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 (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 ¹⁸F-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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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^{1–4} and its response to pharmacological treatment.^{5–9} As most antipsychotics used to treat the positive symptoms of schizophrenia act on D2 receptors,^{10–17} a better understanding of how genetic variation may affect D2/3 receptor availability (binding potential, BP_{ND}, a ratio of specifically bound D2/3 tracer to its nondisplaceable concentration) could be useful in aiding clinicians in prescribing more targeted treatments. The C957T (rs6277) single-nucleotide polymorphism (SNP) of the *DRD2* gene warrants particular attention given that the C allele has been associated with schizophrenia in Caucasians in a meta-analysis of nearly 7000 participants (3000 schizophrenia cases).¹⁸ Understanding the functional consequences of the C957T SNP has implications beyond schizophrenia as this SNP has also been associated with an increased risk for substance use disorders.^{19–21} However, to date only one research group has reported on the effect of this SNP on D2/3 BP_{ND}.^{22–24} Another *DRD2* SNP, -141C Ins/Del (rs1799732), has also been reported to impact striatal D2/3 BP_{ND} with Jonsson *et al.*²⁵ finding heightened BP_{ND} in Del carriers, but replication has been difficult, with Pohjalainen *et al.*²⁶ finding no significant effect (and, if anything, higher BP_{ND} in Ins/Ins versus Ins/Del individuals) despite a similarly sized sample and use of the same radiotracer, ¹¹C-raclopride. To date, more attention has been

paid to the impact of the Taq1A SNP in the *ANKK1* gene (rs1800497) with several groups reporting that A1 carriers have lowered striatal D2/3 BP_{ND} relative to A2 homozygotes.^{25,27,28} However, sample sizes in these studies have generally been small and the results have not been consistent across all studies.^{29–31} Nevertheless, based on the positive findings in the literature, many researchers have used Taq1A as a proxy for D2 receptor status (or more loosely as an index of general dopamine functioning^{32–35}).

Given that Taq1A polymorphism does not occur within the *DRD2* gene itself, researchers have speculated that polymorphisms in Taq1A may associate with other SNPs in the *DRD2* gene that are the real drivers of expression of the receptor *in vivo*.³⁶ The C957T and -141C Ins/Del polymorphisms are in strong linkage disequilibrium with Taq1A^{21,37,38} and have themselves been associated with striatal D2/3 BP_{ND}.^{22,23,25} Despite the data suggesting that these SNPs are strongly linked, few studies have systematically investigated the effect of C957T, -141C Ins/Del, and Taq1A in isolation and combination on D2/3 BP_{ND}. Characterizing the functional effect of these SNPs on D2/3 BP_{ND} has implications for better understanding the mechanisms through which they exert their demonstrated influence on motivated behaviors including learning and decision making.^{39–41}

Furthermore, as past research has implicated C957T,^{22,23} Taq1A^{25,27,28,42} and -141C Ins/Del²⁵ in striatal BP_{ND}, the use of multilocus SNP scores that combine the effects of each *DRD2*

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variant might be useful to understand whether these SNPs have additive effects on BP_{ND}. Multilocus dopaminergic scores have been used in a number of behavioral/clinical and functional magnetic resonance imaging studies,^{43–48} 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 BP_{ND}. 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 BP_{ND}, 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 BP_{ND} using ¹¹C-FLB-457 and found an effect of C957T,²⁴ 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,⁴⁹ 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 BP_{ND} in a sample of 84 healthy subjects.

MATERIALS AND METHODS

Subjects

Our data set consisted of 84 total participants (ages 18–37, $m=24.17 \pm 5.05$; 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 1⁵⁰ 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 synthesis 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.⁵¹

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.⁵³ Putamen and cerebellum reference regions of interest (ROIs) were created from the WFU Pickatlas⁵⁴ 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 model⁵⁵ performed in PMOD software (PMOD Technologies, Zurich, Switzerland) to estimate Fallypride binding potential (BP_{ND}, a ratio of specifically bound Fallypride to its nondisplaceable concentration). Specifically PMOD's

PXMOD tool was used to estimate BP_{ND} voxelwise using a published basis function fitting approach.⁵⁶

Subject-specific BP_{ND} images were then warped to MNI space using the saved FSL transforms to create MNI-normalized BP_{ND} 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.^{58–61} We initially performed independent sample *T*-tests in SPM8 comparing BP_{ND} 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.^{25,27} We also tested for a linear effect of A2 allele dosage given previously published data.⁶² 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 BP_{ND} given previously published ¹¹C-raclopride PET data.^{22,23,25,27} 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.*⁶³ and used in prior PET studies,^{64–66} 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 BP_{ND}. To clarify the results, we investigated BP_{ND} extracted from the anatomical striatal ROIs⁶³ in *post hoc* analyses when significant effects were observed in the striatum during the primary voxelwise analyses. In supplemental analyses, we also extracted BP_{ND} from anatomical masks of extrastriatal regions (see Supplementary Information for details).⁶⁷ We also calculated η^2 effect sizes (controlling for age and sex) and 95% confidence intervals for BP_{ND} obtained from both our striatal and extrastriatal ROIs across genotype groups to allow for comparisons with previously published findings.⁴²

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 -141C Ins/Del group ($\chi^2=8.02$, $P=0.091$), 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.⁶⁸ Covarying for participant ethnicity (Caucasian, African American, Asian, or Hispanic), however, did not alter the statistical significance or lack thereof of any reported results.

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

SNP	n	Age (s.d.)	Age F, P	Sex (% male)	Sex χ^2 , P	Ethnicity (% Caucasian)	Ethnicity χ^2 , P
C957T			2.23, 0.11		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			1.30, 0.28		1.11, 0.57		9.27, 0.010
A2A2	48	24.2 (5.0)		45.8		77.1	
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		1.84, 0.40		2.17, 0.34
InsIns	59	24.1 (4.6)		44.1		74.1	
InsDel	21	24.7 (6.6)		57.1		57.1	
DelDel	4	22.3 (2.9)		25.0		75.0	

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 BP_{ND}. Results from a regression analysis run in SPM8 identified areas where Fallypride BP_{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 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. BP_{ND}, nondisplaceable binding potential; FDR, false discovery rate; SNP, single-nucleotide polymorphism.

C957T and Fallypride BP_{ND}

Controlling for participant age and sex in our voxelwise analysis, we found two large striatal clusters in which BP_{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) $k(\text{voxel } \#) = 528$, $T = 4.48$, $P_{\text{FDR}} = 0.018$, 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 BP_{ND} differed significantly in the putamen and ventral striatum (VS, Supplementary Table S1, Supplementary Material). We found no support for C957T effects on extra-striatal BP_{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.*²⁴ reported extra-striatal 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 BP_{ND}, we further tested for an effect of C957T on extra-striatal ROIs. However, we found no significant differences in BP_{ND} in these extra-striatal ROIs (Supplementary Table S2).

Taq1A and Fallypride BP_{ND}

Investigating the effect of Taq1A on Fallypride BP_{ND}, an A2A2 > A1A1 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 BP_{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 BP_{ND} after applying a small volume correction within our striatal ROI. We also found no evidence for genotype effects in BP_{ND} in ROI analysis of the three striatal subregions (Supplementary Table S3).

-141C Ins/Del and Fallypride BP_{ND}

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

Figure 2. Multilocus DRD2 SNP effects on BP_{ND}. C957T T+Ins/Ins score (alone on left, displayed in hot yellow colors) and C957T T+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 T+Ins/Ins multilocus score affects BP_{ND} in midbrain/thalamus and midbrain/pons (evident in coronal and sagittal 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 sagittal slice). Data are displayed using a $P < 0.005$, uncorrected threshold. BP_{ND}, nondisplaceable binding potential; SNP, single-nucleotide polymorphism.

DRD2 multilocus analyses: C957T alone explains BP_{ND} effect in striatum

Given that all of the DRD2 SNPs that we examined are believed to be in high linkage disequilibrium,^{21,37,38} we tested whether the addition of either Taq1A or -141C Ins/Del genotype, or both, increased the prediction of BP_{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_{ND} (Supplementary Table S5). The addition of Ins/Del Ins/Ins vs Del Carrier status to C957T T allele dose (T allele #+Ins/Ins status (0,1)) increased the spatial extent of right striatal voxels in which BP_{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 ($P_{FDR} = 0.002$ 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; $P_{FDR} 0.018$ to 0.043 at -20, 8, -8). In addition, the combined C957T+Ins/Del score was associated with higher BP_{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 ($P_{FDR} = 0.087$), 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/thalamus area was identified ($k = 144$, $T = 3.70$ at 8, -22, -4) but did not reach significance ($P_{FDR} = 0.53$; Figure 3, Supplementary Figure S1).

Investigating the effect of a combined multilocus score with number of C957T T alleles, 141C Ins/Ins status (1,0) and number of Taq1A A2 alleles on Fallypride BP_{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/Ins

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_{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_{ND}) in large portions of the striatum. Our results replicate the previous observation with ¹¹C-raclopride PET that C957T T allele dosage is related to higher BP_{ND} in the striatum.^{22,23} 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 (¹⁸F-Fallypride) than Hirvonen et al.^{22,23} (¹¹C-raclopride), further suggesting the robustness of the effect. We also report for the first time the effect of C957T in predicting D2/3 BP_{ND} in specific subregions of the striatum^{63–66} 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 [¹⁸F]-Fallypride over [¹¹C]-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 ¹¹C-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,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 *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).³⁷ 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*.

Figure 3. Fallypride BP_{ND} increases with increasing T allele number in the ventral striatum and putamen. Graphs depict Fallypride BP_{ND} (covarying for age and sex) from anatomically based striatal ROIs as defined by Mawlawi *et al.*⁶³ plotted by C957T genotype. Significant linear effects of T allele dosage were observed for right ($\beta = 0.32$, $P = 0.004$) and left ($\beta = 0.27$, $P = 0.015$) putamen as well as right ($\beta = 0.22$, $P = 0.039$) and left ($\beta = 0.35$, $P = 0.001$) ventral striatum. Note minimum BP_{ND} is set at 10 in all figures to better display group differences. Error bars reflect s.e.m. ** $P < 0.01$, * $P < 0.05$, # $P < 0.10$ reflect significance of across genotype group differences in BP_{ND}, controlling for age and sex. BP_{ND}, nondisplaceable binding potential; ROI, region of interest; SNP, single-nucleotide polymorphism; VS, ventral striatum.

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 BP_{ND} 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 BP_{ND} (Ins/Ins > 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 minor²⁵ or no effect²⁶ of Ins/Del genotype on striatal BP_{ND}. Specifically, Jonsson *et al.*²⁵ observed a small ($P = 0.024$; Cohen's $d \sim 0.69$) effect of -141C Ins/Del with Del Carriers having higher striatal D2/3 BP_{ND}, 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 BP_{ND} but the direction of difference was similar to what we observed (higher for InsIns).²⁶ 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 BP_{ND} 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 (BP_{ND}) in Ins/Ins individuals fits with *in vitro* data using two human-derived cell lines, including Y-79 cells demonstrated to express functional D2 receptors,⁶⁹ which show that the Del variant in -141C results in reduced transcriptional efficiency of the DRD2 gene.¹

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

Although a Taq1A A2/A2 > A1 Carriers effect on striatal BP_{ND} has been observed in a recent meta-analysis of five studies⁴² 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 BP_{ND} in our voxelwise analysis. Furthermore, our ROI analysis found only a very small A2/A2 > A1 BP_{ND} 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-analysis⁴² with the confidence interval including zero, suggesting that the effect was not robust.

The authors of the meta-analysis point 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 BP_{ND}. Importantly, when we controlled for sex and age effects in our ROI analyses, we observed no effect of Taq1A on striatal BP_{ND} (min $P=0.24$; max $\eta^2=0.017$, $d\approx0.26$; Supplementary Table S3). Earlier studies observing Taq1A effects have often not controlled for these potential confounds on BP_{ND}. Furthermore, not all imaging studies have found effects of Taq1A on BP_{ND} 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 multiplication 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 BP_{ND} 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.^{32,33}

Although this study utilized the D2/3 tracer ¹⁸F-Fallypride (vs ¹¹C-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 ¹⁸F-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 ¹¹C-NMB, which is relatively insensitive to endogenous DA levels, did observe an effect of Taq1A on striatal BP_{ND}.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 BP_{ND}.⁴² That paper also did not examine effects for SNPs other than Taq1A, and thus did not address whether C957T status affects striatal BP_{ND}. Clearly, further work is needed to determine the biological processes underlying differences in BP_{ND} 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^{21,37} or between C957T and Taq1A.³⁸ To follow up on this work, we used LDmatrix⁷⁴ 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 BP_{ND} 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 BP_{ND} effects. C957T alone accounted for most of the genetic variance in striatal BP_{ND} 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).⁷⁶ We also observed a smaller cluster in midbrain/thalamus ($k=144$, at 8, -22, -4, $P_{FDR}=0.53$). Given that variation in Fallypride BP_{ND} in midbrain and thalamus has been associated with schizophrenia,⁷⁷ further investigation of genetic polymorphisms that affect BP_{ND} 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, BP_{ND}, 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^{2–4,18} 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 (BP_{ND}) 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,⁸⁵ 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} 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 BP_{ND} we observed in the striatum of CC individuals fits with a wealth of data suggesting substance-dependent individuals display lower D2/3 BP_{ND}.^{93,94} Furthermore our C957T BP_{ND} effects were strongest in the VS (accounting for 13 and 17% of the variance in right and left VS BP_{ND}, 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,⁹⁶ 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^{45–48} 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 groups⁶⁸ 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' sex¹⁹ 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 allele dosage is positively related to striatal D2/3 receptor availability (BP_{ND}) 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 BP_{ND} 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 BP_{ND}, above the C957T effect. These findings demonstrate that DRD2 SNPs beyond Taq1A, specifically C957T, impact individual differences in striatal D2/3 BP_{ND}. 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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