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The impact of common dopamine D2 receptor gene polymorphisms on D2/3 receptor availability: C957T as a key determinant in putamen and ventral striatum

Dopamine function is broadly implicated in multiple neuropsychiatric conditions believed to have a genetic basis. Although a few positron emission tomography (PET) studies have investigated the impact of single-nucleotide polymorphisms (SNPs) in the dopamine D2 receptor gene (DRD2) on D2/3 receptor availability (binding potential, \( BP_{ND} \)), these studies have often been limited by small sample size. Furthermore, the most commonly studied SNP in D2/3 \( BP_{ND} \) (Taq1A) is not located in the DRD2 gene itself, suggesting that its linkage with other DRD2 SNPs may explain previous PET findings. Here, in the largest PET genetic study to date \((n = 84)\), we tested for effects of the C957T and -141C Ins/Del SNPs (located within DRD2) as well as Taq1A on \( BP_{ND} \) of the high-affinity D2 receptor tracer \(^{18}F\)-Fallypride. In a whole-brain voxelwise analysis, we found a positive linear effect of C957T T allele status on striatal \( BP_{ND} \) bilaterally. The multilocus genetic scores containing C957T and one or both of the other SNPs produced qualitatively similar striatal results to C957T alone. The number of C957T T alleles predicted \( BP_{ND} \) in anatomically defined putamen and ventral striatum (but not caudate) regions of interest, suggesting some regional specificity of effects in the striatum. By contrast, no significant effects arose in cortical regions. Taken together, our data support the critical role of C957T in striatal D2/3 receptor availability. This work has implications for a number of psychiatric conditions in which dopamine signaling and variation in C957T status have been implicated, including schizophrenia and substance use disorders.

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variant might be useful to understand whether these SNPs have additive effects on BPND. Multilocus dopaminergic scores have been used in a number of behavioral/c clinical and functional magnetic resonance imaging studies, but have surprisingly not been conducted in dopamine imaging. A multilocus approach provides an added advantage of determining the relative impact of each SNP on D2/3 BPND. Furthermore, given that the majority of the previous studies used the positron emission tomography (PET) radiotracer 11C-raclopride, which is not able to image extrastriatal BPND, little is known about the impact of these DRD2 SNPs on D2/3 receptor availability outside the striatum. Although one paper has investigated extrastriatal D2/3 BPND using 11C-FLB-457 and found an effect of C957T, it was limited by low numbers of CC (n = 7) and TT (n = 8) individuals in the analysis. Considering that there is evidence that striatal and extrastriatal D2/3 receptors are differentially regulated, further exploration of the effects of DRD2 SNPs on receptor availability across the brain is needed. In the present study, we used 18F-Fallypride, which is a D2/3 receptor tracer with favorable affinity to measure both striatal and extrastriatal receptors. We assessed the impact of C957T, Taq1A and -141C Ins/Del SNPs and multilocus effects of these SNPs in combination on D2/3 BPND in a sample of 84 healthy subjects.

**MATERIALS AND METHODS**

**Subjects**

Our data set consisted of 84 total participants (ages 18-37, m = 24.17 ± 5.05; 53.6% female; 69% Caucasian) who participated in three PET studies in the Zald Affective Neuroscience lab over the period of 10 years. Participants gave written informed consent, as approved by the Vanderbilt University Institutional Review Board. Participants had no known past or present neurological or psychiatric diagnoses, no history of substance use disorders and no current use of psychoactive medications or substances as assessed by Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders administered at screening.

**PET imaging**

[18F]-Fallypride ([S]-N-[(1-allyl-2-pyrrolidinyl)methyl]-5-(3-[18F]fluoropropyl)-2,3-dimethoxybenzamide) was produced in the radiochemistry laboratory attached to the PET unit at Vanderbilt University Medical Center, following synthetic and quality control procedures described in the US Food and Drug Administration IND 47,245. All the data were collected on the same GE Discovery STE PET scanner. Serial scan acquisition was started simultaneously with a 5.0 mCi (185 MBq) slow bolus injection of DA D2/3 tracer [18F]-Fallypride (specific activity ≈ 300 Ci mmol⁻¹). Computed tomographic scans were collected for attenuation correction before each of the three emission scans, which together lasted approximately 3.5 h with two breaks for subject comfort. Acquisition times for the dynamic PET scans were the same across all studies and have been reported previously.

**PET data processing**

After decay correction and attenuation correction, PET scan frames were corrected for motion using SPM8 (ref. 52) with the last dynamic image frame of the first series serving as the reference image. The mean PET image created from the realignment was then registered to each subject’s high-resolution T1 magnetic resonance image (FLIRT, 6 degrees of freedom), which was nonlinearly registered to MNI space (FNIRT) in FSL. Putamen and cerebellum reference regions of interest (ROIs) were created from the WFU Pickatlas with the cerebellum modified such that the anterior one-fourth of the ROI along with voxels within 5 mm of cortex were excluded to prevent contamination of the PET signal from nearby areas such as midbrain or occipital cortex. These ROIs were then warped to each subject’s PET space using the FLIRT and FNIRT FSL transform matrices (MNI → T1 → PET) and used in a simplified reference tissue model performed in PMOD software (PMOD Technologies, Zurich, Switzerland) to estimate Fallypride binding potential (BPND), a ratio of specifically bound Fallypride to its nondisplaceable concentration. Specifically PMOD’s PXMOD tool was used to estimate BPND voxelwise using a published basis function fitting approach.

Subject-specific BPND images were then warped to MNI space using the saved FSL transforms to create MNI-normalized BPND images (resampled to 2 mm isotropic voxels). These MNI-normalized images were then analyzed (using an explicit MNI brain mask) in SPM8 to test for their relation to SNPs in the DRD2 gene.

**Genotyping of DRD2 SNPs**

Blood samples from each subject were genotyped for Taq1A (rs1800497), C957T (rs6277) and -141C Ins/Del (rs1799732) SNPs via Sequenom analysis performed at Vanderbilt University’s VANTAGE Genomics Core (see ref. 57 for detailed Sequenom genotyping methods).

**PET analyses for DRD2 SNP effects**

In all the analyses, we controlled for age and sex as these have been found to affect dopamine signaling. We initially performed independent sample t-tests in SPM8 comparing BPND for Taq1A A2A2 versus A1 Carriers as well as -141C Ins/Ins vs Del Carriers as these groupings have often been used when analyzing these two SNPs. We also tested for a linear effect of A2 allele dosage given previously published data. For the C957T SNP, we tested for linear effects of T allele dosage via a multiple regression analysis in SPM with number of T alleles as our independent variable of interest. We had a priori hypotheses that the three SNPs would affect striatal BPND, given previously published 11C-raclopride PET data. Therefore, we also applied a small volume correction in all SPM8 analyses that consisted of a bilateral striatal ROI composed of caudate, putamen and ventral striatum as defined in Mawlawi et al. and used in prior PET studies, thus limiting significance testing to the striatum by masking the SPM T images in follow-up analyses. We also explored the effects of additive multilocus scores comprising our DRD2 SNPs (weighted as in previously published PET studies or based on our own single SNP analyses when our data did not conform to previous reports, which was the case with the Ins/Del SNP) via multiple regression of allele dose with Fallypride BPND. To clarify the results, we investigated BPND extracted from the anatomical striatal ROIs in post hoc analyses when significant effects were observed in the striatum during the primary voxelwise analyses. In supplemental analyses, we also extracted BPND from anatomical masks of extrastriatal regions (see Supplementary Information for details). We also calculated effect sizes (controlling for age and sex) and 95% confidence intervals for BPND obtained from both our striatal and extrastriatal ROIs across genotype groups to allow for comparisons with previously published findings.

**RESULTS**

DRD2 SNP distributions and associations

All SNPs were present in expected ratios and did not violate Hardy–Weinberg equilibrium (max χ² = 4.94, min P = 0.09 for Ins/Del; see Table 1). There were significant differences in the Taq1A distribution across the C957T individuals (χ² = 14.66, df = 4, P = 0.005) with A1A1 being exclusively present in CC individuals and the majority of TT individuals expressing A2A2 (79%, 11/14). There was a trend toward differences in Taq1A distributions across -141C Ins/Del group (χ² = 8.02, P = 0.091), but this was undoubtably driven by the lack of individuals with two copies of either rare alleles (Del (~5%) and A1 (~7%)). When comparing distributions of Taq1A A1 Carriers vs A2A2, no difference in Ins/Del genotype distribution was present (χ² = 0.31, df = 2, P = 0.86). There was, however, a significant difference in C957T distribution across Ins/Del individuals (χ² = 12.77, df = 4, P = 0.012) with all TT individuals expressing Ins/Ins (14/14) and CC individuals being majority Del/Del (75%, 3/4).

Importantly, there were no significant differences in sex distributions or age across our genotype groups (Table 1), whereas differences in ethnicity across C957T and Taq1A were expected given previously reported allelic distributions by ethnic group. Covarying for participant ethnicity (Caucasian, African American, Asian, or Hispanic), however, did not alter the statistical significance or lack thereof of any reported results.
C957T as a key determinant of striatal $B_{\text{ND}}$

CT Smith et al.

Table 1. Demographics of DRD2 SNPs and allelic distributions by sex and ethnicity

<table>
<thead>
<tr>
<th>SNP</th>
<th>n</th>
<th>Age (s.d.)</th>
<th>Sex ($%$ male)</th>
<th>Ethnicity (% Caucasian)</th>
<th>Ethnicity $\chi^2$, P</th>
</tr>
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<tbody>
<tr>
<td>C957T</td>
<td>2.23</td>
<td>1.31</td>
<td>1.11</td>
<td>21.51</td>
<td>&lt; 0.001</td>
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<tr>
<td></td>
<td>0.11</td>
<td>0.52</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>30</td>
<td>23.1 (4.6)</td>
<td>53.3</td>
<td>40.0</td>
<td></td>
</tr>
<tr>
<td>CT</td>
<td>40</td>
<td>24.2 (5.0)</td>
<td>40.0</td>
<td>82.1</td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>14</td>
<td>26.5 (5.1)</td>
<td>50.0</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td>Taq1A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A2A2</td>
<td>48</td>
<td>24.2 (5.0)</td>
<td>45.8</td>
<td>77.1</td>
<td>0.010</td>
</tr>
<tr>
<td>A1A2</td>
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<td>23.5 (4.6)</td>
<td>43.3</td>
<td>69.0</td>
<td></td>
</tr>
<tr>
<td>A1A1</td>
<td>6</td>
<td>27.2 (6.9)</td>
<td>66.7</td>
<td>16.7</td>
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<tr>
<td>-141C Ins/Del</td>
<td>0.34</td>
<td></td>
<td>1.84</td>
<td>74.1</td>
<td>0.34</td>
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<td></td>
<td>0.68</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ins/Ins</td>
<td>59</td>
<td>24.1 (4.6)</td>
<td>44.1</td>
<td>74.1</td>
<td></td>
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<tr>
<td>InsDel</td>
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<td>24.7 (6.6)</td>
<td>57.1</td>
<td>57.1</td>
<td></td>
</tr>
<tr>
<td>Del/Del</td>
<td>4</td>
<td>22.3 (2.9)</td>
<td>25.0</td>
<td>75.0</td>
<td></td>
</tr>
</tbody>
</table>

Demographic breakdowns of age, sex and ethnicity across the three DRD2 single-nucleotide polymorphisms (SNPs) investigated. Although age and sex did not differ across the SNPs, they were controlled for in all the analyses. Although the Taq1A and C957T allelic distributions differed by ethnic group (as expected based on previous work), the addition of ethnicity as a covariate did not alter the significance of any reported genetic results.

Figure 1. C957T T allele dosage is associated with increased striatal $B_{\text{ND}}$. Results from a regression analysis run in SPM8 identified areas where Fallypride $B_{\text{ND}}$ was positively correlated with number of T alleles in the C957T SNP. Large clusters were observed in the striatum with both left and right clusters surviving an FDR cluster-level correction for multiple comparisons. A small (\(k = 39\)) midbrain/thalamic cluster (peak at \(x = 26, y = 14, z = -4\)) is visible on the axial slice. In all figures, data are displayed in neurological convention (image on left represents left side of brain). Data are displayed using a $P < 0.005$, uncorrected threshold. $B_{\text{ND}}$, nondisplaceable binding potential; FDR, false discovery rate; SNP, single-nucleotide polymorphism.

C957T and Fallypride $B_{\text{ND}}$

Controlling for participant age and sex in our voxelwise analysis, we found two large striatal clusters in which $B_{\text{ND}}$ increased along with the number of C957T T alleles. The clusters, one in each hemisphere, both reached cluster-level false discovery rate (FDR) significance: (i) $k$ (voxel #) = 528, $T = 4.48$, $P_{\text{FDR}} = 0.018$, peak at 26, 10, −4; and (ii) $k$ = 516, $T = 3.86$, $P_{\text{FDR}} = 0.018$, peak at −24, 4, −2 (Figure 1). The left striatal cluster also extended down into the ventral striatum. These results were supported by anatomically based striatal ROI analysis, which showed that $B_{\text{ND}}$ differed significantly in the putamen and ventral striatum (VS, Supplementary Table S1, Supplementary Material). We found no support for C957T effects on extrastriatal $B_{\text{ND}}$ in our voxelwise analysis, except for a very small set of voxels in the midbrain/thalamus (\(k = 39\); Figure 1). Because Hirvonen et al.\(^\text{24}\) reported extrastriatal C957T effects using a priori cortical ROIs, which can be more sensitive to group effects due to their use of a more stable regional aggregate of $B_{\text{ND}}$, we further tested for an effect of C957T on extrastriatal ROIs. However, we found no significant differences in $B_{\text{ND}}$ in these extrastriatal ROIs (Supplementary Table S2).

Taq1A and Fallypride $B_{\text{ND}}$

Investigating the effect of Taq1A on Fallypride $B_{\text{ND}}$, an A2A2 > A1 Carrier T-test run in SPM resulted in no significant clusters, even at cluster-level $P < 0.05$ uncorrected. We also tested for a linear effect of A2 dose on $B_{\text{ND}}$ (A1A1 < A1A2 < A2A2) via regression in SPM. Again, no significant clusters were identified. In neither case did we identify significant effects of Taq1A on $B_{\text{ND}}$ after applying a small volume correction within our striatal ROI. We also found no evidence for genotype effects in $B_{\text{ND}}$ in ROI analysis of the three striatal subregions (Supplementary Table S3).

141C Ins/Del and Fallypride $B_{\text{ND}}$

When comparing -141C Ins/Del Carriers with Ins/Ins, no significant clusters were present in the Del Carriers > Ins/Ins $B_{\text{ND}}$ analysis (where a previous effect had been observed on striatal $B_{\text{ND}}$\(^\text{25}\)). The opposite contrast, Ins/Ins > Del Carriers, resulted in two modest clusters, but neither were significant after correcting for multiple comparisons: right sub-gyrall/orbitofrontal cortex ($k = 264$, $T = 3.63$ at 24, 28, −8, $P_{\text{FDR}}$ cluster level = 0.32) and midbrain/pons ($k = 111$, $T = 3.38$ at 0, −26, −28, $P_{\text{FDR}}$ cluster level = 0.82). We observed no significant $B_{\text{ND}}$ effects in the striatum even after applying a small volume correction. We also found no evidence for genotype differences in $B_{\text{ND}}$ across striatal ROIs (Supplementary Table S4).

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Figure 2. Multilocus DRD2 SNP effects on BP_{ND} C957T+Ins/Ins score (alone on left, displayed in hot yellow colors) and C957T+Ins/Ins+Taq1A A2 dose score (center, displayed in cool colors) effects on Fallypride BP_{ND} are displayed as T-scores. Beyond the striatum, the C957T+Ins/Ins multilocus score affects BP_{ND} in midbrain/thalamus and midbrain/pons (evident in coronal and saggital slices). The addition of A2 dosage information does not alter the multilocus score’s relationship to striatal and thalamic BP_{ND} appreciatively (overlap of both multilocus effects in white/pink). Taq1A A2 dose also has no effect on the deeper midbrain/pons genetic effect observed with the C957T+Ins/Ins multilocus score (see saggital slice). Data are displayed using a P < 0.05, uncorrected threshold. BP_{ND}, nondisplaceable binding potential; SNPs, single-nucleotide polymorphism.

**DISCUSSION**

C957T T allele is associated with heightened striatal D2/3 receptor availability

Here, we demonstrate that increasing number of C957T T alleles are associated with heightened D2/3 receptor availability (BP_{ND}) in large portions of the striatum. Our results replicate the previous observation with {^{11}}C-raclopride PET that C957T T allele dosage is related to higher BP_{ND} in the striatum. Such replications are critical in PET studies because the expense and inconveniences of PET radioligand research leave most studies substantially underpowered for genetic analysis. However, the directness of the links between genes for a given receptor and PET assessment of those same receptors makes SNPs such as C957T (in the DRD2 gene itself) more reasonable targets for genomic neuroimaging than most candidate polymorphisms. It is notable that we observed the C957T effect with a different D2/3 radiotracer (^{18}F-Fallypride) than Hirvonen et al. {^{22,23}} ({^{11}}C-raclopride), further suggesting the robustness of the effect. We also report for we believe the first time the effect of C957T in predicting D2/3 BP_{ND} in specific subregions of the striatum and found support for the T allele being associated with higher bilateral putamen and ventral striatum BP_{ND} (but only restricted impact in the caudate).

C957T and extrastriatal D2/3 receptor availability

A primary advantage of {^{18}}F-Fallypride over {^{11}}C-raclopride as a tracer is its ability to measure extrastriatal D2 receptors. We therefore sought to replicate the findings of Hirvonen et al. {^{24}} who found that C alleles were associated with higher {^{11}}C-FLB-457 binding in anatomically defined extrastriatal regions. However, our voxelwise analysis did not identify any significant extrastriatal clusters, and we found no evidence for differences in BP_{ND} in extrastriatal ROIs chosen to approximate those of Hirvonen et al. {^{24}} Although qualitatively BP_{ND} in some cortical ROIs was higher with the C allele, as found by Hirvonen et al. {^{24}} they did not reach statistical significance. Thus, C957T is not exerting a homogeneous global influence over both striatal and extrastriatal regions. This is consistent with evidence that individual differences in the striatal and cortical D2 BP_{ND} are at least partially dissociable, which in turn suggests that some genetic and environmental influences on D2 receptor expression and functioning should be expected to be different across regions.

Reconciling PET and *in vitro* data on C957T

One reason why our replication of the prior striatal findings of Hirvonen et al. {^{22,23}} is important is that the direction of the C957T effect in the striatum is opposite of what would be predicted based on *in vitro* data where the T allele in the synonymous C957T SNP in CHO-K1 cells is associated with less DRD2 protein synthesis and less stable DRD2 mRNA (due to folding). The source of the discrepancy between the *in vitro* data and the striatal PET data is unclear. The CHO-K1 cell line used is nonhuman in origin (from hamsters), does not normally express DRD2, and may potentially be a poor proxy for human cells that naturally express D2 receptors in striatum (medium spiny neurons). Taken together, the human PET data strongly suggest that it is a mistake to extrapolate the *in vitro* finding of Duan et al. {^{37}} to human striatal D2 receptor expression *in vivo.*
Moderate effect of Ins/Del SNP on striatal and midbrain/pons D2/3 receptor availability

The potential role of DRD2 SNPs in affecting D2/3 BPND in extrastriatal subcortical regions will require further study, as our results are somewhat equivocal and did not reach conservative levels of statistical significance. Our voxelwise data suggest the -141C Ins/Del SNP may affect BPND (Ins/Ins vs Del carriers) in the midbrain/pons even though it had little effect in the striatum ($\eta^2 = 0.007$, $d = 0.17$ from ROI analysis, Supplementary Table S4). Previous work has found only minor or no effect of Ins/Del genotype on striatal BPND. Specifically, Jonsson et al. observed a small ($P = 0.024$; Cohen’s $d = −0.69$) effect of -141C Ins/Del with Del Carriers having higher striatal D2/3 BPND, opposite to the effect we observe here. Their data, however, were collected across two different PET scanners, which could have introduced systematic variance in the data (see the ‘Lack of Robust Effect of Taq1A’ section below). A similarly sized raclopride PET study observed no significant effect of Ins/Del on striatal BPND but the direction of difference was similar to what we observed (higher for Ins/Ins).

One reason for this discrepancy may be that neither study reported data from the different striatal subdivisions. In contrast to the ventral striatum and putamen, we observed slightly higher BPND in the caudate of Del Carriers, suggesting that averaging across striatal subdivisions may mask the SNP’s effects. Finally, we note that our voxelwise results of increased D2/3 receptor availability (BPND) fits with in vitro data using two human-derived cell lines, including Y-79 cells demonstrated to express functional D2 receptors, which show that the Del variant in -141C results in reduced transcriptional efficiency of the DRD2 gene.

Lack of robust effect of Taq1A on D2/3 receptor availability

Although a Taq1A A2/A2 vs A1 Carriers effect on striatal BPND has been observed in a recent meta-analysis of five studies and our dataset had ~80% power to observe the mean effect size of $d = 0.57$, we found no effect of Taq1A genotype on Fallypride BPND in our voxelwise analysis. Furthermore, our ROI analysis found only a very small A2/A2 > A1 BPND effect (Hedges $g = 0.12$, 95% confidence interval: −0.21, 0.28) in the striatum that was around 20% of that reported in the meta-analysis with the confidence interval including zero, suggesting that the effect was not robust.
Thus, there is strong empirical evidence that C957T is linked with Del (literature.32,33) functioning more generally as has been the case in some of the Supplementary Table S3). Earlier studies observing Taq1A effects have often not controlled for these potential confounders on BPND. Furthermore, not all imaging studies have found effects of Taq1A on BPND including the study with the largest sample size to date (peak at 2, −26, −28, P_{FDR} = 0.087) not present in the C957T analysis alone. The location of this cluster ventral to the dopaminergic midbrain as well as the failure of the effect to reach significance when controlling for multiple comparisons make it difficult to draw conclusions about Ins/Del in this region (Supplementary Figure S1). We also observed a smaller cluster in midbrain/thalamus (k = 144, at 8, −22, −4, P_{FDR} = 0.53). Given that variation in Fallypride BPND in midbrain and thalamus has been associated with schizophrenia,77 further investigation of genetic polymorphisms that affect BPND in these regions could aid in understanding risk for the disease. In addition, it is notable that individual differences inthalamic D2/3 receptor availability have been associated with differences in responses to dopaminergic drugs.79 Thus, genetic variants that affect DRD2 in the thalamus (or its subregions) may have implications for determining optimum pharmacological treatments. However, these extrastrial findings should be interpreted with some caution until they are replicated.

Linkage of DRD2 SNPs

In our data, individuals expressing the Taq1A A2 allele were more likely to also express the C957T T and Ins/Del Ins alleles. Others have reported strong linkage disequilibrium between C957T, -141C Ins/Del and Taq1A.31,37 or between C957T and Taq1A.38 To follow up on this work, we used LDmatrix.4 to search the 1000 Genomes population database across all HapMap ethnic stratifications, and found linkage disequilibrium to be much higher between C957T and Taq1A (D’ = 0.76) and C957T and -141C Ins/Del (D’ = 0.84) than between Taq1A and -141C Ins/Del (D’ = 0.12). Thus, there is strong empirical evidence that C957T is linked with two other SNPs where D2/3 BPND effects have been observed with PET/PECT42,75 and, therefore, may have driven some of the effects observed with Taq1A (or -141C Ins/Del) in past studies. Given that most previous Taq1A studies did not report C957T status, it is not possible to determine the effects of one SNP from another in those studies. We note, however, that despite the observed linkage disequilibrium, we only observed modest, nonsignificant effects for Taq1A in the present study, suggesting that linkage disequilibrium only partially accounts for past Taq1A findings.

Multilocus DRD2 score effects on D2/3 receptor availability

When probing for additional effects of Taq1A and Ins/Del to our observed main effects of C957T, we found little evidence for additional explanatory power for either SNP on our BPND effects. C957T alone accounted for most of the genetic variance in striatal BPND whether we focused our analyses on clusters identified from our multilocus score regression analyses or anatomically defined putamen and ventral striatum ROIs. However, we identified in our C957T+Ins/Del multilocus score analysis a midbrain/pons cluster (peak at 2, −26, −28, P_{FDR} = 0.087) not present in the C957T analysis alone. The location of this cluster ventral to the dopaminergic midbrain as well as the failure of the effect to reach significance when controlling for multiple comparisons make it difficult to draw conclusions about Ins/Del in this region (Supplementary Figure S1). We also observed a smaller cluster in midbrain/thalamus (k = 144, at 8, −22, −4, P_{FDR} = 0.53). Given that variation in Fallypride BPND in midbrain and thalamus has been associated with schizophrenia,77 further investigation of genetic polymorphisms that affect BPND in these regions could aid in understanding risk for the disease. In addition, it is notable that individual differences in thalamic D2/3 receptor availability have been associated with differences in responses to dopaminergic drugs.79 Thus, genetic variants that affect DRD2 in the thalamus (or its subregions) may have implications for determining optimum pharmacological treatments. However, these extrastrial findings should be interpreted with some caution until they are replicated.

C957T, BPND and psychiatry

Our findings have implications for a variety of dopamine-linked psychiatric disorders. The C allele of C957T is more prevalent in patients with schizophrenia3,4,18 and affects a variety of learning processes79–82 as well as executive function.53,84 However, despite the C957T effects observed here, differences in striatal D2/3 receptor availability (BPND) have not been consistently observed in contrasts of schizophrenics and healthy controls.77 This could reflect the difficulty of measuring D2/3 receptor levels in patients who may possess heightened DA synthesis capacity,35 which may impact both competition of radiotracers with endogenous dopamine,86,87 and long-term regulation of D2/3 expression. Furthermore, additional short- and long-term impacts of antipsychotic medications on D2/3 receptor expression12,14,15 and dopamine regulation may impact PET measures in these patients. It is also conceivable that in the context of schizophrenia, C957T alters the impact of endogenous or exogenous perturbations of the dopamine system on D2/3 receptors. As such, it warrants particular attention in treatment research. Interestingly, the C allele has previously been associated with weight gain during treatment with antipsychotics.88

Furthermore, C957T has been associated with behavioral impulsivity89,90 whose effects increase with aging91 and reward sensitivity,92 which may explain why the C allele has been associated with increased risk for alcohol dependence.19 The lower D2/3 BPND we observed in the striatum of CC individuals fits with a wealth of data suggesting substance-dependent individuals display lower D2/3 BPND.93,94 Furthermore our C957T BPND effects were strongest in the VS (accounting for 13 and 17% of the variance in right and left VS BPND, respectively), a key area involved in reward processing and dopamine release associated with drugs of abuse.95 There is some evidence that the C957T and Ins/Del SNPs predict quit rates in smokers treated with either
bupropion or nicotine replacement therapy, illustrating the potential utility of using these genetic measures to target effective therapies. Although the mechanistic relationship between C957T and D2 receptor signaling still needs to be determined, the literature suggests that understanding this SNP (and others) may greatly aid us in treating individuals with a variety of psychiatric disorders.

Limitations
Although this study is larger than any other PET study to date examining gene effects on D2 receptor availability, we note that all such studies (including the present one) are underpowered. Although we used a common approach to index multilocus genetic effects and a more sophisticated approach, such as haplotype analysis, might offer additional insights, but require much larger samples due to the relative low minor allele frequency of the Taq1A (0.20) and -141C Ins/Del (0.09) SNPs. Furthermore, although we confirmed that our results remained consistent when controlling for ethnicity, it is possible that the results are stronger in particular ethnic groups, which we could not test for owing to small subject numbers with this further division of the data set. Indeed, the relative frequencies of the SNPs we investigated vary across ethnic groups and so the ethnic composition of this (69% Caucasian) and other studies warrants consideration when interpreting results. In addition, at least one study has suggested C957T effects may vary by individuals’ sex and although we controlled for it in our analyses, we were not well powered to test for sex by genotype interactions.

CONCLUSION
Our results replicate and extend previous work showing C957T T allele dosage is positively related to striatal D2/3 receptor availability (BPND) with significant effects observed in both the putamen and ventral striatum. Furthermore, we show that variation in this SNP explains a much larger portion of variability in striatal BPND than either the -141C Ins/Del or Taq1A alleles. By contrast, Taq1A alone or in combination with the other two tested DRD2 SNPs was not associated with striatal BPND, above the C957T effect. These findings demonstrate that DRD2 SNPs beyond Taq1A, specifically C957T, impact individual differences in striatal D2/3 BPND. To the extent that DRD2 relevant genes are interpreted as proxies in place of actual receptor assays, these data suggest that C957T is preferable to either -141C Ins/Del or Taq1A alleles.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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