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Common Variation in the DOPA Decarboxylase (DDC) Gene and Human Striatal DDC Activity *In Vivo*

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The synthesis of multiple amine neurotransmitters, such as dopamine, norepinephrine, serotonin, and trace amines, relies in part on DOPA decarboxylase (DDC, AADC), an enzyme that is required for normative neural operations. Because rare, loss-of-function mutations in the *DDC* gene result in severe enzymatic deficiency and devastating autonomic, motor, and cognitive impairment, *DDC* common genetic polymorphisms have been proposed as a source of more moderate, but clinically important, alterations in DDC function that may contribute to risk, course, or treatment response in complex, heritable neuropsychiatric illnesses. However, a direct link between common genetic variation in *DDC* and DDC activity in the living human brain has never been established. We therefore tested for this association by conducting extensive genotyping across the *DDC* gene in a large cohort of 120 healthy individuals, for whom DDC activity was then quantified with [^{18}F]-FDOPA positron emission tomography (PET). The specific uptake constant, K_i, a measure of DDC activity, was estimated for striatal regions of interest and found to be predicted by one of five tested haplotypes, particularly in the ventral striatum. These data provide evidence for *cis*-acting, functional common polymorphisms in the *DDC* gene and support future work to determine whether such variation might meaningfully contribute to DDC-mediated neural processes relevant to neuropsychiatric illness and treatment.

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INTRODUCTION

DOPA decarboxylase (DDC; aromatic L-amino acid decarboxylase) is a pyridoxal 5′-phosphate-reliant enzyme that facilitates the synthesis of several key neuroactive biogenic amines in the brain. DDC-mediated decarboxylation represents the last committed step in both dopamine and serotonin synthesis, the penultimate step in norepinephrine synthesis, and a critical process in the synthesis of several centrally expressed trace amines, including 2-phenylethylamine, for which it is rate limiting (Zhu and Juorio, 1995). The essential nature of this enzyme for central nervous system (CNS) physiology is evident in cases of rare, loss-of-function mutations in the *DDC* gene that lead to a devastating neurodevelopmental syndrome (DDC deficiency, OMIM #608643). In this condition, dramatic DDC enzymatic insufficiency results in a constellation of severe autonomic, motor, and cognitive impairments that manifest early in life (Brun *et al*, 2010).

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The hypothesis that more common *DDC* genetic variation might induce subtler but nonetheless biologically meaningful alterations in DDC enzymatic activity has formed the basis for many previous investigations. In clinical genetic association studies, for example, *DDC* variation has been linked to age of onset in schizophrenia (Borglum *et al*, 2001), and implicated in risk for a range of other conditions, including attention-deficit hyperactivity disorder (ADHD) (Guan *et al*, 2009; Lasky-Su *et al*, 2008), autism (Toma *et al*, 2012), nicotine dependence (Ma *et al*, 2005), and migraine (Corominas *et al*, 2010). Similarly, recent reports have linked single-nucleotide polymorphisms (SNPs) in *DDC* with clinically relevant neurocognitive and behavioral phenotypes, such as alerting attention (Zhu *et al*, 2013), suicidal behavior (Giegling *et al*, 2008), and alcohol consumption patterns (Pan *et al*, 2013). In Parkinson's disease, an illness involving nigrostriatal dopaminergic neuronal degeneration, L-3,4-dihydroxyphenylalanine (L-DOPA) is a gold-standard treatment whose efficacy is thought to depend largely on its conversion to dopamine by DDC. Preliminary data indicate that common *DDC* polymorphisms may lead to alterations in therapeutic response to L-DOPA (Devos *et al*, 2014). However, whether common genetic variation in *DDC* is in fact predictive of DDC activity measured in the living human brain remains untested.

The positron emission tomography (PET) radiopharmaceutical L-3,4-Dihydroxy-6-[^{18}F]fluorophenylalanine

([^{18}F]-FDOPA) is a fluorinated analog of L-DOPA and therefore a DDC substrate whose specific uptake in the brain and subsequent vesicular storage as its decarboxylation product, [^{18}F]fluorodopamine, is driven by DDC activity and can be reliably estimated *in vivo* (Gjedde *et al*, 1991; Hoshi *et al*, 1993). Twin studies indicate that a substantial portion of interindividual variation in striatal DDC activity is heritable (Stokes *et al*, 2013). Developing an understanding of the precise genetic factors underlying this heritability has become increasingly important in view of accumulating evidence supporting PET-quantified striatal DDC activity as a significant correlate of heritable neuropsychiatric illnesses. Exaggerated striatal DDC activity has been a replicated finding not only in groups of individuals with schizophrenia (Howes *et al*, 2012; Meyer-Lindenberg *et al*, 2002) but also in those with clinical or genetic risk factors for schizophrenia (Egerton *et al*, 2013; Howes *et al*, 2011; Huttunen *et al*, 2008), suggesting it may be an endophenotype (Gottesman and Gould, 2003) valuable for molecular discovery and biological validation of identified risk genes. Preliminary reports have noted abnormalities of [^{18}F]-FDOPA uptake in the striatum of individuals with autism spectrum conditions (Nieminen-von Wendt *et al*, 2004), alcohol dependence (Kumakura *et al*, 2013), and ADHD (Ludolph *et al*, 2008) but await further corroboration. Here, in a large cohort of healthy volunteers, we employed [^{18}F]-FDOPA PET and extensive genotyping across *DDC* to determine whether and what portion of interindividual variation in striatal DDC activity might be explained by common *DDC* polymorphisms.

MATERIALS AND METHODS

Subjects

A total of 120 Caucasian healthy volunteers (ages 20–55 years, mean 34.8 ± 10.4 years; 61 women) participated after providing informed consent as approved by the National Institute of Mental Health Institutional Review Board and National Institutes of Health (NIH) Radiation Safety Committee. Participants were recruited from the local community and had no psychiatric, neurological, or major medical illness, including any substance use disorder, as determined by clinician-administered standardized clinical interview (SCID) (First *et al*, 1996), structural MRI, routine laboratory tests, qualitative urine toxicology (including amphetamines, cocaine, opiates, cannabinoids, sedative hypnotics), medical history, and physical examination.

Genetics

A total of 23 markers with minor allele frequencies $>5\%$ were selected from across the *DDC* gene providing coverage of HapMap annotated common variants between 20 kb upstream and 2 kb downstream of *DDC* in the CEU sample (at $r^2 > 0.7$ by 2- and 3-marker tagging as implemented by Haploview). All markers were intronic variants, except for rs11575575, a downstream SNP, and rs11575542, a non-synonymous coding variant resulting in an arginine-to-glutamine amino acid change.

All subjects were genotyped using the TaqMan 5′ exonuclease assay (Applied Biosystems, Foster City, CA). *DDC* haplotypes were generated using PHASE software (http://stephenslab.uchicago.edu/software.html). Haplotypes with frequencies of at least 5% were selected for study, and the estimated number of each of the haplotypes carried by each individual were calculated based on the probabilities provided by PHASE. Hardy–Weinberg equilibrium exact testing was performed in R (Graffelman, 2014; Wigginton *et al*, 2005).

Neuroimaging

PET sessions were conducted in a fasting state (at least 6 h) to prevent competitive inhibition of radiotracer transport across the blood–brain barrier by ingested large amino acids. Caffeine and nicotine were not permitted for 4 h before scanning, and the absence of any psychotropic or other confounding substance exposure was confirmed before PET procedures. To reduce peripheral radiotracer metabolism, a 200 mg dose of carbidopa was administered by mouth 1 h before [^{18}F]-FDOPA injection. Volunteers underwent PET imaging with a General Electric Advance 3D PET camera (32 planes, 6.5 mm FWHM) while wearing a thermoplastic mask to limit head motion and remaining in an awake, resting state. An 8-min transmission scan for attenuation correction and—immediately following intravenous injection of 7–16 mCi (mean mass 3.16 ± 0.78 mg, specific activity 1082.96 ± 358.23 mCi/mmol) [^{18}F]-DOPA—a 90-min series of 27 dynamically acquired emission scans were collected for each session.

Filtered back-projection reconstruction was performed with corrections for radioactivity decay, dead time, and scatter after injection, and a registered attenuation correction algorithm was applied that included realignment for the purpose of motion correction. All emission scans were individually realigned to the twenty-first frame with a rigid body transformation using FLIRT (http://fsl.fmrib.ox.ac.uk/fsl/) to correct for interscan motion. Because the first three frames have relatively lower signal with which to guide registration, these images were realigned to the reference frame by adopting the same transformation matrix as the fourth frame. Using the mean of these frames as a target, each individual's separately acquired, nonparametric non-uniform intensity normalized (Sled *et al*, 1998), T1-weighted structural MRI image was coregistered to his or her PET data with SPM5 (http://www.fil.ion.ucl.ac.uk/spm/). Freesurfer-assisted MRI segmentation (http://freesurfer.net/) followed by manual editing using ITKSnap (Yushkevich *et al*, 2006), which was conducted blinded to genetic status, delineated three striatal regions of interest (ROIs) representing known areas of highest tracer uptake signal-to-noise ratios: bilateral dorsal caudate, bilateral dorsal putamen, and bilateral ventral striatum. A standard reference region, the cerebellum (Calne *et al*, 1985), was defined in a manner similar to ROIs above and excluded both the vermis, an area of potential specific binding, as well as lateral/superior regions abutting the transverse venous sinuses.

ROI and reference time-activity curves were extracted from the emission frames, and the kinetic rate constant, K_i, representing specific tracer uptake and a measurement of DDC activity, was calculated for each ROI with PMOD software (http://www.pmod.com/), using the previously validated, noninvasive graphical linearization method established by Patlak and Gjedde (Hoshi *et al*, 1993; Patlak and Blasberg, 1985). For *post hoc*, voxel-wise analyses, ANTS

Table 1 Genotyped Markers and Identified Common Haplotypes

SNP	Haplotype 1	Haplotype 2	Haplotype 3	Haplotype 4	Haplotype 5
rs11575575	G	G	G	T	G
rs2060762	G	A	G	G	G
rs11575542	G	G	G	G	G
rs4947535	A	T	A	T	A
rs11575536	C	C	C	C	T
rs11761683	T	C	C	T	T
rs11238134	C	A	A	C	C
rs745043	C	A	C	C	C
rs10899736	A	G	G	G	G
rs11575404	T	T	C	T	T
rs3807558	C	C	C	C	C
rs11575387	A	A	A	C	A
rs17634958	G	A	G	G	G
rs6592961	G	G	A	A	G
rs6950777	G	A	G	G	G
rs998850	G	C	C	G	C
rs17133877	G	G	G	G	G
rs3779078	G	A	G	G	G
rs2044859	T	C	T	T	C
rs2876830	G	G	A	G	G
rs4947644	C	T	T	C	T
rs6593010	A	G	G	A	A
rs6593011	C	C	C	C	A

software (http://stnava.github.io/ANTs/) was used to warp each individual's PET data into standardized Montreal Neurological Institute (MNI) space using the transform between the individual's coregistered MRI and an MNI space DARTEL-generated target image derived from 240 healthy subjects. To improve signal-to-noise ratios, voxel-wise smoothing (10 mm isotropic Gaussian kernel) was applied as implemented in SPM. Estimation of K_i for each voxel in the striatum was then conducted on the anatomically normalized PET data with PMOD software, using the native-space cerebellar reference region time-activity curve as the input function.

Statistical Analyses

Statistical analyses for demographic and ROI K_i association testing were performed in SPSS (http://www-01.ibm.com/software/analytics/spss/) with standard nonparametric tests and general linear modeling. To better characterize effects, *post hoc* statistical analyses of voxel-wise K_i were conducted in SPM within high signal voxels (1 SD above the whole-brain mean voxel value) and with an uncorrected threshold of $p<0.005$.

RESULTS

Haplotypes and Demographics

Five common haplotypes were identified (Table 1). The frequencies for haplotypes 1–5 were: 35%, 15%, 11%, 7%, and 5%, respectively. None of the haplotypes showed distribution differences across sex or handedness, except haplotype 3 that showed evidence for unequal distribution in males *vs* females (Mann–Whitney $U=2112.5$, $p=0.020$). None of the haplotypes showed association with age (all p's $\geqslant 0.1$). None of the studied SNPs showed deviation from Hardy–Weinberg equilibrium (all p's >0.1).

Neuroimaging

Estimation of five simple multivariate general linear models (one for each haplotype predictor) with regional striatal K_i values (caudate, putamen, ventral striatum) as the dependent variables revealed a specific and significant effect of the estimated number of haplotype 4 copies carried (Pillai's trace $=0.1$; $F(3, 116)=4.31$; $p=0.006$). None of the other haplotypes approached significance (all p's >0.2). Follow-up univariate tests indicated that this finding was strongest for the ventral striatum ($F(1, 118)=5.28$; $p=0.023$) and dorsal putamen ($F(1, 118)=3.98$; $p=0.048$), but was not present for dorsal caudate ($p>0.9$). Repeat multivariate and follow-up univariate testing of haplotype 4 effects on K_i were conducted with haplotype 4 recoded as a binary variable (carrier (estimated number of copies >0.5) *vs* noncarrier (all others)), and the results were nearly identical (multivariate, ventral striatum univariate, putamen univariate p's $=$ 0.006, 0.024, and 0.061 respectively). A simple multivariate linear analysis confirmed no significant relationship between the amount of activity injected and the K_i parameter

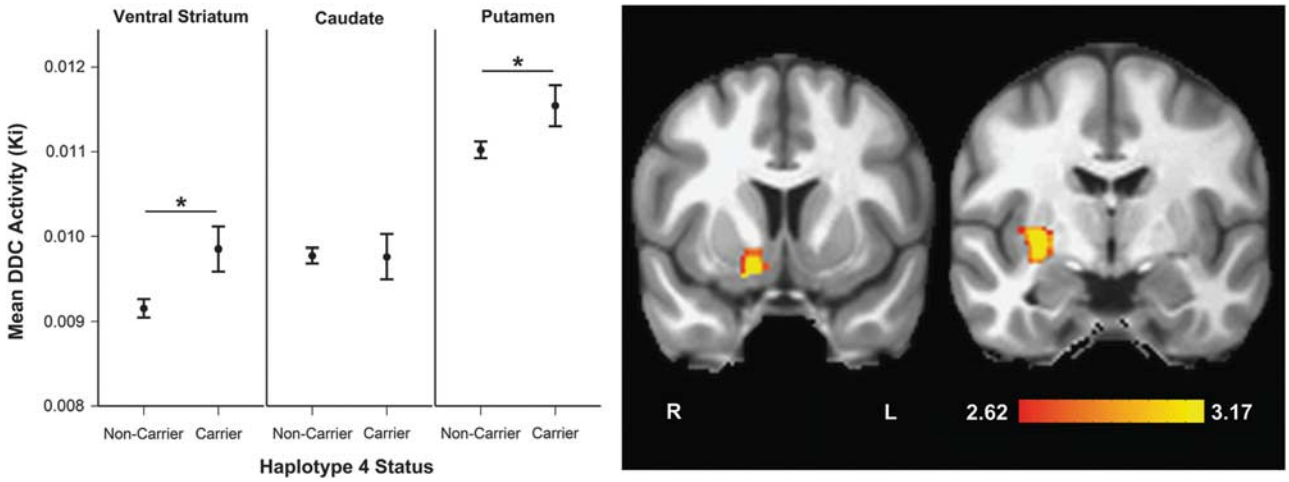

Figure 1 DDC haplotype 4 effects. The graph shows region of interest (ROI) analyses with mean DDC activity values and standard errors for each ROI by haplotype 4 status. Multivariate Pillai's trace = 0.1; $F(3, 116) = 4.31$; $p = 0.006$. Asterisks indicate $p < 0.05$ for univariate comparisons. Coronal slices show results of *post hoc* voxel-wise analysis indicating locations of DDC effects in the striatum (at MNI Y = 9 for ventral striatum and Y = −6 for caudate). Colored clusters represent areas where more copies of the DDC haplotype predicted greater DDC activity. Color bar indicates *t*-values, and data are displayed by radiological convention at a voxel-wise, uncorrected threshold of $p < 0.005$.

(Pillai's trace = 0.016; $F(3, 116) = 0.62$; $p = 0.60$). In addition, a simple linear regression analysis confirmed no significant relationship between the estimated number of haplotype 4 copies carried and terminal frame cerebellar activity ($p > 0.3$), a result that was unchanged when including the amount of activity injected in the model.

The *post hoc* voxel-wise analysis suggested that the strongest effects of haplotype 4 localized to foci in right medial ventral striatum and mid postcommissural putamen (peak voxel MNI coordinates: x, y, z = 16.5, 1.5, −24; $p = 2.4 \times 10^{-4}$ and 30, −6, −3; $p = 4.9 \times 10^{-4}$ respectively), where the haplotype predicted greater uptake rates (Figure 1, Table 2).

DISCUSSION

The present work provides novel evidence that common variation in the *DDC* gene predicts measureable differences in striatal DDC activity. Unlike rare, loss-of-function mutations responsible for clinical DDC deficiency syndrome, *DDC* haplotype 4 was associated with moderate and clinically silent effects within the normal range of DDC functioning as indexed by specific [^{18}F]-FDOPA uptake. In previous studies, striatal DDC activity, so measured, shows variability across healthy individuals that is both heritable and linked to cognitive, personality, and neuropsychiatric illness risk (Borglum *et al*, 2001; Corominas *et al*, 2010; Guan *et al*, 2009; Lasky-Su *et al*, 2008; Ma *et al*, 2005; Pan *et al*, 2013; Toma *et al*, 2012; Zhu *et al*, 2013); the present data suggest the possibility that this variability may be under genetic control via *cis*-acting polymorphisms. Whether the haplotypic effects denoted here might mechanistically contribute to or, alternatively, lend noise to some of these past association findings cannot be confidently determined by these results, but both possibilities highlight the importance of understanding the sources of variance in this widely used measure.

The most robust genetic association was localized to the ventral striatum, a region closely allied with limbic circuitry and dysfunctional in disorders affecting motivated behaviors, such as addictions, mood disorders, and schizophrenia (Everitt and Robbins, 2005; Haber and Knutson, 2009; Russo and Nestler, 2013; Schlagenhauf *et al*, 2014). Patient studies will be required to determine whether the identified haplotype may be of particular significance in these conditions. By the same token, the observed haplotype effects were not diffusely observed (eg, caudate ROI analyses did not show a statistically significant effect), suggesting regional specificity in the impact of this genetic variation, as has been observed with other dopamine-related proteins, in which gene polymorphisms predict the expression of certain splice variants in select brain structures (Kunii *et al*, 2014). This regional specificity may reflect engagement of unique molecular mechanisms (eg, preferential DDC isoform transcription) induced by particular intracellular signaling characteristics of dopaminergic neurons projecting to ventral striatum and putamen or, alternatively, it may be that the impact of *DDC* sequence variation on DDC activity in select caudate projecting neurons is negated or dwarfed by differential local regulatory constraints.

Haplotype 4 has not previously been shown to be functional at the enzymatic level, and further work is needed to understand the extent to which it may not only bias striatal [^{18}F]-FDOPA measurements, which are of crucial importance for a range of basic and clinical PET research applications, but also affect clinically meaningful outcomes. For instance, in conditions treated with L-DOPA, a DDC substrate, DDC enzymatic properties may be important determinants of L-DOPA dose–response relationships, as hypothesized in recent work in Parkinson's disease that identified preliminary associations between common genetic variation and motor responses to L-DOPA therapy (Devos *et al*, 2014). In that study, the major allele of rs921451 predicted greater clinical response but not peripheral pharmacokinetic measurements. Haplotype 4 contains the major allele of rs6593010, which is in linkage disequilibrium with rs921451 in the CEU HapMap sample ($r^2 = 0.805$; data not shown). The direction of those findings appears to agree

Table 2 DDC Activity (K_i) by Haplotype 4 Status

Region	Haplotype 4 status	DDC activity (K_i) Mean	SEM	SD
Ventral striatum	Noncarrier	9.15×10^{-3}	1.09×10^{-4}	1.12×10^{-3}
	Carrier	9.85×10^{-3}	2.63×10^{-4}	1.02×10^{-3}
Caudate	Noncarrier	9.78×10^{-3}	9.12×10^{-5}	9.34×10^{-4}
	Carrier	9.76×10^{-3}	2.64×10^{-4}	1.02×10^{-3}
Putamen	Noncarrier	1.10×10^{-2}	9.85×10^{-5}	1.01×10^{-3}
	Carrier	1.15×10^{-2}	2.43×10^{-4}	9.41×10^{-4}
Ventral striatum peak	Noncarrier	3.00×10^{-3}	4.56×10^{-5}	4.67×10^{-4}
	Carrier	3.42×10^{-3}	1.18×10^{-4}	4.57×10^{-4}
Putamen peak	Noncarrier	7.61×10^{-3}	8.79×10^{-5}	9.01×10^{-4}
	Carrier	8.38×10^{-3}	2.89×10^{-4}	1.12×10^{-3}

Mean, standard error (SEM), and SD values for K_i measured for each ROI and peak ventral striatum and putamen foci (see Figure 1) from the voxel-wise analyses. Peak foci values for each subject were defined as the mean K_i within a sphere, centered at the peak voxel coordinates and of radius 4.5 mm.

(ie, greater central DDC activity would be assumed to facilitate greater L-DOPA treatment response); however, as the haplotype is not uniquely defined by this marker, any inferences about genetically driven L-DOPA efficacy based on the present findings from healthy individuals must await future studies of *DDC* genetics in Parkinson's disease and treatment response.

As DDC is not only involved in monoaminergic synthesis, but is also the rate-limiting enzyme for several neuro- and vaso-active trace amines, such as 2-phenylethylamine, tyramine, and tryptamine that have been implicated in multiple neurological and psychiatric conditions, these data may have clinical relevance beyond Parkinson's disease therapeutics (Berry, 2007). Accordingly, *DDC* has been identified as a candidate risk gene for multiple CNS conditions (Corominas *et al*, 2010; Guan *et al*, 2009; Lasky-Su *et al*, 2008; Ma *et al*, 2005; Toma *et al*, 2012). However, past association studies have proceeded without evidence that common polymorphisms in *DDC* are associated with differential DDC protein function. Here, we provide such evidence that offers some guidance for future work. For instance, a four-marker *DDC* haplotype well aligned with haplotype 4, but not with the other haplotypes studied, has been linked recently with autism (Toma *et al*, 2012) and may merit follow-up experiments.

Although the markers tested provide substantial coverage of the common polymorphisms in the *DDC* gene, these data are not exhaustive and cannot exclude other *cis*-acting, untested variants. By the same token, in view of the relatively limited frequency of haplotype 4 and the measured K_i variance, it is clear that this genetic factor is not the only important source of interindividual variability in striatal [^{18}F]-FDOPA uptake, and additional potential sources should be investigated. Though this haplotype was independent of age and sex in our cohort, the current work does not exclude the possibility that other nongenetic variables, such as menstrual cycle phase or adequacy of pyridoxal 5′-phosphate levels, affected the reported results, and independent replication to confirm these findings is warranted. The Gjedde–Patlak modeling of the macroparameter, K_i, requires negligible differences in K_1, the rate constant reflecting tracer perfusion and extraction from the arterial compartment, between regions of interest and reference. The remote possibility that there exists a spurious or systematic relationship between regional K_1 across individuals and *DDC* haplotype, therefore, poses a potential limitation of the current approach. Arterial sampling was not conducted in this large cohort, precluding empirical comparisons between K_i measurements obtained with the present methods and similar parameters derived using an arterial blood input function. However, past research has identified a strong correlation between reference region delineated K_i (also referred to as k_3^s elsewhere) and estimates of k_3, the rate constant reflecting tracer entrance to the specific compartment, using arterial sampling and kinetic modeling (Hoshi *et al*, 1993). In addition, because the expression of *DDC* in the cerebellum is extremely low in post-mortem assays (eg, as documented by the Allen Brain Atlas; http://www.brain-map.org), it is not expected that *cis*-acting variation would affect tracer kinetics in this region, as supported by the lack of haplotype 4 effects on terminal frame cerebellar activity in the current data. Thus, results are unlikely to reflect genetically driven reference region biases. Finally, it is not known by what mechanism haplotype 4—or variation with which it is in linkage disequilibrium—is associated with greater *in vivo* enzymatic activity, a question that can be best answered with future molecular studies.

Despite these caveats, by offering novel biological evidence for functional effects of common *DDC* polymorphisms in the living human brain, this study identifies a genetic source of variation in a heritable and widely relied upon neurochemical phenotype and lays groundwork upon which to pursue hypotheses linking this candidate gene and aspects of neuropsychiatric conditions with ventral striatal involvement.

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