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Prediction of striatal D2 receptor binding by *DRD2/ANKK1* TaqIA allele status

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Abstract

In humans, the A1 (T) allele of the dopamine (DA) D2 receptor/ankyrin repeat and kinase domain containing 1 (*DRD2/ANKK1*) TaqIA (rs1800497) single nucleotide polymorphism has been associated with reduced striatal DA D2/D3 receptor (D2/D3R) availability. However, radioligands used to estimate D2/D3R are displaceable by endogenous DA and are non-selective for D2R, leaving the relationship between TaqIA genotype and D2R *specific* binding uncertain. Using the positron emission tomography (PET) radioligand, (*N*-[¹¹C]methyl)benperidol ([¹¹C]NMB), which is highly selective for D2R over D3R and is not displaceable by endogenous DA, the current study examined whether *DRD2/ANKK1* TaqIA genotype predicts D2R specific binding in 2

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Author roles

SAE, RB, and TH wrote the manuscript. SAE, RB, LLG, NSCF, JMK, KJB, SMM, JSP, DMB, and TH contributed to study design and methods. All authors reviewed and edited the manuscript.

Conflict of Interest

KJB: ACADIA Pharmaceuticals (advisory board, speakers bureau, research funding), Auspex Pharmaceuticals (consultant), Psyadon, Inc. (research funding), Neurocrine Biosciences, Inc. (research funding), and U. S. patent # 8,463,552 and patent application # 13/890,198.

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independent samples. Sample 1 ($n = 39$) was composed of obese and non-obese adults; sample 2 ($n = 18$) was composed of healthy controls, unmedicated individuals with schizophrenia, and siblings of individuals with schizophrenia. Across both samples, A1 allele carriers (A1+) had 5-12% less striatal D2R specific binding relative to individuals homozygous for the A2 allele (A1-), regardless of body mass index or diagnostic group. This reduction is comparable to previous PET studies of D2/D3R availability (10-14%). The pooled effect size for the difference in total striatal D2R binding between A1+ and A1- was large (0.84). In summary, in line with studies using displaceable D2/D3R radioligands, our results indicate that *DRD2/ANKK1* TaqIA allele status predicts striatal D2R specific binding as measured by D2R-selective [^{11}C]NMB. These findings support the hypothesis that *DRD2/ANKK1* TaqIA allele status may modify D2R, perhaps conferring risk for certain disease states.

GRAPHICAL ABSTRACT

We investigated the difference in striatal dopamine D2 receptor binding, as measured by PET with (*N*-[^{11}C]methyl)benperidol ([^{11}C]NMB), between A1 allele carriers (A1+) and individuals homozygous for the A2 allele (A1-) of the *DRD2/ANKK1* TaqIA single nucleotide polymorphism. In Study 1, A1+ had 5-12% less striatal [^{11}C]NMB binding than A1-.

Keywords

rs1800497; PET; dopamine

Introduction

The role of striatal dopamine (DA) signaling in substance abuse and psychiatric disorders has yet to be fully characterized. Positron emission tomography (PET) studies with displaceable DA D2/D3 receptor (D2/D3R) radioligands show that low striatal D2/D3R availability may be associated with impulsivity (Clark et al., 2012), addiction to alcohol (Volkow et al., 1996; Martinez et al., 2005), substance abuse (Volkow et al., 1990; Volkow et al., 2001; Martinez et al., 2004; Fehr et al., 2008), and obesity (Wang et al., 2001; Haltia et al., 2007; de Weijer et al., 2011) whereas high D2/D3R availability has been associated with risk for schizophrenia (Laruelle, 1998), although this finding has not been replicated (Howes et al., 2012; Kambeitz et al., 2014). Similarly, the A1 (T) allele of the single nucleotide polymorphism (SNP) TaqIA (rs1800497), located in the ankyrin repeat and kinase domain containing 1 (*ANKK1*) 10 kb downstream from the DA D2 receptor (*DRD2*) gene (Grandy et al., 1989), is associated with addictive behavior including gambling (Comings et al., 1996), substance abuse (Noble et al., 1993; Persico et al., 1996; Lawford et al., 2000; Chen

et al., 2004; Messas et al., 2005), and binge eating (Davis et al., 2012) and with obesity (Noble et al., 1994; Spitz MR, 2000; Thomas et al., 2001; Stice et al., 2008; Duran-Gonzalez et al., 2011) while the A2 (C) allele is associated with risk for schizophrenia (Parsons et al., 2007; Dubertret et al., 2010; Arab and Elhawary, 2015).

Individual variability in striatal D2/D3R availability is significantly heritable, as detected by a twin study (Borg et al., 2015). In addition, the similar pattern of associations between *DRD2/ANKK1* TaqIA variants and D2/D3R availability with psychiatric and drug abuse risk has led to speculation that *DRD2*'s role in these disorders may be mediated by D2R. Indeed, the A1 allele of the *DRD2/ANKK1* TaqIA A1 variant has been associated with lower striatal D2/D3R availability relative to the A2 allele in several postmortem (Noble et al., 1991; Thompson et al., 1997; Ritchie and Noble, 2003; Gluskin and Mickey, 2016) and *in vivo* PET studies (Pohjalainen et al., 1998; Jonsson et al., 1999; Hirvonen et al., 2009a; Savitz et al., 2013; Gluskin and Mickey, 2016). However, a SPECT study (Laruelle et al., 1998) and two PET studies (Brody et al., 2006; Wagner et al., 2014) did not find this association, likely due to study of diseased populations (Gluskin and Mickey, 2016). To date, studies have used PET radioligands (e.g. [¹¹C]raclopride (Thompson et al., 1997; Pohjalainen et al., 1998; Jonsson et al., 1999; Brody et al., 2006; Hirvonen et al., 2009a; Savitz et al., 2013; Wagner et al., 2014), [³H]spiperone (Noble et al., 1991; Ritchie and Noble, 2003)) and the SPECT radioligand [¹²³I]IBZM (Laruelle et al., 1998), which do not discriminate between D2R and D3R and whose binding is affected by synaptic dopamine concentrations, leaving the link between *DRD2/ANKK1* TaqIA genotype and D2R *specific* binding unclear. The novel PET radioligand (*N*-[¹¹C]methyl)benperidol ([¹¹C]NMB) specifically binds to D2R in a reversible manner, does not undergo agonist-mediated internalization, is resistant to displacement by endogenous DA, and is selective for D2R over D3R by 200-fold (Moerlein et al., 1997; Karimi et al., 2011). Thus, [¹¹C]NMB is an ideal radioligand to use for the study of D2R binding under various conditions including disease states and genotype status.

The current studies examined whether *DRD2/ANKK1* TaqIA allele status is associated with striatal D2R *specific* binding. We analyzed data from two independent studies that employed PET with [¹¹C]NMB and included human participants genotyped for the *DRD2/ANKK1* TaqIA variant. Based on previous evidence (Pohjalainen et al., 1998; Jonsson et al., 1999; Savitz et al., 2013; Gluskin and Mickey, 2016), we hypothesized that A1 allele carriers (A1+) would have lower striatal D2R binding than individuals homozygous for A2 (A1−). We also performed meta-analyses to generate pooled effect sizes that reflect the ability of *DRD2/ANKK1* TaqIA allele status to predict D2R specific binding across striatal regions.

Materials and Methods

Participants

For Study 1, participants were recruited for a study of obesity and D2R from the St. Louis region via a research volunteer database, flyers, and word of mouth. Data from these individuals regarding the relationship between obesity and striatal D2R were previously presented (Eisenstein et al., 2013; Eisenstein et al., 2015b; Eisenstein et al., 2015a). Obese ($n = 24$) and non-obese ($n = 20$) individuals, aged 18-40 years, were eligible for the study based on strict inclusion and exclusion criteria (Eisenstein et al., 2013). Exclusion criteria

included a diagnosis of type 2 diabetes (based on oral glucose tolerance test), history of psychiatric or neurological diagnoses, tobacco or illegal substance use, and dopaminergic medication use. Handedness was obtained by self-report. A subset of participants (24 obese and 16 non-obese) were genotyped for the *DRD2/ANKK1* TaqIA (rs1800497) polymorphism and completed PET neuroimaging. Some participants were not genotyped because we did not have biological specimens from which to extract DNA ($n = 4$).

For Study 2, healthy controls (HC; $n = 10$), siblings of individuals with schizophrenia (SIB; $n = 10$), and individuals with schizophrenia or schizoaffective disorder (SCZ; $n = 3$) were recruited for a study of schizophrenia, reward behavior, and D2R. Participants (age range 18-50 years) were recruited by word of mouth, flyers, and during recruiting visits to clinics and mental health centers in St. Louis, MO. A trained research assistant administered the Structured Clinical Interview for the DSM-IV (First et al., 2002) to all participants to determine the lifetime and current history of Axis I disorders. Exclusionary criteria included DSM-IV (American Psychiatric Association, 2000) diagnosis of substance abuse or dependence, either currently or within the last 6 months; neurological disorder; history of concussion or head injury; pregnancy; claustrophobia; presence in body of non-removable metallic objects or implanted medical electronic devices; mental retardation; positive drug urine test; and positive alcohol breathalyzer reading. HC must not have had lifetime or family history of psychotic disorders; current mood or anxiety disorder except for specific phobia but may have had a past Axis I disorder except for a psychotic disorder. The exclusionary criteria for SIB were identical to that of HC except that the participant must have had a sibling with confirmed diagnosis of schizophrenia or schizoaffective disorder. SIB were unrelated to SCZ who completed this study. SCZ must have met DSM-IV criteria for diagnosis of schizophrenia or schizoaffective disorder. Participants must have voluntarily abstained from medications such as DA agonists and antagonists and other psychotropic drugs for at least 4 weeks. During each study visit, evidence of alcohol use during the last 24 hr and recent use of drugs of abuse was obtained by breathalyzer and from urine sample drug test, respectively. Handedness was obtained by self-reported preferred hand for writing. A total of 8 HC, 8 SIB, and 2 SCZ were genotyped for the *DRD2/ANKK1* TaqIA (rs1800497) polymorphism and completed PET neuroimaging. Some participants were not genotyped because they participated in the study after genotyping was carried out ($n = 2$) or we did not have biological specimens from which to extract DNA ($n = 3$).

Participants in both studies provided written informed consent prior to participation. The study protocols were approved by the Washington University School of Medicine (WUSM) Human Research Protection Office and the Radioactive Drug Research Committee, and carried out in accordance with the principles expressed in the Declaration of Helsinki.

DNA Extraction and Genotyping

Blood (Study 1) and saliva (Study 2) were obtained from participants and DNA was extracted. Participants were genotyped for the *DRD2/ANKK1* TaqIA (rs1800497) polymorphism (A1/A2; T/C) by the Sequenom Technology Core at WUSM, the Molecular Psychiatry Core, and the Adipocyte Biology and Molecular Nutrition Core at WUSM using mass-spectrometry (Study 1), pyrosequencing (Study 1 and Study 2), and a pre-designed

Taqman SNP genotyping assay (Study 1; Applied Biosystems; Waltham, MA), respectively. Subjects were categorized as A1 allele carriers (A1+) or A2 allele homozygotes (A1-).

Magnetic resonance imaging and PET Imaging

For Study 1, the methods used to obtain magnetic resonance image (MRI) and PET scans were reported (Eisenstein et al., 2013). Briefly, MRI scans were obtained on the Siemens MAGNETOM Tim Trio 3T using a 3-D MP-RAGE sequence (sagittal orientation, TR = 2400 ms, TE = 3.16 ms, flip angle = 8 degrees, slab thickness = 176 mm, FOV = 256 × 256 mm, voxel dimensions = 1 × 1 × 1 mm) and PET scans were obtained on the Siemens CTI ECAT/EXACT HR+. The radioligand [¹¹C]NMB was prepared using an automated system previously described (Moerlein et al., 2004; Moerlein et al., 2010). Radiochemical purity of [¹¹C]NMB was 96% and specific activity was 1000 Ci/mmol (39 TBq/mmol). Participants received 6.4-18.1 mCi [¹¹C]NMB intravenously.

For Study 2, structural magnetic resonance T1-weighted anatomical images were obtained with the Siemens Biograph mMR PET/MR scanner using a 3-D MP-RAGE sequence (sagittal orientation, TR=2400 ms, TE=2.67 ms, flip angle=7 degrees, slab thickness=192 mm, FOV=256×256 mm; voxel dimensions= 1×1×1 mm). PET images were acquired simultaneously with the radioligand [¹¹C]NMB. [¹¹C]NMB was prepared as described for Study 1. Radiochemical purity of [¹¹C]NMB was 95% and specific activity was 1000 Ci/mmol (36 TBq/mmol). Participants received 5.9-18.8 mCi [¹¹C]NMB intravenously.

MR and PET image processing has been previously described in detail (Eisenstein et al., 2012; Eisenstein et al., 2013). *A priori* regions of interest (ROIs) including dorsal and ventral areas of the striatum (putamen, caudate, and nucleus accumbens (NAc)) were identified using FreeSurfer (Fischl et al., 2002) on the MP-RAGE MR images for each participant. Dynamic PET images were co-registered to each other and to the MP-RAGE image for each individual as previously described (Eisenstein et al., 2012). ROIs and the cerebellar reference region were resampled in the same atlas space and decay-corrected tissue activity curves were obtained from the dynamic PET data for every ROI. For both studies, *a priori* regions of interest (ROIs) included putamen, caudate, nucleus accumbens (NAc), dorsal striatum (putamen + caudate), and total striatum (putamen + caudate + NAc). D2R non-displaceable binding potentials (BP_{NDs}) were obtained for each ROI with the Logan graphical method with whole cerebellum as a reference region (Antenor-Dorsey et al., 2008). D2R BP_{NDs} for each ROI were averaged across left and right hemispheres to reduce the number of comparisons for primary analyses and because we did not have *a priori* hypotheses about laterality effects.

Meta-analyses

Cohen's *d* effect sizes were estimated for **Study 1** and **Study 2**. To obtain pooled effect sizes for differences in D2R specific binding between A1+ and A1-, meta-analyses were performed including Study 1 and Study 2 for each ROI.

Primary Statistical Analyses

Data from each study was analyzed separately due to use of different PET scanners. Hierarchical linear regressions were used to determine whether *DRD2/ANKK1* TaqIA allele status predicted D2R BP_{ND} for each ROI. Step 1 included covariates age, education level, ethnicity (White vs. not), and gender and Step 2 included group (obese vs non-obese or HC vs SIB vs SCZ). Step 3 included allele status (A1+ vs A1-) and Step 4 included the interaction between group and allele status. We calculated BP_{ND} means for each ROI adjusted for age, education level, ethnicity, gender and diagnostic group. We then calculated the percent difference in D2R BP_{ND} between A1+ vs A1- groups. Cohen's *d* effect sizes for each study were calculated using means and standard deviations and number of individuals in each allele group (A1+ and A1-). Meta-analyses were performed with Revman 5.3 software (Cochrane IMS, Oxford, UK). Weighted mean difference (MD) with the corresponding 95% CI was reported as the pooled effect size. I^2 and χ^2 tests determined heterogeneity and $p < 0.10$ was considered significant. Since heterogeneity did not exist across studies ($I^2 = 54\%$, $p = 0.14$), a fixed-effects model was used to calculate pooled effect size. Forest plots were generated with $p < 0.05$ considered significant.

Results

Participants

In Study 1, 40 individuals were genotyped for the *DRD2/ANKK1* TaqIA (rs1800497) polymorphism. For unknown reasons, one obese, A1/A2 participant's D2R binding values were greater than 2.5 (2.55-3.14 across all ROIs) standard deviations above the A1+ group mean and was thus excluded from analyses. Only one individual was homozygous for the A1 allele. Therefore, this participant's data was pooled with that from A1/A2 individuals for comparisons to individuals homozygous for A2. The final dataset included 14 obese and 7 non-obese A1+ and 9 obese and 9 non-obese A1-. The distributions of obese and non-obese were not different between A1+ and A1- ($\chi^2 = 1.11$, $p = 0.29$). Handedness distribution did not differ between A1+ and A1- ($\chi^2 = 1.81$, $p = 0.18$). Participant demographics are presented in **Table 1**.

In Study 2, 8 HC, 8 SIB, and 2 unmedicated SCZ were genotyped for the *DRD2/ANKK1* TaqIA (rs1800497) polymorphism and had PET D2R binding data. At the PET imaging visit, SCZ had not taken antipsychotic medications for 9 months and did not display overt signs of psychopathology. There were no individuals homozygous for the A1 allele. 6 HC and 3 SIB were A1+ and 2 HC, 5 SIB, and 2 SCZ were A1-. Group distribution was not different between A1+ and A1- ($\chi^2 = 4.50$, $p = 0.11$). Handedness distribution did not differ between A1+ and A1- ($\chi^2 = 0$, $p = 1$). Participant demographics are presented in **Table 1**.

Genotyping Quality Control

Across both studies *DRD2/ANKK1* TaqIA genotype did not deviate from Hardy-Weinburg Equilibrium in either sample (Study 1: $\chi^2 = 2.8$, $p = 0.10$; Study 2: $\chi^2 = 2.0$, $p = 0.16$) or within the African American (AA) and Caucasian (C) subsamples (AA, Study 1: $\chi^2 = 3.0$, $p = 0.08$; Study 2: $\chi^2 = 1.3$, $p = 0.25$; C, Study 1: $\chi^2 = 0.9$, $p = 0.34$; Study 2: $\chi^2 = 0.7$, $p = 0.39$). Distribution of allele frequencies did not differ between AA and C in either study.

(Study 1: $\chi^2 = 0.71$, $p = 0.40$; Study 2: $\chi^2 = 0.22$, $p = 0.64$) or among groups (obese/non-obese or HC/SIB/SCZ) across either study (Study 1: $\chi^2 = 1.1$, $p = 0.29$; Study 2: $\chi^2 = 4.5$, $p = 0.11$).

Prediction of Striatal D2R Binding by *DRD2/ANKK1* TaqIA Allele Status

In Study 1, *DRD2/ANKK1* TaqIA allele status predicted D2R binding in total striatum and nucleus accumbens (both $\beta = 0.29$, $p = 0.05$; both $R^2 = 0.07$; **Fig 1a-b, Table 2**) and in dorsal striatum at trend-level significance ($p = 0.09$, **Table 2**). *DRD2/ANKK1* TaqIA allele status and D2R binding in putamen and caudate were not significantly related (both $\beta = 0.23$, $p = 0.11$, **Table 2**). Across ROIs, D2R binding was lower in A1+ relative to A1- by 5-8%. Neither a main effect of obesity group (obese/non-obese) nor an interactive effect with *DRD2/ANKK1* TaqIA allele status was observed for D2R binding in any ROI (all $p = 0.13$, **Table 2**). D2R BP_{ND} means (S.D.s), percent difference in binding, and estimated Cohen's d effect sizes are presented in **Table 3**.

In Study 2, *DRD2/ANKK1* TaqIA allele status predicted D2R binding in total striatum, dorsal striatum, and putamen (all $\beta = 0.41$, $p = 0.05$; all $R^2 = 0.11$; **Fig 1c-d, Table 4**). *DRD2/ANKK1* TaqIA allele status did not significantly predict caudate or NAc D2R binding (both $p = 0.09$, **Table 4**). Across all ROIs, D2R binding was lower in A1+ relative to A1- by 8-12%. Diagnostic group (HC, SCZ, SIB) did not significantly predict D2R binding in any ROI (all $p = 0.13$) but the relationship was near significant for caudate ($p = 0.07$), in which SIB (mean BP_{ND} (S.D.) = 3.8 (0.6)) and SCZ (mean BP_{ND} (S.D.) = 3.9 (0.1)) tended to have greater D2R binding relative to HC (mean BP_{ND} (S.D.) = 3.4 (0.6)). It should be noted, however, that SCZ had a small sample size ($n = 2$). Group and *DRD2/ANKK1* TaqIA allele status did not interact to affect D2R binding in any ROI (all $p = 0.44$). D2R BP_{ND} means (S.D.s), percent difference in binding, and estimated Cohen's d effect sizes are presented in **Table 3**.

Meta-Analyses

The pooled analyses of Study 1 and Study 2 revealed that D2R specific binding was significantly lower in A1+ relative to A1- across all striatal ROIs (**Figs 2, 3**).

Discussion

We show in two independent studies that *DRD2/ANKK1* TaqIA (rs1800497) allele status predicts striatal D2R *specific* binding in humans, such that A1+ had lower D2R binding relative to A1-. This relationship did not depend on group membership (i.e. non-obese vs obese or controls versus psychosis). Our findings extend previous reports linking *DRD2/ANKK1* TaqIA allele status to D2/D3R availability in postmortem striatal tissue (Noble et al., 1991; Thompson et al., 1997; Ritchie and Noble, 2003; Gluskin and Mickey, 2016) and *in vivo* molecular imaging data in humans (Pohjalainen et al., 1998; Jonsson et al., 1999; Hirvonen et al., 2009a; Savitz et al., 2013; Gluskin and Mickey, 2016). Pooled effect sizes, or weighted mean differences, for total and dorsal striatal differences in D2R specific binding between A1+ and A1- were larger (0.84, 0.69) than that calculated for *in vivo* D2/D3R availability studies (0.57; Gluskin and Mickey, 2016). However, the 95%

confidence intervals for total striatum (0.30, 1.37) in our D2R study overlaps with that of D2/D3R availability studies (0.27, 0.87; Gluskin and Mickey, 2016), indicating that our effect size estimates are consistent with those of previous D2/D3R studies. Of note, *DRD2/ANKK1* TaqIA status accounted for 5-14% of the variance in striatal D2R binding (**Tables 2, 4**), comparable to 7% of striatal D2/D3R availability in previous *in vivo* PET studies (Gluskin and Mickey, 2016). Differences in participant characteristics, image analysis methods such as use of arterial input function, outcome measures ($B_{\max}$ versus BP_{ND}), and scanner sensitivity may contribute to variability in strength of the relationship between *DRD2/ANKK1* TaqIA allele status and D2R binding or D2/D3R availability.

Our results contrast with those of Laruelle et al. (1998), in which D2/D3R availability did not differ between A1+ and A1-. However, that study used SPECT, which has lower resolution than PET. In addition, a large proportion of the sample in Laruelle et al. (1998) were patients with schizophrenia, some of whom may have been taking neuroleptics that increase D2/D3R availability in schizophrenia (Silvestri et al., 2000). Two other previous studies did not find differences in baseline D2/D3R availability between A1+ and A1- (Brody et al., 2006; Wagner et al., 2014) but these included diseased populations including smokers (Brody et al., 2006) and traumatic brain injured individuals (Wagner et al., 2014). Variability in D2/D3R availability measurement due to small sample sizes may have contributed to null findings in these studies. In addition, recent meta-analysis of these D2/D3R studies revealed that the difference in D2/D3R availability between A1+ and A1- is robust in healthy individuals but not in diseased individuals (Gluskin and Mickey, 2016), suggesting that disease may modify this association. Intriguingly, *extrastriatal* D2/D3R availability, as measured by PET with [^{11}C]FLB457, was *elevated* in A1+ relative to A1- (Hirvonen et al., 2009b), indicating that there may be differential regulation of D2/D3R across brain regions by *DRD2/ANKK1* TaqIA allele status. Therefore future studies may investigate the effects of disease on the relationship between TaqIA allele status and D2R specific binding as well as differences in *extrastriatal* D2R specific binding as measured by PET with [^{11}C]NMB between A1+ and A1-.

There was no significant effect of group on D2R binding in either study. Nor did group interact with TaqIA allele status to predict striatal D2R binding. We have not previously found there to be differences in striatal D2R between an overlapping sample of non-obese and obese individuals (Eisenstein et al., 2013). In the case of study 2, the SCZ group was not large enough to fairly compare striatal D2R binding to HC and SIB. Neither study is truly large enough to investigate the interaction between group and allele status on striatal D2R binding. Therefore, caution should be used in interpreting the null results of these studies. Rather, we emphasize our main finding that when group (and other covariates) was controlled for, striatal D2R binding was lower in A1+ individuals compared to A1- individuals in 2 independent studies.

The mechanism by which the TaqIA variant, which resides in a noncoding region 10 kb downstream from the *DRD2* gene (Grandy et al., 1989), influences D2R binding remains unknown. Strong linkage disequilibrium with one or more functional variants, including the *DRD2* intronic SNPs rs2283265 and rs1076560 (Zhang et al., 2007) and the *ANKK1* missense SNP rs7118900 (Hoenicka et al., 2010), may influence receptor expression

(Comings et al., 1991; O'Hara et al., 1993). A likely candidate is the C957T variant (rs6277), which disrupts mRNA stability and synthesis of D2R (Duan et al., 2003) and is in linkage disequilibrium with the TaqIA SNP (Duan et al., 2003). The C957T variant relates to decreased D2/D3R availability as measured *in vivo* with [¹¹C]raclopride (Hirvonen et al., 2004). However, the C957T variant appears to affect striatal D2/D3R availability by changing receptor affinity while the TaqIA A1 polymorphism contributes to variability in D2/D3R availability by changing $B_{\max}$ (Hirvonen et al., 2009a). The TaqA1 A1 allele may be instead be in linkage disequilibrium with a functional variant that affects presynaptic DA signaling such as decreased inhibition of striatal DA synthesis (Duan et al., 2003; Laakso et al., 2005), which may displace D2/D3R radioligand binding. However, since endogenous DA does not displace [¹¹C]NMB and we observed lower D2R binding in A1+, it is more likely that the TaqIA allele is in linkage disequilibrium with a functional variant in the *DRD2* or *ANKK1* gene that directly affects D2R binding.

Limitations of the currently described studies include small sample sizes and heterogeneity of sample composition. Therefore, our power to detect group differences in D2R binding or allele status and interactions between group and allele status was low. Differences in PET scanner characteristics precluded combining data from the two studies for analyses, which would have provided more power. Nonetheless, we still detected relationships between *DRD2/ANKK1* allele status and striatal D2R specific binding in the predicted direction and with small to large effect sizes, independent of group membership and PET scanner used. Finally, none of the studies described, including ours, had enough data from healthy A1+ homozygotes to actually test the hypothesis that D2/D3R availability or D2R specific binding is lower in these individuals relative to A1+/A1- and A1-/A1-. To test this hypothesis, given the rare occurrence of homozygosity for A1+, future studies must intentionally select enough healthy participants with A1/A1 to have enough power to detect differences in striatal D2/D3R availability or D2R specific binding between A1/A1, A1/A2, and A2/A2.

In summary, the two independent studies described here showed that *DRD2/ANKK1* TaqIA allele status relates to individual differences in striatal D2R *specific* binding, such that A1+ individuals had greater binding relative to A1-. The use of the novel D2R specific radioligand [¹¹C]NMB with insensitivity to displacement by endogenous DA facilitated measurement of D2R specific binding, in contrast to D2/D3R radioligands such as [¹¹C]raclopride which lacks the same specificity and may be displaced by varying levels of endogenous DA (Moerlein et al., 1997; Karimi et al., 2011). Therefore these studies replicate and extend previous findings from postmortem (Noble et al., 1991; Thompson et al., 1997; Ritchie and Noble, 2003) and PET studies (Pohjalainen et al., 1998; Jonsson et al., 1999; Savitz et al., 2013) performed with D2/D3R radioligands. Our findings also support the hypothesis that the A1 allele (or linked functional variant) may influence risk for substance abuse and psychiatric disorders via D2R, which can be formally tested with mediation analyses.

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Figure 1.

In Study 1, *DRD2/ANKK1* TaqIA (rs1800497) allele status predicted D2R specific binding in *a*) striatum (putamen + caudate + nucleus accumbens) and *b*) nucleus accumbens. The data from the A1/A1 individual was pooled with data from A1/A2 for statistical analysis. In Study 2, *DRD2/ANKK1* TaqIA (rs1800497) allele status predicted D2R specific binding in *c*) striatum and *d*) putamen. D2R BP_{ND}, dopamine D2 receptor non-displaceable binding potential; NAc, nucleus accumbens; HC, healthy control; SIB, sibling of individual with schizophrenia; SCZ, individual with schizophrenia or schizoaffective disorder. *, **, p 0.05, = 0.01

Figure 2.

Forest plots of the pooled effect sizes for reduced *a*) striatal and *b*) dorsal D2R specific binding in A1+ relative to A1– individuals according to study. In both cases, the pooled effect sizes were significant. Size of square is proportional to weight of mean. CI, confidence interval; df, degrees of freedom; IV, inverse variance (statistical method).

Figure 3.

Forest plots of the pooled effect sizes for reduced D2R specific binding, as measured by [^{11}C]NMB, in A1+ relative to A1- individuals in Study 1 and Study 2 in *a*) putamen, *b*) caudate, and *c*) nucleus accumbens. Pooled effect sizes were significant for each ROI. Size of square is proportional to weight of mean. CI, confidence interval; df, degrees of freedom; IV, inverse variance (statistical method).

Table 1

Participant demographics for Studies 1 and 2. Mean (S.D.) shown.

Study 1			
	Non-obese (<i>n</i> = 16)	Obese (<i>n</i> = 23)	
Age (years)	28.8 (5.6)	32.3 (6.2)	
Education (years)	16.2 (1.4)	15.0 (1.9)	
BMI (kg/m ²)	22.2 (2.1)	40.2 (4.9)	
Gender	11 F/5 M	19 F/ 4 M	
Ethnicity	13 Caucasian, 1 African American, 1 Hispanic, 1 Other	12 Caucasian, 11 African American	
Handedness	14 right, 2 non-right	23 right	
Allele distribution	7 A1/A2, 9 A2/A2	1 A1/A1, 13 A1/A2, 9 A2/A2	
Study 2			
	Healthy Control (<i>n</i> = 8)	Sibling (<i>n</i> = 8)	Schizophrenia (<i>n</i> = 2)
Age (years)	35.5 (10.3)	33.3 (7.6)	36 (5.7)
Education (years)	12.6 (1.5)	14.9 (1.6)	12.5 (0.7)
Gender	3 F/5 M	5 F/3 M	1 F/1 M
Ethnicity	4 Caucasian, 4 African American	5 Caucasian, 3 African American	2 African American
Handedness	6 right, 2 non-right	7 right, 1 non-right	1 right, 1 non-right
Allele distribution	6 A1/A2; 2 A2/A2	3 A1/A2, 5 A2/A2	2 A2/A2

Table 2

Summary of hierarchical multiple linear regression analyses for prediction of striatal dopamine D2 receptor specific binding by *DRD2/ANKK1* Taq1A (rs1800497) allele status in Study 1.

Step 1			Step 2			Step 3			Step 4		
Striatum (N = 39)											
Variable	B	SEB	β	B	SEB	β	B	SEB	β	B	SEB
Education	.14	.10	.21	.16	.11	.24	.16	.10	.25	.16	.10
Age	-.07	.03	-.37	-.08	.03	-.39	-.08	.03	-.42	-.08	.03
Gender	.59	.39	.21	.54	.40	.19	.27	.40	.10	.27	.42
White or not	.44	.35	.18	.49	.36	.20	.46	.34	.19	.46	.36
Group				-.25	.37	-.10	-.42	.36	-.18	-.44	1.1
Allele status							.68	.33	.29*	.66	1.0
Group × allele status										.01	.69
R ²		.38			.39			.46			.46
F for change in R ²		5.2, p < 0.01			0.45, p = 0.51			4.3, p = 0.05			0, p = 0.98
Dorsal Striatum (N = 39)											
Variable	B	SEB	β	B	SEB	β	B	SEB	β	B	SEB
Education	.10	.09	.18	.12	.09	.21	.12	.09	.22	.12	.09
Age	-.06	.03	-.35	-.06	.03	-.38	-.07	.03	-.40	-.07	.03
Gender	.42	.34	.18	.36	.35	.15	.16	.36	.07	.13	.37
White or not	.37	.31	.18	.42	.31	.21	.40	.31	.19	.44	.32
Group				-.27	.32	-.13	-.40	.32	-.20	-.81	1.0
Allele status							.51	.29	.26†	.15	.90
Group × allele status										.26	.61
R ²		.32			.33			.39			.39
F for change in R ²		4.0, p = 0.01			0.67, p = 0.42			3.1, p = 0.09			0.18, p = 0.67
Putamen (N = 39)											
Variable	B	SEB	β	B	SEB	β	B	SEB	β	B	SEB
Education	.03	.04	.10	.04	.04	.15	.04	.04	.16	.04	.04

	Step 1			Step 2			Step 3			Step 4		
Age	-.04	.01	-.47	-.04	.01	-.50	-.04	.01	-.52	-.04	.01	-.52
Gender	.27	.16	.24	.23	.16	.20	.14	.17	.13	.13	.17	.11
White or not	.11	.14	.11	.15	.14	.15	.14	.14	.14	.14	.15	.15
Group				-.18	.15	-.18	-.23	.15	-.24	-.43	.46	-.44
Allele status							.22	.14	.23	.05	.41	.05
Group $\times$ allele status		.38			.40			.45		.12	.28	.29
R^2												.46
F for change in R^2		5.2, $p < 0.01$			1.4, $p = 0.25$			2.8, $p = 0.11$				0.20, $p = 0.66$
Caudate ($N = 39$)												
Variable	B	$SE\ B$	β	B	$SE\ B$	β	B	$SE\ B$	β	B	$SE\ B$	β
Education	.07	.06	.22	.08	.06	.24	.08	.06	.25	.08	.26	.25
Age	-.02	.02	-.23	-.02	.02	-.24	-.03	.02	-.27	-.03	.02	-.27
Gender	.15	.21	.11	.13	.21	.10	.02	.22	.01	0	.23	0
White or not	.26	.18	.22	.28	.19	.24	.26	.19	.22	.28	.20	.24
Group				-.09	.20	-.08	-.17	.20	-.15	-.38	.62	-.33
Allele status							.29	.18	.26	.10	.55	.09
Group $\times$ allele status										.14	.37	.28
R^2		.24			.25			.31			.31	
F for change in R^2		2.7, $p = 0.04$			0.22, $p = 0.64$			2.6, $p = .11$				0.14, $p = .71$
Nucleus Accumbens ($N = 39$)												
Variable	B	$SE\ B$	β	B	$SE\ B$	β	B	$SE\ B$	β	B	$SE\ B$	β
Education	.04	.03	.27	.04	.03	.26	.04	.03	.28	.04	.02	.27
Age	-.01	.01	-.30	-.01	.01	-.29	-.02	.01	-.32	-.02	.01	-.31
Gender	.17	.09	.26	.18	.10	.27	.11	.10	.17	.14	.10	.21
White or not	.07	.08	.13	.07	.09	.12	.06	.08	.11	.03	.08	.05
Group				.02	.09	.03	-.02	.09	-.04	.37	.26	.64
Allele status							.17	.08	.30*	.51	.23	.91
Group $\times$ allele status										-.25	.16	-1.0
R^2		.37			.37			.45			.49	

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	Step 1	Step 2	Step 3	Step 4
<i>F</i> for change in R^2	5.0, $p < 0.01$	0.1, $p = 0.83$	4.5, $p = 0.04$	2.4, $p = 0.13$

* $p = 0.05$;
 $\dagger$ $p = 0.09$

Table 3Mean (S.D.) striatal D2 receptor specific binding (BP_{ND}) by *DRD2/ANKK1* TaqIA allele status.

	<i>N</i>	BP _{ND} Unadjusted	BP _{ND} Adjusted	Percent difference in Adjusted BP _{ND} between A1 ⁺ and A1 ⁻	Estimated Effect Size (Cohen's <i>d</i>)
Study 1					
Total Striatum					
Total Sample					
A1+	21	9.74 (0.80)	9.75 (0.99)	6.5%	0.69
A1-	18	10.45 (1.46)	10.43 (0.99)		
Non-obese					
A1+	7	9.92 (0.86)			
A1-	9	10.47 (1.53)			
Obese					
A1+	14	9.65 (0.79)			
A1-	9	10.43 (1.48)			
Dorsal Striatum					
Total Sample					
A1+	21	7.81 (0.70)	7.81 (0.88)	6.1%	0.58
A1-	18	8.32 (1.23)	8.32 (0.89)		
Non-obese					
A1+	7	7.86 (0.69)			
A1-	9	8.36 (1.28)			
Obese					
A1+	14	7.78 (0.72)			
A1-	9	8.28 (1.26)			
Putamen					
Total Sample					
A1+	21	4.02 (0.35)	4.02 (0.40)	5.2%	0.54
A1-	18	4.24 (0.59)	4.24 (0.41)		
Non-obese					
A1+	7	3.98 (0.28)			
A1-	9	4.25 (0.68)			
Obese					
A1+	14	4.03 (0.39)			
A1-	9	4.23 (0.54)			
Caudate					
Total Sample					
A1+	21	3.79 (0.41)	3.79 (0.54)	7.1%	0.54
A1-	18	4.08 (0.70)	4.08 (0.54)		
Non-obese					
A1+	7	3.87 (0.43)			
A1-	9	4.12 (0.65)			

	<i>N</i>	BP_{ND} Unadjusted	BP_{ND} Adjusted	Percent difference in Adjusted BP_{ND} between A1⁺ and A1⁻	Estimated Effect Size (Cohen's <i>d</i>)
Obese					
A1+	14	3.75 (0.40)			
A1-	9	4.05 (0.78)			
Nucleus Accumbens					
Total Sample					
A1+	21	1.93 (0.21)	1.94 (0.24)	8.1%	0.71
A1-	18	2.13 (0.32)	2.11 (0.24)		
Non-obese					
A1+	7	1.86 (0.07)			
A1-	9	2.15 (0.09)			
Obese					
A1+	14	1.86 (0.20)			
A1-	9	2.14 (0.39)			
Study 2					
Total Striatum					
Total Sample					
A1+	9	10.66 (1.70)	10.48 (1.1)	10.7%	1.14
A1-	9	11.54 (1.40)	11.73 (1.1)		
Healthy Control					
A1+	6	10.87 (1.74)			
A1-	2	10.04 (1.51)			
Sibling					
A1+	3	10.26 (1.81)			
A1-	5	12.24 (1.18)			
Schizophrenia					
A1+	0	N/A			
A1-	2	11.31 (0.66)			
Dorsal Striatum					
Total Sample					
A1+	9	7.62 (1.2)	7.48 (0.79)	11.7%	1.25
A1-	9	8.33 (0.95)	8.47 (0.79)		
Healthy Control					
A1+	6	7.75 (1.27)			
A1-	2	7.37 (1.11)			
Sibling					
A1+	3	7.35 (1.4)			
A1-	5	8.77 (0.85)			
Schizophrenia					
A1+	0	N/A			
A1-	2	8.20 (0.55)			
Putamen					

	<i>N</i>	BP_{ND} Unadjusted	BP_{ND} Adjusted	Percent difference in Adjusted BP_{ND} between A1⁺ and A1⁻	Estimated Effect Size (Cohen's <i>d</i>)
Total					
Sample					
A1+	9	4.19 (0.57)	4.07 (0.33)	11.3%	1.56
A1-	9	4.47 (0.5)	4.59 (0.33)		
Healthy Control					
A1+	6	4.31 (0.58)			
A1-	2	4.09 (0.61)			
Sibling					
A1+	3	3.94 (0.58)			
A1-	5	4.67 (0.47)			
Schizophrenia					
A1+	0	N/A			
A1-	2	4.33 (0.43)			
Caudate					
Total					
Sample					
A1+	9	3.43 (0.70)	3.41 (0.5)	12.1%	0.94
A1-	9	3.87 (0.48)	3.88 (0.5)		
Healthy Control					
A1+	6	3.44 (0.72)			
A1-	2	3.29 (0.5)			
Sibling					
A1+	3	3.41 (0.84)			
A1-	5	4.10 (0.4)			
Schizophrenia					
A1+	0	N/A			
A1-	2	3.87 (0.12)			
Nucleus Accumbens					
Total Sample					
A1+	9	3.05 (0.45)	3.0 (0.32)	8.0%	0.81
A1-	9	3.21 (0.45)	3.26 (0.32)		
Healthy Control					
A1+	6	3.11 (0.48)			
A1-	2	2.67 (0.39)			
Sibling					
A1+	3	2.91 (0.44)			
A1-	5	3.47 (0.35)			
Schizophrenia					
A1+	0	N/A			
A1-	2	3.10 (0.11)			

BP_{ND}, non-displaceable binding potential; A1⁺, *DRD2/ANKK1* TaqIA (rs1800497) A1 allele carrier; A1[−], *DRD2/ANKK1* TaqIA (rs1800497) A2 allele homozygote; N/A, not applicable.

BP_{ND} adjusted means are adjusted for age, gender, ethnicity, education, and group membership.

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Table 4

Summary of hierarchical multiple linear regression analyses for prediction of striatal D2R specific binding by *DRD2/ANKK1* Taq1A (rs1800497) allele status in Study 2.

Step 1			Step 2			Step 3			Step 4		
Striatum (N = 18)											
Variable	B	SEB	β	B	SEB	β	B	SEB	β	B	SEB
Education	−.20	.18	−.24	−.28	.18	−.33	−.37	.16	−.44	−.28	.21
Age	−.06	.04	−.31	−.05	.04	−.27	−.06	.03	−.29	−.06	.03
Gender	1.8	.72	.58	1.8	.67	.61	2.2	.60	.72	1.9	.70
White or not	1.7	.62	.55	1.9	.60	.62	1.7	.52	.57	1.7	.55
Group				.67	.41	.29	.20	.41	.09	.05	.48
Allele status							2.5	1.1	.41*	.15	3.8
Group × allele status										1.4	2.2
R ²	.58			.66			.77			.78	
F for change in R ²	4.6	4.6, p = 0.02		2.7, p = 0.13			5.1, p = 0.05			0.42, p = 0.53	
Dorsal Striatum (N = 18)											
Variable	B	SEB	β	B	SEB	β	B	SEB	β	B	SEB
Education	−.13	.14	−.21	−.19	.14	−.31	−.26	.12	−.43	−.21	.16
Age	−.05	.03	−.33	−.04	.03	−.29	−.04	.02	−.32	−.05	.03
Gender	1.2	.54	.53	1.2	.51	.55	1.5	.45	.67	1.3	.53
White or not	1.2	.47	.52	1.3	.45	.59	1.2	.39	.54	1.1	.41
Group				.50	.31	.30	.13	.30	.08	.03	.36
Allele status							2.0	.82	.45*	.51	2.8
Group × allele status										.89	1.6
R ²	.55			.63			.76			.77	
F for change in R ²	4.0	p = 0.03		2.6, p = 0.13			5.8, p = 0.04			.30, p = 0.60	
Putamen (N = 18)											
Variable	B	SEB	β	B	SEB	β	B	SEB	p	B	SEB
Education	−.10	.06	−.33	−.11	.06	−.39	−.15	.05	−.52	−.12	.07

	Step 1			Step 2			Step 3			Step 4		
Age	-.02	.01	-.35	-.02	.01	-.33	-.02	.01	-.36	-.03	.01	-.41
Gender	.59	.24	.56	.61	.24	.58	.74	.19	.71	.66	.22	.63
White or not	.58	.20	.55	.62	.21	.59	.57	.16	.54	.53	.17	.51
Group				.14	.14	.18	-.05	.13	-.06	-.11	.15	-.14
Allele status							1.0	.35	.49**	.14	1.2	.07
Group × allele status										.54	.68	.46
R^2		.63		.66			.81				.82	
F for change in R^2		5.4, $p = 0.01$		1.0, $p = 0.33$			8.8, $p = 0.01$				0.64, $p = 0.44$	

Caudate ($N = 18$)												
Variable	B	$SE\ B$	β	B	$SE\ B$	β	B	$SE\ B$	β	B	$SE\ B$	P
Education	-.03	.08	-.10	-.08	.08	-.23	-.11	.07	-.33	-.14	.07	-.41
Age	-.02	.02	-.30	-.02	.02	-.25	-.02	.01	-.27	-.01	.01	-.18
Gender	.57	.33	.47	.61	.30	.50	.74	.28	.60	.90	.26	.74
White or not	.58	.33	.47	.68	.26	.56	.63	.24	.52	.57	.22	.47
Group				.36	.18	.39	.18	.19	.20	.22	.17	.25
Allele status							.95	.52	.39[†]	.63	.48	.26
Group × allele status										.16	.08	.37
R^2		.46		.59			.69				.78	
F for change in R^2		2.7, $p = 0.07$		3.9, $p = 0.07$			3.4, $p = 0.09$				4.1, $p = 0.07$	

Nucleus Accumbens ($N = 18$)												
Variable	B	$SE\ B$	β	B	$SE\ B$	β	B	$SE\ B$	β	B	$SE\ B$	β
Education	-.07	.05	-.29	-.09	.05	-.37	-.11	.05	-.45	-.12	.05	-.50
Age	-.01	.01	-.23	-.01	.01	-.19	-.01	.01	-.21	-.01	.01	-.15
Gender	.60	.20	.69	.62	.19	.72	.69	.18	.80	.77	.19	.89
White or not	.52	.17	.60	.57	.17	.66	.54	.16	.63	.51	.15	.59
Group				.17	.11	.26	.07	.12	.11	.09	.12	.11
Allele status							.52	.34	.30	.36	.34	.21
Group × allele status										.08	.06	.25

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	Step 1	Step 2	Step 3	Step 4
R^2	.62	.68	.74	.78
F for change in R^2	5.4, $p = 0.01$	2.3, $p = 0.16$	2.4, $p = 0.15$	1.9, $p = 0.20$

* $p < 0.05$,
** $p < 0.01$;
† $p = 0.09$