English speakers and gave written informed consent in accordance with protocols approved by the local research ethics commit-

tee. Patients who had a diagnosis of schizophrenia from their clinical team and met DSM-IV criteria for schizophrenia (n=41) were recruited from the South London and Maudsley National Health Service Trust. The DSM-IV diagnosis was made by an experienced psychiatrist (including M.M.P.) using a structured diagnostic interview (Schedules for Clinical Assessment in Neuropsychiatry26 and Schedule for Affective Disorders and Schizophrenia-21). When interview data were missing or incomplete, the diagnosis was determined by means of the Operational Criteria Checklist.56 The Schedules for the Assessment of Positive and Negative Symptoms were used to measure psychopathologic symptoms at the time of imaging. All patients were in a stable clinical state and had previously been treated with antipsychotic medication; however, 5 were not taking antipsychotic medication at the time of imaging. The mean duration of antipsychotic treatment was 12 years. Healthy volunteers (n=44) had no history of mental illness and no first-degree relatives with a psychotic disorder, as assessed by the Family Interview for Genetic Studies. Subjects who met DSM-IV criteria for a substance misuse disorder were excluded.

All subjects were genotyped for DAT at 3′UTR VNTR. This yielded 18 subjects who were 9-repeat carriers (2 of whom were homozygotes) and 26 subjects who were 10-repeat homozygotes in the healthy volunteer group, and 18 who were 9-repeat carriers (2 homozygotes) and 23 who were 10-repeat homozygotes in the patient group. Fisher exact tests or χ² tests (for categorical variables) and analysis of variance tests (for numeric variables) were calculated with SPSS version 15.0 (SPSS Inc, Chicago, Illinois) to detect demographic differences in relation to genotype, diagnosis, and their interaction. There were no significant differences (P>.05) between the patient and control groups in age, ethnicity, or handedness, but patients had a lower mean IQ, fewer years of education, and a higher proportion of males (Table 1). Within the total sample and within each diagnostic group, there were no significant differences (P>.05) between genotype subgroups in any of the demographic variables. No demographic variables showed a significant genotype × diagnosis interaction, except for sex. Within the patient group, the genotype subgroups did not differ significantly (P>.05) in total scores on the Schedule for the Assessment of Positive Symptoms (mean [SD], 6.6 [6.6]) or Schedule for the Assessment of Negative Symptoms (mean [SD], 7.8 [5.1]) scores, nor in the duration (mean [SD], 12.2 [9.5] years), dose (chlorpromazine equivalents; mean [SD], 598.8 [452.3]), or type (first or second generation) of antipsychotic medication.

### GENOTYPING

DNA was extracted from blood or cheek swabs by standard methods.44 Amplification of the 3′UTR VNTR region was performed by a polymerase chain reaction using the forward primer 5′TGGCAGGCACTCTGAGAG3′ (melting temperature, 60.8°C) and the reverse primer 5′GCCATTGGAGATGGGC3′ (melting temperature, 62.3°C). Its products were then separated under UV light after electrophoresis on a 3.5% agarose gel containing ethidium bromide. Genotyping was successful in 88 subjects (98%). Genotype frequencies were similar to frequencies described in the literature. The patient group was in Hardy-Weinberg equilibrium (P>.99; calculated with GENEPOP®), but the control group showed a minor deviation (P=.03), which was apparently due to the presence of a rare homozygous genotype, a 6/6 repeat, in a single individual. Three subjects carrying genotypes with alleles other than the 9-repeat or the 10-repeat allele (as well as 2 subjects for whom genotype calling was unreliable) were not included in the 85-subject sample further analyzed, to reduce allelic heterogeneity.
Main Effect of DAT

<table>
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<tr>
<th>Diagnosis</th>
<th>Controls (n=44)</th>
<th>Patients (n=41)</th>
<th>9-Repeat Carriers (n=36)</th>
<th>10/10-Repeat Homozygotes (n=49)</th>
<th>9-Repeat Carriers (n=18)</th>
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<td>32.9 (10.6)</td>
<td>33.3 (10.9)</td>
<td>36.7 (11.9)</td>
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<td>96.8 (16.4)b</td>
<td>108.8 (14.5)</td>
<td>105.8 (19.4)</td>
<td>116.4 (11.2)</td>
<td>119.2 (9.3)</td>
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<td>93.2 (18.0)</td>
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<tr>
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<td>13.6 (2.6)c</td>
<td>14.4 (2.6)</td>
<td>14.5 (3.1)</td>
<td>15.1 (2.5)</td>
<td>15.2 (3.3)</td>
<td>13.5 (2.6)</td>
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<td>35/1</td>
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<td>24/2</td>
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<td>23/13</td>
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<td>15/2/0/1</td>
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</tr>
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</table>

Abbreviations: CPZ, chlorpromazine hydrochloride; DAT, dopamine transporter; L, left; NA, not applicable; R, right; SANS, Schedule for the Assessment of Negative Symptoms; SAPS, Schedule for the Assessment of Positive Symptoms; UTR, untranslated region; VF, verbal fluency; VNTR, variable number of tandem repeats.

a IQ was assessed by means of the Wechsler Adult Intelligence Scale III (WAIS-III).60 Wechsler Adult Intelligence Scale–Revised (WAIS-R).60 Wechsler Abbreviated Scale of Intelligence–Full-Scale IQ (WASI-FSIQ-4),61 or the Quick Test.62 The WAIS-III correlates highly with both the WAIS-R (93.9%)59 and the WASI-FSIQ-4 (92%).46 The Quick Test has also been shown to yield results comparable to the WAIS (mean [SD] scores: Quick Test, 78 [7], and the WAIS, 83 [6]).

b Significant differences (at P<.05) in demographic features: F=41.02, P<.001.

c Significant differences (at P<.05) in demographic features: F=5.06, P=.03.

d Significant differences (at P<.05) in demographic features: χ²=12.24, P=.001.

e Significant differences (at P<.05) in demographic features: χ²=5.56, P=.01 (DAT × diagnosis).

VERBAL FLUENCY TASK

During a “generation” condition, subjects were visually presented with a series of letters and were required to overtly articulate a word beginning with each letter. This was contrasted with a “repetition” condition in which subjects were presented with the word rest and were required to say “rest” out loud. A blocked design was used, with letter and rest cues presented in blocks of 7 events. The demands of the task were manipulated by presenting 2 different sets of letter cues, termed easy and hard.45 These had previously been shown to be associated with a significant difference in behavioral performance in healthy volunteers.46 The easy condition involved the presentation of letters that are normally associated with relatively large numbers of correct responses and relatively few errors (eg, T, B, S), whereas the hard condition involved letters associated with the generation of fewer correct words and relatively more errors (eg, N, E, G). Five blocks of rest trials alternated with 5 blocks of easy letters or hard letters, resulting in a total of 70 generation and 70 repetition trials. Verbal responses were recorded, permitting the identification of “incorrect” trials in which the subject did not generate any response or generated repetitions, derivatives, or grammatical variations of a previous word. Further details are provided in the eMethods section.

BEHAVIORAL ANALYSIS

The effect of task load, genotype, diagnosis, and their interaction on the level of accuracy of verbal responses (measured by the number of incorrect responses during imaging) was assessed by means of a multivariate 2 × 2 × 2 analysis of variance, with diagnosis and genotype as between-subject factors and task load as a within-subject factor.

SYSTEM

T2*-weighted gradient-echo single-shot echo-planar images were acquired on a 1.5-T, neuro-optimized imaging system (IGE LX System; General Electric, Milwaukee, Wisconsin) at the Maudsley Hospital, London, England. Twelve noncontiguous axial planes (7-mm thickness, 1-mm section skip, 3.75 × 3.75-mm voxel size in plane, and 64 × 64-mm matrix size in plane) parallel to the anterior commissure–posterior commissure line were collected during 1100 milliseconds in a “clustered” acquisition (echo time, 40 milliseconds; flip angle, 70°), which permitted articulatory responses to be made when images were not being acquired, minimizing the effects of head movement on the blood oxygen level–dependent signal.46 Immediately after each acquisition, a letter was presented (remaining visible for 730 milliseconds; height, 7 cm; subtending a 0.4° field of view), and a single overt verbal response was made during the silent portion (duration, 2900 milliseconds) of each repetition (repetition time, 4000 milliseconds), with an image acquired during 1100 milliseconds. Head movement was minimized by a forehead strap. To ensure that subjects heard their responses clearly, their speech was amplified by a computer sound card and then relayed back through an acoustic magnetic resonance imaging sound system and noise-insulated headphones. Further details are provided in the eMethods section.
IMAGE ANALYSIS

Analysis was performed with SPM5 software (http://www.fil.ion.ucl.ac.uk/spm), running under MATLAB 6.5 (MathWorks Inc, Sherborn, Mass). To minimize movement-related artifacts, all volumes from each subject were realigned and unwarped (by means of the first as reference resliced with sinc interpolation), normalized to a standard MINI-305 template, and spatially smoothed with an 8-mm full-width at half-maximum isotropic gaussian kernel. First, the statistical analysis of regional responses was performed in a subject-specific fashion by convolving each onset time with a synthetic hemodynamic response function. To minimize performance confounds, we modeled correct and incorrect trials separately by using an event-related model, yielding 4 experimental conditions: (1) easy generation, (2) hard generation, (3) repetition, and (4) incorrect responses. The last was excluded from the group analysis to control for effects of group differences in task performance. Correct responses among the generation events (35 events in the hard version and 35 in the easy version) were contrasted with 70 repetition events. To remove low-frequency drifts, data were high-pass filtered by using a set of discrete cosine basis functions with a cutoff period of 128 seconds. Parameter estimates were calculated for all brain voxels by means of the general linear model, and contrast images for “easy generation > repetition” and “hard generation > repetition” were computed in a subject-specific fashion. Second, the subject-specific contrast images were entered into a full-factorial 2 × 2 × 2 analysis of variance, with task load as a repeated measurement, to permit inferences at the population level. This allowed us to characterize the impact of the experimental task on brain activation in easy and hard conditions separately within each of the 4 experimental groups (9-repeat carrier controls, 10/10-repeat controls, 9-repeat carrier patients, and 10/10-repeat patients) and test for the main effects of diagnostic group and genotype and their interaction. We modeled task load to minimize error variance but for the main effects of diagnostic group and genotype and their interaction (especially during the hard version) in the precuneus and rostral anterior cingulate gyrus.

RESULTS

Expectedly, there was a significant (P < .05) main effect of task demand on the number of incorrect responses (F = 50.36; P < .001), as there was for diagnosis, with patients making more errors than controls (F = 8.72; P = .004). The main effect of genotype was not significant (F = 1.10; P = .30). There was no significant interaction between task demand, diagnosis, and genotype (F = 1.18; P = .28). However, there was a trend for a diagnosis × genotype interaction (F = 3.56; P = .06), irrespective of task load, reflecting poorer performance in 10/10-repeat than 9-repeat carrier patients but the converse in healthy volunteers, especially during the hard version (Table 1).

NEUROIMAGING DATA

Main Effect of Task

In both diagnostic groups, word generation (irrespective of task difficulty or genotype) was associated with activation in a distributed network that included, bilaterally, the inferior frontal, insular, and dorsal anterior cingulate cortex; the caudate and the thalamus; and the left middle frontal, superior temporal, and inferior parietal cortex (FWE P < .05) (Figure 1). Conversely, repetition was associated with greater engagement of the rostral anterior cingulate gyrus, precuneus, and occipital cortex.

Main Effect of Diagnostic Group

Activation in the left inferior frontal gyrus (−44, 18, 30; Z = 3.4), anterior insula (−34, 14, 8; Z = 3.3), and frontal operculum (−36, 14, 12; Z = 3.6) was greater in patients than in healthy volunteers (P < .001, uncorrected). There
were no areas more activated in healthy volunteers than in patients, and there were no between-group differences in deactivation.

**Main Effect of DAT 3’UTR VNTR Genotype**

Within the regions of interest, the 10/10-repeat group showed greater activation than the 9-repeat carrier group in the left insula ($\eta^2=5.6\%$) and in the right caudate nucleus ($\eta^2=5.4\%$) (right anterior insula $P=.02$; left caudate nucleus $P=.03$, SVC) (Figure 2 and Table 2), irrespective of diagnosis. Inspection of the parameter estimates (plotted in Figure 2A for the left insula) showed that these main effects were driven by relatively strong effects of genotype in the patient group. Also, the focus of maximal significance in the left insula ($−30, 6, 16$) was close to the focus of the cluster where patients showed greater activation than controls ($−34, 14, 8$, as noted earlier). None of the regions of interest showed greater activation in 9-repeat carriers than in 10-repeat homozygotes.

Whole-brain analysis indicated that, irrespective of diagnosis, subjects in the 10/10-repeat group showed greater activation than those in the 9-repeat carrier group ($P<.001$, uncorrected) in the left anterior insula ($−30, 6, 16$; $Z=3.5$; $\eta^2=5.6\%$) and in a right-sided cluster focused at $26, 4, 18$ ($Z=3.6$; $\eta^2=6.6\%$) that included the right caudate (reported for the foregoing region of interest analysis) and the adjacent part of the right insula.

The plot of the parameter estimates was similar to that of the left anterior insula (described in the previous paragraph; Figure 2A). There was also a trend ($P<.001$, uncorrected) for a main effect in the rostral part of the anterior cingulate gyrus bilaterally ($−2, 40, −2$; $Z=3.4$; and $2, 40, −2$; $Z=3.3$; $\eta^2=6.7$). Exploration of the parameter estimates (Figure 2B) showed that this reflected deactivation during word generation (ie, more activation during repetition than generation), which was more pronounced in the 10/10-repeat group than in the 9-repeat carrier group.

**Group × Genotype Interaction**

There was an interaction between the effects of diagnosis and genotype in the left middle frontal gyrus ($\eta^2=6.4\%$) and in the left nucleus accumbens ($\eta^2=4.8\%$) (left middle frontal gyrus $P=.05$; left nucleus accumbens FEW $P=.02$, SVC) (Figure 3 and Table 2). In the former region, there was no statistically significant difference in activation between the DAT 3’UTR VNTR genotypes in healthy volunteers, but in patients the 9-repeat allele was associated with significantly greater activation (left middle frontal gyrus $FWE P=.004$, SVC; $\eta^2=21.5\%$). In the left nucleus accumbens, healthy volunteers with the 10/10-repeat genotype showed more activation than 9-repeat carriers, whereas the opposite applied in the patients. No other brain regions showed a diagnosis × genotype interaction (at $P<.001$, uncorrected).

**Table 2**

<table>
<thead>
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<th>UT Z</th>
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</table>

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Effects of Potentially Confounding Factors on Activation

Linear regression analysis indicated that activation in the areas where there were significant effects of DAT (either irrespective of or dependent on diagnosis) was not related to the dose, type, or duration of antipsychotic treatment, even at a very liberal statistical threshold ($P = .5$, uncorrected). Analyses included sex as a covariate of no interest. Repeating these analyses after covarying independently for IQ, years of education, or ethnicity did not alter the location or the Z score of the reported results.

Our hypothesis that the DAT 3’UTR VNTR genotype would influence task-related activation was confirmed in the left insula and caudate nucleus. In both of these regions, the 10-repeat allele was associated with greater activation than the 9-repeat allele in both healthy and schizophrenic subjects. A whole-brain analysis also demonstrated a trend for the same effect of DAT in the part of the right insula homologous to that identified in the region-of-interest analysis. The insula, especially in the left hemisphere, plays a major role in verbal fluency and articulation.73-76 It is a major termination site of central dopaminergic projections from the brainstem.77 The extent to which the foregoing model of dopaminergic tuning of cortical efficiency is also applicable in the striatum is unclear.

There was a trend for the 10-repeat allele to be associated with greater deactivation during word generation compared to 9-repeat carriers, as noted in previous studies.78-80 The more marked response in individuals with the 10/10-repeat genotype may reflect an effect of lower dopamine activity on the efficiency of cingulate cortical function during the baseline condition, although the concept of the dopaminergic modulation of efficiency is derived from studies of prefrontal cortex during working memory tasks.78-80 The direction of the DAT 3’UTR VNTR’s effect on cingulate...
activation is the same as that of a previous report,\textsuperscript{11} although this was in a more dorsal part of the gyrus during a working memory task.

Consistent with our hypotheses about genotype \texttimes{} diagnosis interactions, there was an interaction in the left middle frontal gyrus. In this region, there was significantly greater activation in patients carrying the 9-repeat than the 10/10-repeat genotype but a similar response in the 2 genotype subgroups in healthy volunteers. The 9-repeat allele is associated with lower gene expression\textsuperscript{15-18} and hence weaker DAT activity and higher dopamine levels in the prefrontal cortex. A decrease in DAT activity associated with the 9-repeat allele may therefore increase synaptic dopamine levels and, thus, D\textsubscript{2} stimulation in the striatum, inhibiting the thalamus and decreasing its excitatory input to the prefrontal cortex, which is thought to be necessary for an optimal signal to noise ratio in the prefrontal cortex. In schizophrenia, increased striatal dopamine activity may be amplified in patients with the 9-repeat allele, further increasing local dopamine levels and inhibition of the thalamus, leading to a marked reduction in the signal to noise ratio in prefrontal pyramidal neurons.\textsuperscript{89} This could account for the increased prefrontal activation we detected in patients with the 9-repeat allele.

A similar interaction was evident in the left nucleus accumbens. In healthy volunteers, this region was more active during verbal repetition than generation in 9-repeat carriers, but there was no difference between the conditions in subjects with the 10/10-repeat genotype. The converse applied in the patient subgroups (Table 2). As discussed in relation to the interaction in the prefrontal cortex, in schizophrenia, increased dopamine activity in the striatum may alter the impact of variation in DAT genotype on local dopamine levels, producing the opposite effect on activation in patients compared with controls.

Because antipsychotics have an antagonistic effect on central dopamine receptors and because all of our patients were receiving antipsychotic drugs, the poten-
tially confounding effects of medication on our findings in patients with schizophrenia must be considered. Antipsychotics can modulate presynaptic dopamine uptake capacity\textsuperscript{90-92} and cortical activation\textsuperscript{93} and may also affect performance of verbal fluency\textsuperscript{94,95} and other cognitive tasks\textsuperscript{92,96-97}, although these findings have not always been replicated.\textsuperscript{98} Moreover, antipsychotic medication may reduce DAT activity via blockade of D\textsubscript{2} receptors.\textsuperscript{99} Therefore, it is possible that differences in the effect of genotype between patients and controls may have been related to effects of medication rather than an effect of schizophrenia. In view of these concerns, we examined the potential effect of dose, type, and duration of antipsychotic treatment on activation in regions where we found significant effects of genotype. There was no evidence that the effects of genotype we observed were related to effects of medication on activation in these regions. Nevertheless, the possibility that the differences in the effects of genotype in patients compared with controls were related to medication as opposed to schizophrenia cannot be excluded without repetition of the present study in medication-naive patients. However, recruiting and performing imaging in a large sample of this type would be logistically difficult.

The network of areas engaged by the verbal fluency task in the present study is consistent with that reported in several previous studies.\textsuperscript{44,96,97,101} Patients showed greater activation than healthy volunteers in the left middle frontal gyrus, frontal operculum, and anterior insula. Many previous comparisons of patients with schizophrenia and volunteers have found differences in left frontal activation during verbal fluency, although some have reported decreases and others, increases.\textsuperscript{44,97,30-53,100-103} This inconsistency may partly reflect differences in sample sizes and the degree to which the effects of impaired task performance in schizophrenia have been controlled for. The present sample was comparatively large, and the effects of differential task performance were minimized by using a paced paradigm, online monitoring of behavioral performance, and restriction of the analysis to images associated with correct responses. Greater prefrontal activation in schizophrenia during cognitive tasks after controlling for differential performance may reflect impaired prefrontal cortical efficiency.\textsuperscript{104}

The effect of the DAT 3’UTR VNTR genotype on verbal fluency performance has not been investigated before, to our knowledge. Although there was no significant main effect of genotype, there was a trend for a diagnosis × genotype interaction, which reflected poorer performance of the hard condition in 10/10-repeat than in 9-repeat carrier patients but the converse in healthy volunteers. Previous studies in healthy subjects suggest that the DAT 3’UTR VNTR genotype does not influence performance on working memory tasks,\textsuperscript{11,27} but that the 10-repeat allele is associated with a higher number of commission errors during the Continuous Performance Test and with impaired selective attention and response inhibition.\textsuperscript{105} The trend for poorer verbal fluency performance in the 10/10-repeat group with schizophrenia might thus reflect an influence of the DAT 3’UTR VNTR genotype (and hence dopamine) on attention and response inhibition, both of which are involved in executing verbal fluency tasks. Because the sample size in the present study was powered to detect differences at the neurophysiologic rather than the neuropsychological level and because the task was paced (reducing task demands), it is possible that more marked effects of DAT genotype on performance would have been evident in a larger sample, using an unpaced version of the paradigm.

We cannot exclude the possibility that the effects of the DAT 3’UTR VNTR genotype were due to other polymorphisms in strong linkage disequilibrium with the 3’UTR VNTR. The latter was selected because it has been shown to have the greatest functional effect on DAT expression.\textsuperscript{13-18} We compared individuals with one or two 9-repeat alleles against those with none (10/10-repeat group) because it was difficult to recruit a sufficient number of individuals homozygous for the 9-repeat allele. This precluded examination of whether the effect of the risk allele is better described by a dominant/recessive or additive model. Although, in most subjects, IQ was assessed by means of the Wechsler Adult Intelligence Scale, different versions of this instrument were used and, in a minority of subjects, IQ was assessed by means of the Quick Test. However, previous studies have shown that the IQ estimates obtained from these scales are highly correlated.\textsuperscript{99-101} Moreover, even if using different instruments had influenced the IQ estimates, it is unlikely to have affected the results because the proportion of subjects assessed with each version was matched across genotype groups. We used an event-related approach in the image analysis, although this is not ideal for data acquired via a block design with fixed interstimulus intervals. Although we modeled correct and incorrect trials separately to minimize the potentially confounding effects of differences in performance accuracy, reaction times were not measured, so the findings could have been influenced by differences in response speed.

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100. Stevens AA, Goldman-Rakic PS, Gore JC, Fulbright RK, Wexler BE. Cortical dysfunction in schizophrenia during auditory word and tone working memory demonstrated by functional magnetic resonance imaging. *Arch Gen Psychiatry*. 1998;55(12):1097-1103.


