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ORIGINAL ARTICLE

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

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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, these studies have often been limited by small sample size. Furthermore, the most commonly studied SNP in D2/3 BPND (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 BPND of the high-affinity D2 receptor tracer [(18F)F-Fallypride]. In a whole-brain voxelwise analysis, we found a positive linear effect of C957T T allele status on striatal BPND 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 BPND 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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INTRODUCTION

Genetic variation in the dopamine (DA) D2 receptor (DRD2) gene or its neighbor, the ankyrin repeat and kinase domain containing 1 (ANKK1) gene, have been associated with risk for schizophrenia and its response to pharmacological treatment. As most antipsychotics used to treat the positive symptoms of schizophrenia – for example, clozapine, olanzapine, and risperidone – have similar efficacy in treating symptoms of negative schizophrenia, the presence or absence of the Taq1A allele can affect the efficacy of these treatments. In a meta-analysis of nearly 7000 participants (3000 schizophrenia cases), we tested for effects of the C957T and -141C Ins/Del SNPs (located within DRD2) as well as Taq1A on BPND of the high-affinity D2 receptor tracer [(18F)F-Fallypride]. In a whole-brain voxelwise analysis, we found a positive linear effect of C957T T allele status on striatal BPND 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 BPND 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/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 > 3000 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 (\(BPND_{FD} \)), 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 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 allelic dose with Fallypride BPND. To clarify the results, we investigated BPND extracted from the anatomical striatal ROIs 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 \(n^2\) 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 \(\chi^2 = 4.94, \min P = 0.09\) for Ins/Del; see Table 1). There were significant differences in the Taq1A distribution across the C957T individuals (\(\chi^2 = 14.66, df = 4, P = 0.005\)) 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 Ins/Del groups (\(\chi^2 = 8.02, P = 0.091\)), but this was undoubtedly 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 (\(\chi^2 = 0.31, df = 2, P = 0.86\)). There was, however, a significant difference in C957T distribution across Ins/Del individuals (\(\chi^2 = 12.77, df = 4, P = 0.012\)) 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.
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.

| SNP     | n   | Age (s.d.) | Age F, P | Sex (%) male | Sex χ², P | Ethnicity (% Caucasian) | Ethnicity χ², P |
|---------|-----|------------|----------|--------------|------------|------------------------|----------------|
| C957T   | 2.23 |            | 1.31, 0.52 |              | 21.51, < 0.001 |                        |                |
| CC      | 30  | 23.1 (4.8) | 53.3     |              | 40.0       |                        |                |
| CT      | 40  | 24.2 (5.0) | 40.0     |              | 82.1       |                        |                |
| TT      | 14  | 26.5 (5.1) | 50.0     |              | 100.0      |                        |                |
| Taq1A   |     |            |          |              |            |                        |                |
| A2A2    | 48  | 24.2 (5.0) | 45.8     |              | 77.1       |                        | 9.27, 0.010    |
| A1A2    | 30  | 23.5 (4.6) | 43.3     |              | 69.0       |                        |                |
| A1A1    | 6   | 27.2 (6.9) | 66.7     |              | 16.7       |                        |                |
| -141C Ins/Del | 0.34, 0.68 | 44.1 | 1.84, 0.40 | 74.1 | 2.17, 0.34 |
| InsIns  | 59  | 24.1 (4.6) | 57.1     |              | 57.1       |                        |                |
| InsDel  | 21  | 24.7 (6.6) | 40.0     |              | 75.0       |                        |                |
| DelDel  | 4   | 22.3 (2.9) | 25.0     |              |            |                        |                |

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 BPND. Results from a regression analysis run in SPM8 identified areas where Fallypride BPND 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 2, −10, −2) 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. BPND, nondisplaceable binding potential; FDR, false discovery rate; SNP, single-nucleotide polymorphism.

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DRD2 multilocus analyses: C957T alone explains BP\textsubscript{ND} effect in striatum

Given that all of the DRD2 SNPs that we examined are believed to be in high linkage disequilibrium,\textsuperscript{2,13,17,38} we tested whether the addition of either Taq1A or -141C Ins/Del genotype, or both, increased the prediction of BP\textsubscript{ND} beyond the observed effects of the C957T SNP. The addition of Taq1A genotype to C957T did not provide additional benefit in predicting Fallypride BP\textsubscript{ND} (Supplementary Table S5). The addition of Ins/Dels in Ins/dels vs Del Carrier status to C957T T allele dose (T allele #+Ins/del status (0,1)) increased the spatial extent of right striatal voxels in which BP\textsubscript{ND} was associated with genotype (k went from 528 to 1019) but not the strength of the association (max T value went from 4.48 to 4.20 (P\textsubscript{FDR} = 0.002 at 26, 8, -4); Supplementary Table S6). The lack of improvement in strength of association was confirmed by ROI analysis (see Supplementary Materials). The left striatum effect decreased in both spatial extent (k went from 516 to 488) and strength (max T value went from 3.86 to 3.41; P\textsubscript{FDR} = 0.018 to 0.043 at -20, 8, -8). In addition, the combined C957T+Ins/del score was associated with higher BP\textsubscript{ND} in a large midbrain/pons area (k = 353, T = 4.30 (global max from this analysis) at 2, -26, -28), though it did not survive corrections for multiple comparisons (P\textsubscript{FDR} = 0.087), and did not conform to the location and shape of a specific anatomical structure (Figure 2; Supplementary Table S6), although it is notable that part of the focus was in the vicinity of the raphe nuclei. Furthermore, a small midbrain/thalamic area was identified (k = 144, T = 3.70 at 8, -22, -4) but did not reach significance (P\textsubscript{FDR} = 0.53; Figure 3, Supplementary Figure S1).

Investigating the effect of a combined multilocus score with number of C957T T alleles, 141C Ins/del status (1,0) and number of Taq1A A2 alleles on Fallypride BP\textsubscript{ND} resulted in qualitatively similar results in the striatum as our C957T analysis alone (Supplementary Table S7). Furthermore, stepwise regression, with age and sex in the first step, and C957T in the second step, revealed that there was no significant improvement in predictive power in the identified left (F-change = 0.333, P = 0.566) or right (F-change = 0.775, P = 0.381) striatum, above the effects of C957T, when Ins/dels status or number of Taq1A A2 alleles were added in the third and fourth steps. We also conducted anatomically based striatal ROI stepwise regression analyses that confirmed that C957T explains more of the variance in BP\textsubscript{ND} than the Taq1A and -141C Ins/Del SNPs, particularly in ventral striatum and putamen (see Supplementary Materials).

**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\textsubscript{ND}) in large portions of the striatum. Our results replicate the previous observation with \textsuperscript{11}C-raclopride PET that C957T T allele dosage is related to higher BP\textsubscript{ND} in the striatum.\textsuperscript{22,23} Such replications are critical in PET studies because the expense and inconveniences of PET radioligand research leave most studies substantially under-powered 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 (\textsuperscript{18}F-Fallypride) than Hirvonen \textit{et al.}\textsuperscript{22,23} (\textsuperscript{11}C-raclopride), further suggesting the robustness of this effect. We also report for the first time the effect of C957T in predicting D2/3 BP\textsubscript{ND} in specific subregions of the striatum\textsuperscript{53-62} and found support for the T allele being associated with higher bilateral putamen and ventral striatum BP\textsubscript{ND} (but only restricted impact in the caudate).

C957T and extrastriatal D2/3 receptor availability

A primary advantage of \textsuperscript{18}F-Fallypride over \textsuperscript{11}C-raclopride as a tracer is its ability to measure extrastriatal D2 receptors. We therefore sought to replicate the findings of Hirvonen \textit{et al.}\textsuperscript{24} who found that C alleles were associated with higher \textsuperscript{11}C-F18-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\textsubscript{ND} in extrastriatal ROIs chosen to approximate those of Hirvonen \textit{et al.}\textsuperscript{24}

Although qualitatively BP\textsubscript{ND} in some cortical ROIs was higher with the C allele, as found by Hirvonen \textit{et al.}\textsuperscript{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\textsubscript{ND} are at least partially dissociable,\textsuperscript{49} 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 \textit{in vitro} data on C957T

One reason why our replication of the prior striatal findings of Hirvonen \textit{et al.}\textsuperscript{22,23} is important is that the direction of the C957T effect in the striatum is opposite of what would be predicted based on \textit{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).\textsuperscript{37} The source of the discrepancy between the \textit{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 \textit{in vitro} finding of Duan \textit{et al.}\textsuperscript{37} to human striatal D2 receptor expression \textit{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 4 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, showing 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 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.
The authors of the meta-analysis pointed out that certain moderators, including age and sex, might explain variation in PET/SPECT studies focused on the relationship between DRD2 genetics and D2/3 BPND. Importantly, when we controlled for sex and age effects in our ROI analyses, we observed no effect of Taq1A on striatal BPND (min $P = 0.24$; max $\eta^2 = 0.017$, $d = 0.26$; Supplementary Table S3). Earlier studies observing Taq1A effects have often not controlled for these potential confounds on BPND. Furthermore, not all imaging studies have found effects of Taq1A on BPND, including the study with the largest sample size to date ($n = 70$)$^{29-31}$ and at least one of the most-cited studies showing an effect has a methodological concern. That study, by Jonsson et al.$^{25}$ consisted of half the sample being run on a different PET system, which they tried to correct for with a systematic multiplicity to their data (bound/free ratio). This approach could have introduced systematic error in the data as the paper does not provide the distribution of the genotypes across the two PET scanners used. Here, we limited our genetic analyses to data collected on the same PET system—a GE Discovery STE. The present study is also the largest ($n = 84$) single PET study on DRD2 genetic effects to date. Our systematic analysis suggests that Taq1A allele status does not robustly affect D2/3 BPND except in specific striatal subdivisions and, thus, raises caution in the use of this SNP as a proxy for global striatal D2 receptor levels (or for DA functioning more generally) as has been the case in some of the literature.$^{22,33}$

Although this study utilized the D2/3 tracer $^{18}$F-Fallypride (vs $^{11}$C-raclopride in most prior studies), we do not have a reason to specifically predict that kinetic properties of the D2/3 tracer used would lead to a different result. That said, Fallypride has higher affinity for D2-like receptors and appears less sensitive to endogenous dopamine levels than raclopride.$^{70,71}$ Thus, if there are indeed significant effects of Taq1A on raclopride binding but not Fallypride binding, it could suggest that Taq1A effects are due to an impact on endogenous dopamine levels, rather than DRD2 affinity or receptor density. In fact, an $^{18}$F-DOPA PET study has implicated the A1 allele of Taq1A (but no effect of C957T or -141C Ins/Del SNPs) with increased dopamine synthesis in the putamen.$^{72}$ Interestingly, a recent PET study using the D2-specific radiotracer $^{11}$C-NMB, which is relatively insensitive to endogenous DA levels, did observe an effect of Taq1A on striatal BPND.$^{73}$ However interpretation of this study is complicated by the fact that only 24 of the 57 participants studied were considered healthy controls and disease state may influence Taq1A effects on BPND.$^{42}$ That paper also did not examine effects for SNPs other than Taq1A, and thus did not address whether C957T status affects striatal BPND. Clearly, further work is needed to determine the biological processes underlying differences in BPND observed with various PET tracers as well as the role of specific DRD2 SNPs on these processes.

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$^{5,17}$ or between C957T and Taq1A$^{18}$ To follow up on this work, we used LDMatrix$^{4}$ to search the 1000 Genomes population database across all HapMap ethnic strata 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/SPECT$^{42,75}$ 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 $P = 2$, $–26$, $–28$, $P_{\text{FDR}} = 0.087$) 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).$^{76}$ We also observed a smaller cluster in midbrain/thalamus ($k = 144$, at $8$, $–22$, $–4$, $P_{\text{FDR}} = 0.53$). 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.$^{78}$ Thus, genetic variants that affect DRD2 in the thalamus (or its subregions) may have implications for determining optimum pharmacological treatments. However, these extrastriatal 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 schizophrenia$^{79-82}$ and affects a variety of learning processes$^{79-82}$ as well as executive function.$^{83,84}$ 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 expression$^{12,14,15}$ 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 impulsivity$^{89,90}$ 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.$^{39}$ There is some evidence that the C957T and Ins/Del SNPs predict quit rates in smokers treated with either...
The authors declare no conflict of interest.

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