Molecular Neurobiology
https://doi.org/10.1007/s12035-019-1543-0

DRD2 Genotype-Based Variants Modulates D2 Receptor Distribution in Ventral Striatum

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Received: 29 November 2018 / Accepted: 27 February 2019

Abstract
Dopaminergic signaling within the striatum is crucial for motor planning and mental function. Neurons within the striatum contain two dopamine D2 receptor isoforms—D2 long and D2 short. The amount of expression for these receptor isoforms is affected by the genotype within two single nucleotide polymorphisms (SNPs), rs2283265 and rs1076560 (both are in high linkage disequilibrium; C > A), found in the *DRD2* gene. However, it is unclear how these SNPs affect the distribution of D2 receptors in vivo within the nigrostriatal dopaminergic system. We aim to elucidate this with PET imaging in healthy young adults using [^{11}C]-(+)-PHNO. Participants were genotyped for the *DRD2* rs2283265 SNP and a total of 20 enrolled: 9 with CC, 6 with CA, and 5 with AA genotype. The main effect of genotype on [^{11}C]-(+)-PHNO binding was tested and we found significant group effect within the ventral striatum. Specifically, CC and CA carriers had higher binding in this region compared to AA carriers. There were no observed differences between genotypes in other regions within the basal ganglia. Our preliminary results implicate that the polymorphism genotype affects the dopaminergic signaling by controlling either the quantity of D2 receptors, D2 affinity, or a combination thereof within the ventral striatum.

Keywords Dopamine D2 receptor · *DRD2* gene · Positron emission tomography · Single nucleotide polymorphism · [^{11}C]-(+)-PHNO radiotracer

Introduction

The dopamine D2 receptor is one of the important classes of dopamine receptors that is expressed mainly within the striatum [1, 2]. These receptors have inhibitory properties that influence the regulation of dopaminergic neural activity and control the synthesis, release, and uptake of dopamine [1, 3]. Previous studies in humans and animal models showed that the D2 receptors within the striatum mediate locomotion [4], cognitive function such as learning, memory, and decision-making [5–7] as well as personality traits, specifically impulsivity and novelty-seeking behavior [8, 9].

Given the significant impact D2 receptors have on central brain function, these receptors are a primary target for pharmacotherapy to treat various psychiatric and motor disorders [1–3]. Especially in the context of treating these disorders, there are, however, limited studies that have distinguished between pre- and post-synaptic D2 receptors which have been understood to differentially modulate activity of striatal neurons [10–12]. Pre-synaptic D2 receptors are understood to play a role in modulating glutamate release, while post-

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Published online: 08 March 2019

synaptic receptors are understood to be involved in the inhibition of GABA [13–15]. In humans, the individual variances in D2 receptor expression are not well explored, but genetic factors show to play an important underlying role.

Twin studies observed the combined influence of genetic inheritance and individual-specific environmental factors on striatal dopamine function using PET [^{18}F]-DOPA (a radioligand that measures aromatic acid decarboxylase activity, which converts DOPA to dopamine) and [^{11}C]-raclopride (a D2/3 antagonist radiotracer). Heritability was found to vary between functional striatal subregions, with the lowest being in the ventral striatum (limbic striatum), implicating that this region is susceptible to environmental influences [16, 17]. In addition to environmental influences, single nucleotide polymorphisms (SNPs) also influence dopamine function. A recent meta-analysis identified rs1800497 SNP that showed to play a strong role in modulating D2 receptor availability [18].

Another set of important SNPs has been identified, rs2283265 and rs1076560 (C > A), in which they are in high linkage disequilibrium with each other and found within the *DRD2* gene. These SNPs modulate the alternative splicing of exon 6 within this gene resulting in two D2 receptor isoforms—a "long" splice variant (D2L) which is primarily post-synaptic, and a "short" variant (D2S) that is mainly a pre-synaptic autoreceptor [10, 14]. The degree of expression of these receptor isoforms depends on the genotype (i.e., CC, CA, or AA) within these two SNPs [19]. CC homozygote carriers are predicted to have higher levels of pre-synaptic D2S autoreceptors in nigrostriatal terminals [14, 19]. In contrast, the minor A allele carriers (CA and AA) predict an increased expression of post-synaptic D2L receptors in medium spiny GABAergic dendrites in the striatum and nucleus accumbens.

DRD2 polymorphism genotypes may play a role in personality traits relating to emotional processing [20] and schizotypy [21]. The importance of these *DRD2* polymorphism genotypes are also heightened clinically where associations between polymorphism genotype and susceptibility to drug dependence was observed [22, 23], while other studies involving schizophrenia [24] and Parkinson's disease [25, 26] revealed that polymorphism genotype impacted medication responsiveness. These clinical studies suggest that these polymorphisms are a sensitive index of endogenous dopaminergic transmission [25].

Few neuroimaging studies have begun to examine rs2283265 and rs1076560 SNP genotype differences. In addition to using functional MRI, a study also used [^{123}I] FP-CIT SPECT to measure dopamine transporter (DAT) binding, and found that relative to CC genotype, minor A allele carriers had significant negative correlation between left striatal binding and functional MRI BOLD signal change of the left supplementary motor area while completing a visually paced motor task [27]. Another SPECT study showed that subjects with the A allele had reductions of [^{123}I] FP-CIT and [^{123}I] IBZM (antagonist D2 radioligand) within the striatum compared to CC carriers. This study also found that the genotype predicts striatal [^{123}I] IBZM and [^{123}I] FP-CIT binding and the direction of the correlation between D2 signaling with prefrontal activity during performance of working memory [28].

These SPECT studies indicate that the *DRD2* polymorphisms play a role in dopaminergic signaling within the basal ganglia. However, due to the higher noise level and low spatial resolution of SPECT, it remains unclear how these polymorphism genotypes affect the distribution of D2 receptors in vivo within the nigrostriatal dopaminergic system. PET imaging offers higher spatial resolution and signal to noise ratio that can offset the limitations of SPECT imaging. [^{11}C]-(+)-Propyl-Hexahydro-Naphtho-Oxazin ([^{11}C]-(+)-PHNO), a PET radioligand, is sensitive in binding to D2/3 receptors in their active and functionally relevant form in the striatum [29, 30] and demonstrates stronger binding signals in the striatum, globus pallidus, substantia nigra, and anterior thalamus compared to other D2/3 receptor radioligands including antagonist [^{11}C]-raclopride [31–34]. To elucidate *DRD2* rs2283265 polymorphism genotype effects on the distribution of D2 receptors in vivo within subregions of the basal ganglia, healthy young adults grouped by their polymorphism genotype were tested using PET imaging with [^{11}C]-(+)-PHNO. We hypothesize that relative to minor A allele carriers, young adults with the CC genotype will show higher D2 receptor availability (i.e., higher [^{11}C]-(+)-PHNO binding) within the basal ganglia as CC carriers are predicted to have higher levels of pre-synaptic D2S autoreceptors compared to A allele carriers [19].

Materials and Methods

Participants

A total of 69 healthy young adults were recruited through postings and advertisements. After screening interview and saliva sampling for genotyping, 20 participated in the imaging component of the study: 9 with CC genotype (mean age = 30.4 ± 3.78 years; 5 males), 6 with CA genotype (mean age = 24.2 ± 3.19 years; 3 males), and 5 with AA genotype (mean age = 25.6 ± 9.13 years; 3 males). Exclusion criteria included self-reported history of psychiatric and/or neurological disorders, any previous exposure to stimulant drugs, pregnancy, and migraines. Participants were also screened for depression and general cognitive function using the Beck Depression Inventory (BDI-II; exclusion criterion score of > 15) and Montreal Cognitive Assessment (MoCA; exclusion criterion score of < 26), respectively. The study was approved by the Center for Addiction and Mental Health research ethics board (protocol reference number 106/2016). After participants

received a complete description of the study, informed consent was obtained prior to beginning any procedures.

Polymorphism Genotyping

Given that *DRD2* rs2283265 and rs1076560 SNPs are in high linkage disequilibrium with each other, we therefore genotyped healthy volunteers only for the *DRD2* rs2283265 SNP. Genomic DNA was extracted from 2 mL saliva collected and preserved in Oragene DNA kits (DNA Genotek, Kanata, ON). Participants were genotyped for the rs2283265 variant in the *DRD2* gene region using 20 ng DNA in a standard TaqMan SNP genotyping amplification protocol (assay ID C__16070796_10; LifeTechnologies, Foster City, CA) scaled down to a total volume of 10 μL. Genotypes were visualized and automatically called on the ViiA7™ Real-Time PCR System (LifeTechnologies, Foster City, CA) using ViiA7™ software v1.2.4. All genotyping results were confirmed by two research personnel.

Imaging Acquisition

Each study participant underwent a single PET and MRI scan. PET scans were acquired on a high-resolution PET/CT Siemens-Biograph HiRez XVI (Siemens Molecular Imaging Knoxville, TN, USA) operating in three-dimensional (3D) mode with an intrinsic in-plane resolution of 4.6 mm full width at half-maximum (FWHM). The radiosynthesis of [^{11}C]-(+)-PHNO has been described in detail elsewhere [35]. To prevent head movement during the PET scan, a thermoplastic facemask was custom-fitted to each participant and attached to a head-fixation system (Tru-Scan Imaging, Annapolis). Prior to the PET scan, a low dose (0.2 mSv) CT scan was performed and used for attenuation correction. For each PET scan, [^{11}C]-(+)-PHNO was injected as a bolus into an intravenous line placed in the antecubital vein. PET emission data were acquired over 90 min at complete resting state and subsequently redefined into 30 frames of progressively increasing duration (15 1-min frames and 15 5-min frames). For each 3D sinogram, data were normalized with attenuation and scatter corrected before applying Fourier rebinning to convert the 3D sinograms into 2D sinograms. The 2D sinograms were then reconstructed into image space using a 2D filtered back projection algorithm, with a ramp filter at Nyquist cutoff frequency. After reconstruction, a Gaussian filter with a 5 mm FWHM was applied and the images calibrated to nCi/cc. The spatial resolution of the reconstructed images was 2 × 2 × 2 mm ($X \times Y \times Z$).

To rule out structural lesions in the brain and to provide anatomical reference for the parametric PET image analysis, a T1-weighted MRI image was obtained from each participant using high-resolution MRI (GE Discovery MR750 3 T; T1-weighted images, fast-spoiled gradient echo with repletion time = 6.7 msec, echo time = 3.0 msec, flip angle = 8 mm, slice thickness = 1 mm, number of excitations = 1, matrix size = 256 × 192).

Image Analysis

Image preprocessing was done using in-house software, Regions of Mental Interest (ROMI) [36]. ROMI works by using Statistical Parametric Mapping (SPM8, Welcome Department of Imaging Neuroscience, London, UK) where each individual's MRI was used to nonlinearly transform a standardized brain template (International Consortium for Brain Mapping/Montreal Neurological Institute 152 MRI) with predefined cortical and subcortical regions of interest (ROIs). The individual ROI template was then further refined based on gray matter probability of the segmented MRI. The refined individual ROIs were aligned and resliced using a normalized mutual information algorithm [37] to match the individual's PET scan. Finally, time activity curves from ROIs within the basal ganglia were obtained from the dynamic [^{11}C]-(+)-PHNO PET images in native space with reference to co-registered MRI images of ROIs.

To derive the nondisplaceable binding potentials (BP_{ND}) of [^{11}C]-(+)-PHNO from basal ganglia ROIs, simplified reference tissue model 2 (SRTM2; [38]) was applied with the cerebellum as a reference region using the PMOD software (version 3.6, PMOD Technologies, Zurich, Switzerland). SRTM with cerebellar input function has been validated to be reliable for quantifying D2/3 receptors with [^{11}C]-(+)-PHNO [39]. The basal ganglia ROIs examined were the caudate, putamen, and ventral striatum which was defined according to Mawlawi and colleagues [40] while the globus pallidus was defined using the WFU-Pickatlas toolbox [41].

Statistical Analysis

SPSS (version 21; Chicago, IL) was used to conduct Pearson's correlation between [^{11}C]-(+)-PHNO BP_{ND} in each ROI on the one hand, and age, BDI, and MoCA score on the other. SPSS was also used to compare the extracted BP_{ND} between the three polymorphism genotypes (i.e., participants with the CC, CA, and AA genotypes) for each of the ROIs using ANCOVA, controlling for sex and BDI score. Post hoc independent sample *t* tests were used to test for differences between genotypes and were corrected for multiple comparisons using false discovery rate (FDR).

Demographic factors were also analyzed to test for differences between groups. An ANOVA and Bonferroni's post hoc testing were performed on age, MoCA scores, BDI, and quality (i.e., mass and specific activity) and quantity (i.e., dose) of injected radioligand across all three participant groups. To test for differences in sex proportions between groups, a chi-squared analysis was performed. For all tests, the alpha level

was set to 0.05 to determine statistical significance while 0.10 was the threshold for trend levels.

Results

The demographic and psychological characteristics for each group are summarized in Table 1. Genotype groups were comparable for age, sex, BDI, and MoCA. Sex ratio across all three groups also did not differ significantly. There were no differences between groups in relation to the radiotracer injected dose, mass, and specific activity.

We observed significant and trending correlations between BDI score and BP_{ND} within the ventral striatum ($r = 0.48$; $p = 0.03$), putamen ($r = 0.49$; $p = 0.03$), caudate ($r = 0.40$; $p = 0.07$), and globus pallidus ($r = 0.42$; $p = 0.06$). However, there were no significant correlations of BP_{ND} with age and MoCA score. Given that BP_{ND} was strongly associated BDI score and that sex is known to have significant effect on dopamine levels in healthy controls [42, 43], we therefore controlled for these two variables in ANCOVA.

Results from ANCOVA revealed a significant genotype main effect of [^{11}C]-(+)-PHNO BP_{ND} within the ventral striatum ($F(2, 15) = 6.69$, $p = 0.008$). Following the pairwise comparisons, the BP_{ND} of the CC and CA group were significantly higher than the AA group ($p = 0.02$; $p = 0.03$, respectively) (Fig. 1). However, for other ROIs examined, there were no significant main effects of [^{11}C]-(+)-PHNO BP_{ND} within the putamen ($F(2, 15) = 2.71$, $p = 0.10$), caudate ($F(2, 15) = 0.90$, $p = 0.43$), and globus pallidus ($F(2, 15) = 0.02$, $p = 0.98$) (Fig. 2).

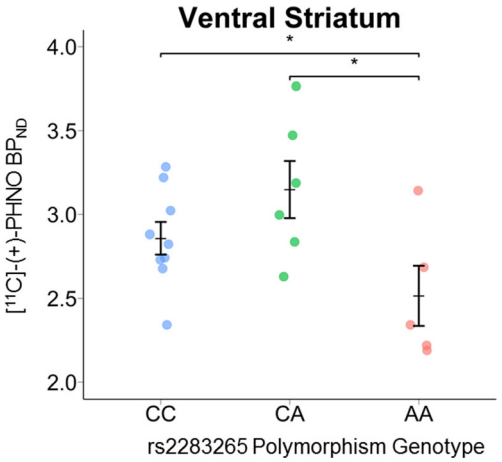

Fig. 1 A scatterplot showing BP_{ND} differences between genotype groups in the ventral striatum. Participants with the AA genotype were significantly lower than the CC ($p = 0.02$) and CA genotype ($p = 0.03$) in this ROI. *$p < 0.05$ (FDR-corrected)

Discussion

To elucidate *DRD2* rs2283265 polymorphism genotype effects on the distribution of D2 receptors in vivo within subregions of the basal ganglia, healthy young adults grouped by their genotype were tested using PET imaging with [^{11}C]-(+)-PHNO. We report a main effect of genotype differences of tracer binding within the ventral striatum and not in other regions including the caudate, putamen, and globus pallidus. Specifically, our hypothesis was confirmed only in the ventral striatum and that carriers of the CC genotype had higher binding compared to AA carriers, but not CA carriers in this region.

The ventral striatum is a region within the basal ganglia rich in D2 receptors and is understood to play a critical role in decision-making and reward processing [44]. Difference in radioligand binding between the CC/CA and AA genotype group in this region can be explained by the quantity of available D2 receptors in this brain region [44, 45], where AA carriers have lower amount of D2 receptors available relative to CC/CA carriers. In addition, differences in D2 affinity for [^{11}C]-(+)-PHNO within the ventral striatum could be another explanation, since this agonist radioligand is expected to preferentially bind to the high-affinity state of the receptor [46–48]

Table 1 Demographic, behavioral, and PET imaging characteristics of participants with the CC, CA, and AA polymorphism genotype

	CC	CA	AA	*p* value
N (M:F)	9 (5:4)	6 (3:3)	5 (3:2)	0.95[a]
Age (years) ± SD (range)	30.4 ± 3.78 (26–38)	24.2 ± 3.19 (20–29)	25.6 ± 9.13 (20–41)	0.10
BDI ± SD	3.78 ± 4.44	7.67 ± 5.72	6.6 ± 5.41	0.33
MoCA ± SD	29 ± 1.5	29 ± 1.1	28.8 ± 0.84	0.95
[^{11}C]-(+)-PHNO dose (mCi) ± SD	9.33 ± 1.33	9.64 ± 0.37	9.73 ± 0.46	0.71
[^{11}C]-(+)-PHNO mass (μg) ± SD	1.93 ± 0.29	1.88 ± 0.31	1.69 ± 0.39	0.44
[^{11}C]-(+)-PHNO specific activity (mCi/μmol) ± SD	1233.90 ± 291.60	1301.41 ± 228.16	1510.82 ± 500.07	0.36

BDI Beck Depression Inventory, *MoCA* Montreal Cognitive Assessment

[a] Pearson's chi-square

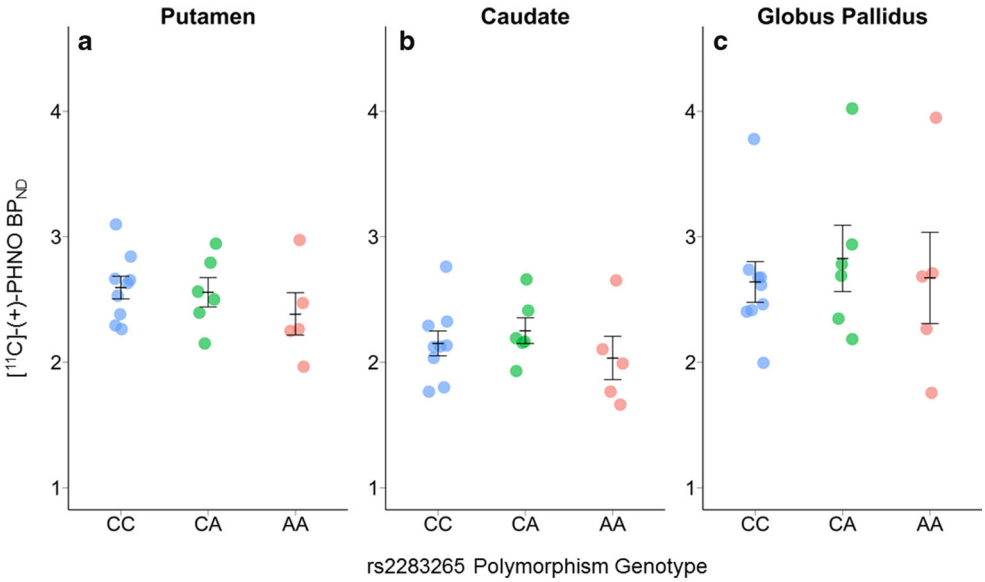

Fig. 2 There were no significant main effects in [^{11}C]-(+)-PHNO BP_{ND} between genotype groups within the putamen ($p = 0.10$); the caudate ($p = 0.43$); and the globus pallidus ($p = 0.98$)

according to the two- and three-state model of receptor activation [48, 49]. Previous studies using [^{11}C]-(+)-PHNO showed relatively higher binding in the ventral striatum and globus pallidus in humans compared to other regions within the basal ganglia [34, 50]. This finding was also consistent in nonhuman primates which showed a 196% uptake of [^{11}C]-(+)-PHNO within the ventral striatum relative to binding within all of the striatum [32]. In conjunction with these previous findings and that polymorphism genotypes modulated [^{11}C]-(+)-PHNO binding in the ventral striatum, it implicates that the SNP affects the dopaminergic signaling by controlling either the quantity of D2 receptors or D2 high-affinity state availability or a combination thereof within this region.

Our results with the ventral striatum contrast with previous molecular imaging studies examining the D2 receptor availability in relation to the rs1076560 *DRD2* polymorphism which is in high linkage disequilibrium with our currently examined polymorphism rs2283265. While one study using [^{123}I] FP-CIT SPECT found no genotype differences in the rs1076560 polymorphism for DAT binding [51], another study using both [^{123}I] FP-CIT and [^{123}I] IBZM (antagonist D2 radioligand) found that A allele carriers had lower binding for both tracers compared to the CC group [28]. More specifically, visualizing the D2 receptors through [^{123}I] IBZM, investigators found that CC carriers had higher binding in the right putamen, but higher binding of DAT in left putamen with [^{123}I] FP-CIT relative to those with the minor A allele [28].

Interestingly, we saw trend level main effect within the putamen but did not reach the level of significance. [^{11}C]-(+)-PHNO in humans have been shown to be sensitive to fluctuations in endogenous dopamine levels compared to other antagonist radioligands [32, 39, 52]. This sensitivity may explain the lack of differential bindings observed between genotype groups within the putamen [53]. Given that going from CC carriers to AA carriers, there is decreasing D2S autoreceptor availability and increasing endogenous dopamine [27, 54], we speculate that such change in [^{11}C]-(+)-PHNO competition with endogenous dopamine and receptor availability results in negligible changes across genotype group particularly within the putamen [53].

Previous studies have examined polymorphism genotype rs2283265 and rs1076560 influence on the expression of D2S and D2L mRNA in postmortem human samples [19, 23]. These studies show that individuals carrying the minor A allele had significantly reduced relative expression of D2S mRNA within the prefrontal cortical and striatal regions compared to CC carriers. Moyer and colleagues [23] specifically showed that the relative expression of D2S mRNA was significantly lower for both CA and AA compared to CC carriers within the prefrontal cortex and putamen, with the level for CA being higher than AA carriers but the difference was not significant. Although our study with [^{11}C]-(+)-PHNO PET imaging cannot directly observe D2S and D2L, we hypothetically should have observed a similar binding pattern in vivo, but instead we observed that only the AA carriers was lower in receptor availability compared to both CC and CA carriers. Our findings suggest that the C allele has a complete dominance as opposed to incomplete dominance heredity. The C allele may be completely masking the phenotype of the A allele as opposed to the CA genotype producing intermediary binding effects within the ventral striatum. In addition to small sample size that could affect our ability to observe CA intermediary binding effects, another explanation could be that CA carriers may have lower D2 receptor availability compared to CC carriers, but have a high proportion of receptors in their high-affinity state yielding negligible difference in radioligand binding between these two genotype groups [55]. These

speculations cannot be confirmed in our study and would need follow-up investigations to examine these relationships within the ventral striatum.

Genotype polymorphisms of rs2283265 and rs1076560 SNPs have important clinical implications in Parkinson's disease and schizophrenia. In patients with schizophrenia and healthy controls, carriers of the minor A allele not only showed impaired working memory performance, they were also associated with reduced relative D2S mRNA expression compared to CC carriers in the postmortem prefrontal cortex [13]. Functional MRI results showed that schizophrenia patients with the CA genotype showed reduced working memory performance—one of the negative symptoms of this disorder—and reduced brain activity where they do not fully engage their prefrontal-striatal resources. From the perspective of Parkinson's disease (PD), a large multisite study showed that patients with the CC genotype have shown the most significant impact on their motor and mental function improvement with the drug rasagiline compared to patients with the minor A allele [26]. PD patients often suffer from behavioral complications such as apathy [56] which is also shared by patients with schizophrenia as one of their negative symptoms [57]. Apathy is commonly described as impaired motivation and reduced goal-directed behavior [57]. Converging evidence from the PD and schizophrenia neuroimaging literature points to the critical role of the ventral striatum as part of the greater circuitry involved in the mesolimbic-mesocortical pathway in dopamine transmission [57, 58]. With polymorphism genotype affecting D2 receptor availability within the ventral striatum as shown in our study, clinical neuroimaging studies are warranted to examine the impact the genotype has on dopaminergic signaling in these clinical groups. These further investigations will potentially help reveal new biomarkers that may help refine biological targets in the treatment of schizophrenia and PD patients suffering complications especially relating to motivation and self-generating purposeful behaviors.

It becomes a question as to whether our results can be attributed to both D2 and D3 receptors since the ventral striatum has both receptors and that [^{11}C]-(+)-PHNO binds to D3 as well as D2 receptors. Based on several observations from previous research, we posit that the genotype difference is attributed to D2 rather than the D3 receptors. For example, a study in humans using the D3 antagonist GSK59880 showed approximately 74% of the [^{11}C]-(+)-PHNO binding signal in the ventral striatum was attributed to D2 while 26% was attributed to D3 receptors [59]. In a similar vein in nonhuman primates, it has been estimated that about 30% of the [^{11}C]-(+)-PHNO signal in this region was occupied by the D3 antagonist BP897 [32]. It is therefore unlikely that our results with [^{11}C]-(+)-PHNO represent altered expression of D3 because its signal within the ventral striatum is small.

We made efforts to avoid potential confounding factors influencing genotype effect on [^{11}C]-(+)-PHNO bindings where we matched age, sex, BDI, and MoCA scores. However, there are limitations to this study that should be addressed. Future replication studies are encouraged with larger sample size with the inclusion of all known dopamine-related polymorphisms to strengthen the present findings. [^{11}C]-(+)-PHNO PET imaging cannot visualize the D2L and D2S independently and it is unclear whether this radioligand preferentially binds to pre-synaptic D2S or post-synaptic D2L receptors. Even though majority of the [^{11}C]-(+)-PHNO signal in the ventral striatum is attributed to the D2 binding, we cannot disentangle the contribution of the D3 receptors and its role cannot be ruled-out. Since dopamine release was not measured, we could not address the full role the genotype has on endogenous dopamine levels. Future studies are needed to address these shortcomings.

Conclusion

Our preliminary results reveal an association between *DRD2* genotype-based variants of the rs2283265 polymorphism and [^{11}C]-(+)-PHNO binding in the ventral striatum in healthy young adults. Specifically, AA carriers had lower BP_{ND} compared participants with the CC and CA genotype. This finding implicates that the genotype controls dopaminergic signaling by modulating either the D2 affinity or the quantity of D2 receptors, or a combination thereof within the ventral striatum. Since this study provides a foundational understanding of how this polymorphism genotype affects the D2 receptor distribution in healthy adults, future neuroimaging studies are needed to explore how the distribution patterns are related with individual behavioral factors and changes in pathological conditions such as schizophrenia and PD.

Acknowledgments The authors thank the Psychiatric Neurogenetics Laboratory team for performing the genotyping; and Laura Nguyen, Anusha Ravichandran, Colin Cole, and Alvina Ng for their technical assistance.

Authors' Contributions Study conception and design: MV and APS
Data acquisition: MV and YK
Data analysis: MV, SSC, and PR
Data interpretation: MV and APS
Manuscript drafting: MV
Manuscript review and critique for important intellectual content: MV, SSC, MM, RC, PR, JK, YK, AM, and APS
Approved version to be published: MV and APS

Funding information Dr. Antonio Strafella was supported by the Canadian Institutes of Health Research (CIHR) (MOP 136778) and the Canada Research Chair program. Mikaeel Valli was supported by the CIHR's Canada Graduate Scholarship, Master's Program. Dr. Robert Chen was supported by the Catherine Manson Chair in Movement Disorders.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethical Approval All procedures performed in this study were in accordance with the standards of the Center for Addiction and Mental Health research ethics board (protocol reference number 106/2016) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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