Received: 15 April 2016 | Revised: 26 May 2016 | Accepted: 27 May 2016
DOI 10.1002/syn.21916

ORIGINAL ARTICLE

WILEY SYNAPSE

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

Sarah A. Eisenstein[1,2] | Ryan Bogdan[3] | Latisha Love-Gregory[4] | Nadia S. Corral-Frías[1] | Jonathan M. Koller[1] | Kevin J. Black[1,2,5,6] | Stephen M. Moerlein[2,7] | Joel S. Perlmutter[2,5,8] | Deanna M. Barch[1,2,3] | Tamara Hershey[1,2,3,5]

[1]Department of Psychiatry, Washington University in St. Louis, St. Louis, MO 63110
[2]Department of Radiology, Washington University in St. Louis, St. Louis, MO 63110
[3]Department of Psychological & Brain Sciences, Washington University in St. Louis, St. Louis, MO 63130
[4]Department of Medicine, Washington University in St. Louis, St. Louis, MO 63110
[5]Department of Neurology, Washington University in St. Louis, St. Louis, MO 63110
[6]Department of Neuroscience, Washington University in St. Louis, St. Louis, MO 63110
[7]Department of Biochemistry, Washington University in St. Louis, St. Louis, MO 63110
[8]Programs in Physical Therapy and Occupational Therapy, Washington University in St. Louis, St. Louis, MO 63110

Correspondence
Sarah A. Eisenstein, Department of Psychiatry, Campus Box 8225, Washington University in St. Louis, St. Louis, MO 63110.
Email: eisensteins@npg.wustl.edu

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 nonselective for D2R, leaving the relationship between TaqIA genotype and D2R *specific* binding uncertain. Using the positron emission tomography (PET) radioligand, (*N*-[^{11}C]methyl)benperidol ([^{11}C]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 two independent samples. Sample 1 ($n = 39$) was composed of obese and nonobese 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 to 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.

KEYWORDS
dopamine, PET, rs1800497

1 | INTRODUCTION

The role of striatal dopamine (DA) signaling in substance abuse and psychiatric disorders has yet to be fully characterized. Postiron 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 (Martinez et al., 2005; Volkow et al., 1996), substance abuse (Fehr et al., 2008; Martinez et al., 2004; Volkow et al., 1990, 2001), and obesity (de Weijer et al., 2011; Haltia et al., 2007; Wang et al., 2001) 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, Abi-Dargham, Kapur, & Howes, 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 (Chen et al., 2004; Messas et al., 2005; Noble et al., 1993; Persico, Bird, Gabbay, & Uhl, 1996; Lawford et al., 2000), and binge eating

© 2016 Wiley Periodicals, Inc.

(Davis et al., 2012) and with obesity (Duran-Gonzalez et al., 2011; Noble et al., 1994; Spitz, 2000; Stice, Spoor, Bohon, & Small, 2008; Thomas, Critchley, Tomlinson, Cockram, & Chan, 2001) while the A2 (C) allele is associated with risk for schizophrenia (Arab & Elhawary, 2015; Dubertret et al., 2010; Parsons et al., 2007).

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 (Gluskin & Mickey, 2016; Noble, Blum, Ritchie, Montgomery, & Sheridan, 1991; Ritchie & Noble, 2003; Thompson et al., 1997) and *in vivo* PET studies (Gluskin & Mickey, 2016; Hirvonen et al., 2009a; Jonsson et al., 1999; Pohjalainen et al., 1998; Savitz et al., 2013). However, a SPECT study (Laruelle, Gelernter, & Innis, 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 & Mickey, 2016). To date, studies have used PET radioligands (e.g. [^{11}C]raclopride (Brody et al., 2006; Hirvonen et al., 2009a; Jonsson et al., 1999; Pohjalainen et al., 1998; Savitz et al., 2013; Thompson et al., 1997; Wagner et al., 2014), [^{3}H]spiperone (Noble et al., 1991; Ritchie & Noble, 2003)) and the SPECT radioligand [^{123}I]IBZM (Laruelle et al., 1998), which do not discriminate between D2R and D3R and whose binding is affected by synaptic DA concentrations, leaving the link between *DRD2/ANKK1* TaqIA genotype and D2R *specific* binding unclear. The novel PET radioligand (*N*-[^{11}C]methyl) benperidol ([^{11}C]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 (Karimi et al., 2011; Moerlein, Perlmutter, Markham, & Welch, 1997). Thus, [^{11}C]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 [^{11}C]NMB and included human participants genotyped for the *DRD2/ANKK1* TaqIA variant. Based on previous evidence (Gluskin & Mickey, 2016; Jonsson et al., 1999; Pohjalainen et al., 1998; Savitz et al., 2013), 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.

2 | MATERIALS AND METHODS

2.1 | 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, 2015a, 2015b). Individuals with obesity ($n = 24$) and without obesity ($n = 20$), aged 18 to 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 nonobese) 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 nonremovable 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 or 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 eight HC, eight SIB, and two 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

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

Study 1			
	Nonobese ($n = 16$)	Obese ($n = 23$)	
Age (yr)	28.8 (5.6)	32.3 (6.2)	
Education (yr)	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 nonright	23 right	
Allele distribution	7 A1/A2, 9 A2/A2	1 A1/A1, 13 A1/A2, 9 A2/A2	
Study 2			
	Healthy control ($n = 8$)	Sibling ($n = 8$)	Schizophrenia ($n = 2$)
Age (yr)	35.5 (10.3)	33.3 (7.6)	36 (5.7)
Education (yr)	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 nonright	7 right, 1 nonright	1 right, 1 nonright
Allele distribution	6 A1/A2; 2 A2/A2	3 A1/A2, 5 A2/A2	2 A2/A2

carried out in accordance with the principles expressed in the Declaration of Helsinki.

2.2 | 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 at WUSM, and the Adipocyte Biology and Molecular Nutrition Core at WUSM using mass-spectrometry (Study 1), pyrosequencing (Study 1 and Study 2), and a predesigned Taqman SNP genotyping assay (Study 1; Applied Biosystems; Waltham, MA), respectively. Subjects were categorized as A1 allele carriers (A1+) or A2 allele homozygotes (A1−).

2.3 | 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 three-dimensional MP-RAGE sequence (sagittal orientation, TR = 2,400 ms, TE = 3.16 ms, flip angle = 8°, 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 [^{11}C]NMB was prepared using an automated system previously described (Moerlein, Perlmutter, Welch, 2004; Moerlein et al., 2010). Radiochemical purity of [^{11}C]NMB was ≥96% and specific activity was ≥1,000 Ci/mmol (39 TBq/mmol). Participants received 6.4 to 18.1 mCi [^{11}C]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 = 2,400 ms, TE = 2.67 ms, flip angle = 7°, slab thickness = 192 mm, FOV = 256 × 256 mm; voxel dimensions = 1 × 1 × 1 mm). PET images were acquired simultaneously with the radioligand [^{11}C]NMB. [^{11}C]NMB was prepared as described for Study 1. Radiochemical purity of [^{11}C]NMB was ≥95% and specific activity was ≥1,000 Ci/mmol (36 TBq/mmol). Participants received 5.9 to 18.8 mCi [^{11}C]NMB intravenously.

MR and PET image processing has been previously described in detail (Eisenstein et al., 2012, 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 nondisplaceable binding potentials (BP_{ND}s) were obtained for each ROI with the Logan graphical method with whole cerebellum as a reference region (Antenor-Dorsey, Markham, Moerlein, Videen, & Perlmutter, 2008). D2R BP_{ND}s 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.

2.4 | 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

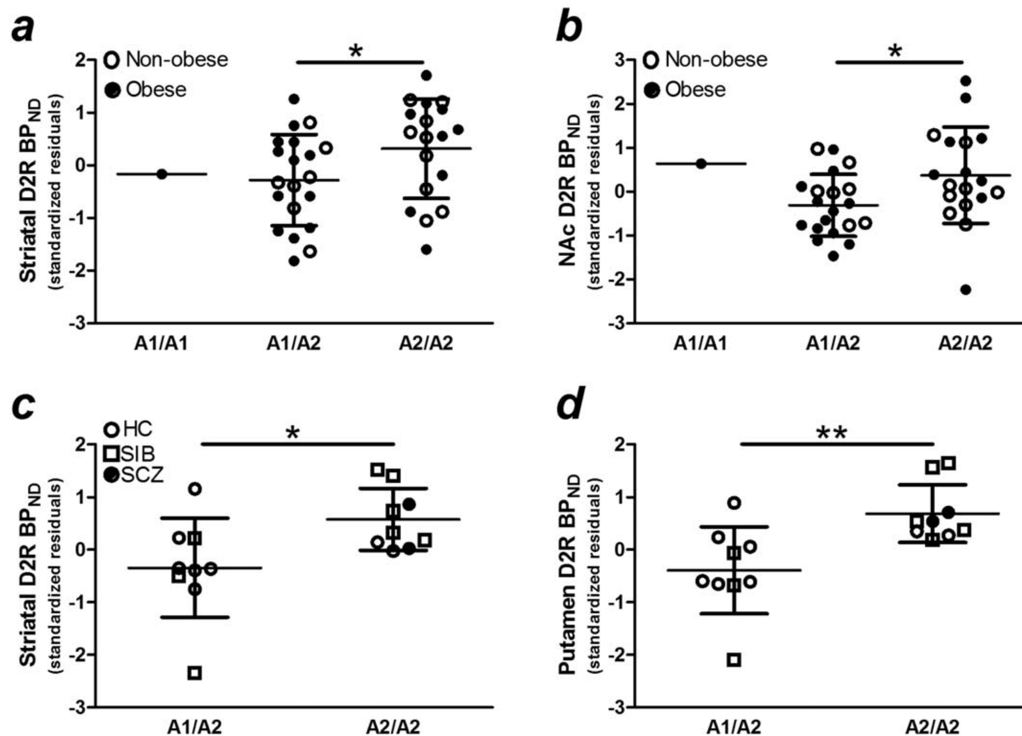

FIGURE 1 In Study 1, *DRD2/ANKK1* TaqIA (rs1800497) allele status predicted D2R specific binding in (a) striatum (putamen + caudate + nucleus accumbens) and (b) nucleus accumbens. The data from the A1/A1 individual was pooled with data from A1/A2 for statistical analysis. In Study 2, *DRD2/ANKK1* TaqIA (rs1800497) allele status predicted D2R specific binding in (c) striatum and (d) putamen. D2R BP_{ND}, dopamine D2 receptor nondisplaceable binding potential; NAc, nucleus accumbens; HC, healthy control; SIB, sibling of individual with schizophrenia; SCZ, individual with schizophrenia or schizoaffective disorder. *, ** $p \leq .05$, $= .01$.

between A1+ and A1−, meta-analyses were performed including Study 1 and Study 2 for each ROI.

2.5 | 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. nonobese 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+ versus 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 $p < .10$ was considered significant. Since heterogeneity did not exist across studies ($I^2 \leq 54\%$, $p \geq .14$), a fixed-effects model was used to calculate pooled effect size. Forest plots were generated with $p < .05$ considered significant.

3 | RESULTS

3.1 | 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 (2.6–3.1 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 nonobese A1+ and 9 obese and 9 nonobese A1−. The distributions of obese and nonobese were not different between A1+ and A1− ($\chi^2 = 1.11$, $p = .29$). Handedness distribution did not differ between A1+ and A1− ($\chi^2 = 1.81$, $p = .18$). Participant demographics are presented in Table 1.

In Study 2, eight HC, eight SIB, and two 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. Six HC and three SIB were A1+ and two HC, five SIB, and two SCZ were A1−. Group distribution was not different between A1+ and A1− ($\chi^2 = 4.50$, $p = .11$). Handedness distribution did not differ between A1+ and A1− ($\chi^2 = 0$, $p = 1$). Participant demographics are presented in Table 1.

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*	*SE B*	*β*	*B*	*SE B*	*β*	*B*	*SE B*	*β*	*B*	*SE B*	*β*
Education	.14	.10	.21	.16	.11	.24	.16	.10	.25	.16	.10	.25
Age	−.07	.03	−.37	−.08	.03	−.39	−.08	.03	−.42	−.08	.03	−.42
Gender	.59	.39	.21	.54	.40	.19	.27	.40	.10	.27	.42	.10
White or not	.44	.35	.18	.49	.36	.20	.46	.34	.19	.46	.36	.19
Group				−.25	.37	−.10	−.42	.36	−.18	−.44	1.1	−.19
Allele status							.68	.33	**.29***	.66	1.0	.28
Group × allele status										.01	.69	.01
R^2		.38			.39			.46			.46	
F for change in R^2		5.2, $p < .01$			0.45, $p = .51$			4.3, $p = .05$			0, $p = .98$	
Dorsal striatum ($N = 39$)												
Variable	*B*	*SE B*	*β*	*B*	*SE B*	*β*	*B*	*SE B*	*β*	*B*	*SE B*	*β*
Education	.10	.09	.18	.12	.09	.21	.12	.09	.22	.12	.09	.22
Age	−.06	.03	−.35	−.06	.03	−.38	−.07	.03	−.40	−.07	.03	−.41
Gender	.42	.34	.18	.36	.35	.15	.16	.36	.07	.13	.37	.06
White or not	.37	.31	.18	.42	.31	.21	.40	.31	.19	.44	.32	.21
Group				−.27	.32	−.13	−.40	.32	−.20	−.81	1.0	−.40
Allele status							.51	.29	**.26****	.15	.90	.08
Group × allele status										.26	.61	.30
R^2		.32			.33			.39			.39	
F for change in R^2		4.0, $p = .01$			0.67, $p = .42$			3.1, $p = .09$			0.18, $p = .67$	
Putamen ($N = 39$)												
Variable	*B*	*SE B*	*β*	*B*	*SE B*	*β*	*B*	*SE B*	*β*	*B*	*SE B*	*β*
Education	.03	.04	.10	.04	.04	.15	.04	.04	.16	.04	.04	.16
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	.15	.15	.15
Group				−.18	.15	−.18	−.23	.15	−.24	−.43	.46	−.44
Allele status							.22	.14	.23	.05	.41	.05
Group × allele status										.12	.28	.29
R^2		.38			.40			.45			.46	
F for change in R^2		5.2, $p < .01$			1.4, $p = .25$			2.8, $p = .11$			0.20, $p = .66$	
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 × allele status										.14	.37	.28
R^2		.24			.25			.31			.31	
F for change in R^2		2.7, $p = .04$			0.22, $p = .64$			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*

TABLE 2 *(Continued)*

	Step 1			Step 2			Step 3			Step 4		
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 × allele status										−.25	.16	−1.0
R^2		.37			.37			.45			.49	
F for change in R^2	5.0, $p < .01$			0.1, $p = .83$			4.5, $p = .04$			2.4, $p = .13$		

*$p \leq .05$; **$p = .09$.

TABLE 3 Mean (*SD*) striatal D2 receptor specific binding (BP_{ND}) by *DRD2/ANKK1* TaqIA allele status

	N	BP_{ND} unadjusted	BP_{ND} adjusted	Percent difference in adjusted BP_{ND} between $A1^+$ and A1−	Estimated effect size (Cohen's *d*)
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)		
Nonobese					
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)		
Nonobese					
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)		
Nonobese					
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)			

TABLE 3 *(Continued)*

	N	BP_{ND} unadjusted	BP_{ND} adjusted	Percent difference in adjusted BP_{ND} between $A1^+$ and A1−	Estimated effect size (Cohen's *d*)
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)		
Nonobese					
A1+	7	3.87 (0.43)			
A1−	9	4.12 (0.65)			
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)		
Nonobese					
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)			

TABLE 3 *(Continued)*

	N	BP_{ND} unadjusted	BP_{ND} adjusted	Percent difference in adjusted BP_{ND} between $A1^+$ and A1−	Estimated effect size (Cohen's *d*)
Putamen					
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}, nondisplaceable 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.

3.2 | Genotyping quality control

Across both studies *DRD2/ANKK1* TaqIA genotype did not deviate from Hardy-Weinburg Equilibrium in either sample (Study 1: $\chi^2 = 2.8$, $p = .10$; Study 2: $\chi^2 = 2.0$, $p = .16$) or within the Black (B) and White (W) subsamples (B, Study 1: $\chi^2 = 3.0$, $p = .08$; Study 2: $\chi^2 = 1.3$, $p = .25$; W, Study 1: $\chi^2 = 0.9$, $p = .34$; Study 2: $\chi^2 = 0.7$, $p = .39$). Distribution of allele frequencies did not differ between B and W in either study (Study 1: $\chi^2 = 0.71$, $p = .40$; Study 2: $\chi^2 = 0.22$, $p = .64$) or

TABLE 4 Summary of hierarchical multiple linear regression analyses for prediction of striatal dopamine 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*	*SE B*	*β*	*B*	*SE B*	*β*	*B*	*SE B*	*β*	*B*	*SE B*	*β*
Education	−.20	.18	−.24	−.28	.18	−.33	−.37	.16	−.44	−.28	.21	−.34
Age	−.06	.04	−.31	−.05	.04	−.27	−.06	.03	−.29	−.06	.03	−.34
Gender	1.8	.72	.58	1.8	.67	.61	2.2	.60	.72	1.9	.70	.64
White or not	1.7	.62	.55	1.9	.60	.62	1.7	.52	.57	1.7	.55	.55
Group				.67	.41	.29	.20	.41	.09	.05	.48	.02
Allele status							2.5	1.1	**.41***	.15	3.8	.03
Group × allele status										1.4	2.2	.42
R^2		.58			.66			.77			.78	
F for change in R^2		4.6, $p = .02$			2.7, $p = .13$			5.1, $p = .05$			0.42, $p = .53$	
Dorsal striatum ($N = 18$)												
Variable	*B*	*SE B*	*β*	*B*	*SE B*	*β*	*B*	*SE B*	*β*	*B*	*SE B*	*β*
Education	−.13	.14	−.21	−.19	.14	−.31	−.26	.12	−.43	−.21	.16	−.34
Age	−.05	.03	−.33	−.04	.03	−.29	−.04	.02	−.32	−.05	.03	−.36
Gender	1.2	.54	.53	1.2	.51	.55	1.5	.45	.67	1.3	.53	.61
White or not	1.2	.47	.52	1.3	.45	.59	1.2	.39	.54	1.1	.41	.52
Group				.50	.31	.30	.13	.30	.08	.03	.36	.02
Allele status							2.0	.82	**.45***	.51	2.8	.12
Group × allele status										.89	1.6	.36
R^2		.55			.63			.76			.77	
F for change in R^2		4.0, $p = .03$			2.6, $p = .13$			5.8, $p = .04$			.30, $p = .60$	
Putamen ($N = 18$)												
Variable	*B*	*SE B*	*β*	*B*	*SE B*	*β*	*B*	*SE B*	*β*	*B*	*SE B*	*β*
Education	−.10	.06	−.33	−.11	.06	−.39	−.15	.05	−.52	−.12	.07	−.40
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, $p = .01$			1.0, $p = .33$			8.8, $p = .01$			0.64, $p = .44$	
Caudate ($N = 18$)												
Variable	*B*	*SE B*	*β*	*B*	*SE B*	*β*	*B*	*SE B*	*β*	*B*	*SE B*	*β*
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, $p = .07$			3.9, $p = .07$			3.4, $p = .09$			4.1, $p = .07$	
Nucleus accumbens ($N = 18$)												
Variable	*B*	*SE B*	*β*	*B*	*SE B*	*β*	*B*	*SE B*	*β*	*B*	*SE B*	*β*

TABLE 4 *(Continued)*

	Step 1			Step 2			Step 3			Step 4		
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
R^2		.62			.68			.74			.78	
F for change in R^2		5.4, $p = .01$			2.3, $p = .16$			2.4, $p = .15$			1.9, $p = .20$	

*, **$p \leq .05$, .01; ***$p = .09$.

among groups (obese/nonobese or HC/SIB/SCZ) across either study (Study 1: $\chi^2 = .1$, $p = .29$; Study 2: $\chi^2 = 4.5$, $p = .11$).

3.3 | 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 $\beta \geq 0.29$, $p \leq .05$; both $\Delta R^2 \geq 0.07$; Figure 1a,b, Table 2) and in dorsal striatum at trend-level significance ($p = .09$, Table 2). *DRD2/ANKK1* TaqIA allele status and D2R binding in putamen and caudate were not significantly related (both $\beta \leq 0.23$, $p = .11$, Table 2). Across ROIs, D2R binding was lower in A1+ relative to A1− by 5–8%). Neither a main effect of obesity group (obese/nonobese) nor an interactive effect with *DRD2/ANKK1* TaqIA allele status was observed for D2R binding in any ROI (all $p \geq .13$, Table 2). D2R BP_{ND} means (SDs), 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 $\beta \geq 0.41$, $p \leq .05$; all $R^2 \geq 0.11$; Figure 1c,d, Table 4). *DRD2/ANKK1* TaqIA allele status did not significantly predict caudate or NAc D2R binding (both $p \geq .09$, Table 4). Across all ROIs, D2R binding was lower in A1+ relative to A1− by 8 to 12%. Diagnostic group (HC, SCZ, SIB) did not significantly predict D2R binding in any ROI (all $p \geq .13$) but the relationship was near significant for caudate ($p = .07$), in which SIB (mean BP_{ND} (SD) = 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 $p \geq .44$). D2R BP_{ND} means (SDs), percent difference in binding, and estimated Cohen's *d* effect sizes are presented in Table 3.

3.4 | 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 (Figures 2 and 3).

4 | DISCUSSION

We show in two independent studies that *DRD2/ANKK1* TaqIA (rs1800497) allele status predicts striatal D2R *specific* binding in

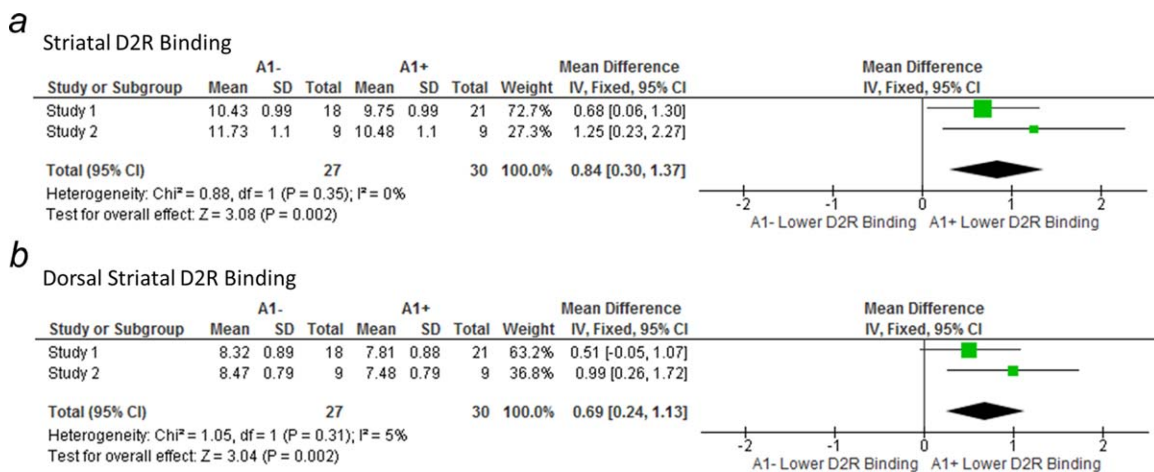

FIGURE 2 Forest plots of the pooled effect sizes for reduced (a) striatal and (b) dorsal D2R specific binding in A1+ relative to A1− individuals according to study. In both cases, the pooled effect sizes were significant. Size of square is proportional to weight of mean. CI, confidence interval; *df*, degrees of freedom; IV, inverse variance (statistical method).

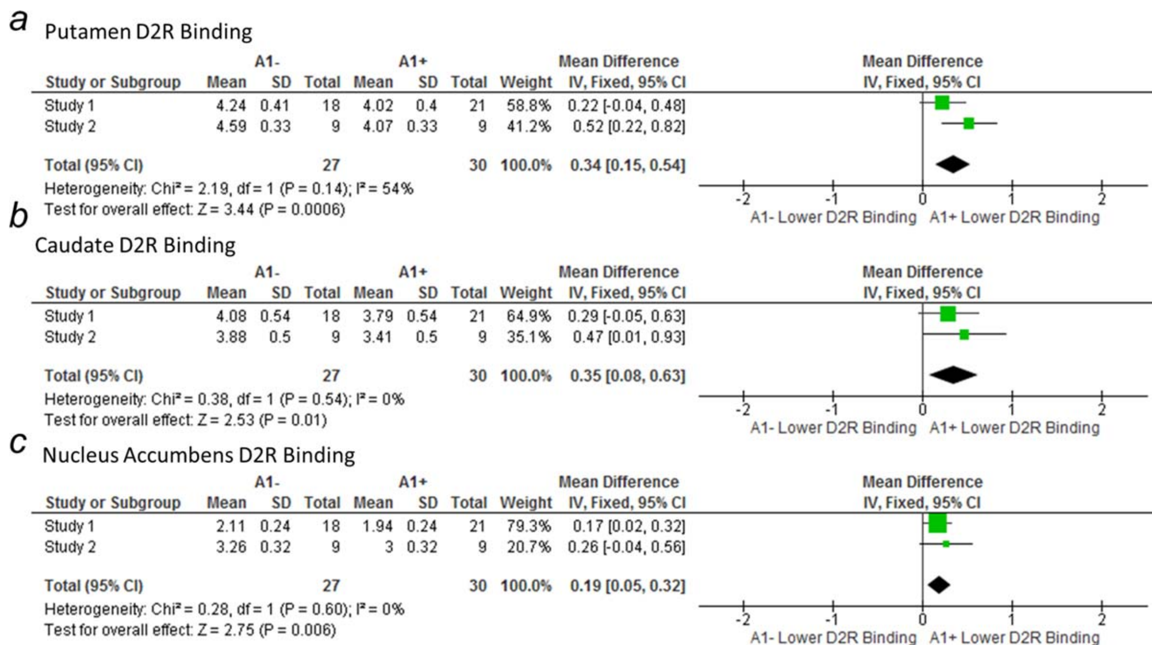

FIGURE 3 Forest plots of the pooled effect sizes for reduced D2R specific binding, as measured by [^{11}C]NMB, in A1+ relative to A1− individuals in Study 1 and Study 2 in (a) putamen, (b) caudate, and (c) nucleus accumbens. Pooled effect sizes were significant for each ROI. Size of square is proportional to weight of mean. CI, confidence interval; *df*, degrees of freedom; IV, inverse variance (statistical method).

humans, such that A1+ had lower D2R binding relative to A1−. This relationship did not depend on group membership (i.e. nonobese vs. obese or controls vs. psychosis). Our findings extend previous reports linking *DRD2/ANKK1* TaqIA allele status to D2/D3R availability in post-mortem striatal tissue (Gluskin & Mickey, 2016; Noble et al., 1991; Ritchie & Noble, 2003; Thompson et al., 1997) and *in vivo* molecular imaging data in humans (Gluskin & Mickey, 2016; Hirvonen et al., 2009a; Jonsson et al., 1999; Pohjalainen et al., 1998; Savitz et al., 2013). 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 & 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 & 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 to 14% of the variance in striatal D2R binding (Tables 2 and 4), comparable to 7% of striatal D2/D3R availability in previous *in vivo* PET studies (Gluskin & 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, Gelernter, & Innis (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 & Mickey, 2016), suggesting that disease may modify this association. Intriguingly, *extra*striatal 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 *extra*striatal 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 individuals without obesity and with obesity (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 with A1− individuals in two 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 [^{11}C]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 [^{11}C]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 [^{11}C]NMB with insensitivity to displacement by endogenous DA facilitated measurement of D2R specific binding, in contrast to D2/D3R radioligands such as [^{11}C]raclopride which lacks the same specificity and may be displaced by varying levels of endogenous DA (Karimi et al., 2011; Moerlein et al., 1997). Therefore these studies replicate and extend previous findings from postmortem (Noble et al., 1991; Ritchie & Noble, 2003; Thompson et al., 1997) and PET studies (Jonsson et al., 1999; Pohjalainen et al., 1998; 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.

ACKNOWLEDGMENTS

We thank Emily Bihun, Samantha Ranck, Melissa Cornejo, Arthur Schaffer, and Danielle Kelly for their help with recruiting participants. We thank Heather Lugar and Jerrel Rutlin for their help with processing MR scans. The studies presented in this work were conducted using the scanning and special services in the MIR Center for Clinical Imaging Research located at the Washington University Medical Center.

FUNDING INFORMATION

This work was supported by a National Alliance for Research on Schizophrenia and Affective Disorders Young Investigator Award to SAE, the National Institutes of Health (R01 DK085575 to T.H., R01 MH066031 to D.M.B., UL1 TR000448, R01 NS41509 to J.S.P.; R01 NS075321 to J.S.P.; R01 NS058714 to J.S.P.; T32 MH014677 to N.S.C.F.; P30 DK056341 to the Washington University Nutrition Obesity Research Center; P30 DK020579 to the Washington University Diabetes Research Center), Barnes Jewish Hospital Foundation (Eliot Stein Family Fund and Parkinson Disease Research Fund) to J.S.P.; the American Parkinson Disease Association (APDA) Advanced Research Center at Washington University to J.S.P., the Greater St. Louis Chapter of the APDA to J.S.P., the McDonnell Center for Systems Neuroscience New Resource Proposal Award to S.A.E., and the Gregory B. Couch Award to D.M.B. R.B. received additional funding from the Klingenstein Third Generation Foundation and NIH (R01 AG045231).

AUTHOR ROLES

S.A.E., R.B., and T.H. wrote the manuscript. S.A.E., R.B., L.L.G., N.S.C.F., J.M.K., K.J.B., S.M.M., J.S.P., D.M.B., and T.H. contributed to study design and methods. All authors reviewed and edited the manuscript.

CONFLICT OF INTEREST

K.J.B.: 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. JMK: U. S. patent #8,463,552 and patent application #13/890,198; D.M.B.: Roche (consultant), Takeda Pharmaceuticals U.S.A., Inc. (consultant), Pfizer (consultant), Amgen (consultant).

REFERENCES

Antenor-Dorsey, J. A., Markham, J., Moerlein, S. M., Videen, T. O., & Perlmutter, J. S. (2008). Validation of the reference tissue model for estimation of dopaminergic D2-like receptor binding with [18F](N-methyl) benperidol in humans. *Nuclear Medicine and Biology*, *35*, 335–341.

Arab, A. H., & Elhawary, N. A. (2015). Association between ANKK1 (rs1800497) and LTA (rs909253) Genetic Variants and Risk of Schizophrenia. *Biomedical Research International*, *2015*, 821827-

American Psychiatric Association. (2000). *Diagnostic and statistical manual of mental disorders* (4th ed.). Washington, DC: American Psychiatric Association.

Borg, J., Cervenka, S., Kuja-Halkola, R., Matheson, G. J., Jonsson, E. G., Lichtenstein, P., ... Farde, L. (2015). Contribution of non-genetic factors to dopamine and serotonin receptor availability in the adult human brain. *Molecular Psychiatry*. advance online publication; doi: 10.1038/mp.2015.147.

Brody, A. L., Mandelkern, M. A., Olmstead, R. E., Scheibal, D., Hahn, E., Shiraga, S., ... McCracken, J. T. (2006). Gene variants of brain dopamine pathways and smoking-induced dopamine release in the ventral caudate/nucleus accumbens. *Archives of General Psychiatry*, *63*, 808–816.

Chen, C. K., Hu, X., Lin, S. K., Sham, P. C., Loh el, W., Li, T., ... Ball, D. M. (2004). Association analysis of dopamine D2-like receptor genes and methamphetamine abuse. *Psychiatric Genetics*, *14*, 223–226.

Clark, L., Stokes, P. R., Wu, K., Michalczuk, R., Benecke, A., Watson, B. J., ... Lingford-Hughes, A. R. (2012). Striatal dopamine D(2)/D(3) receptor binding in pathological gambling is correlated with mood-related impulsivity. *Neuroimage*, *63*, 40–46.

Comings, D. E., Rosenthal, R. J., Lesieur, H. R., Rugle, L. J., Muhleman, D., Chiu, C., ... Gade, R. (1996). A study of the dopamine D2 receptor gene in pathological gambling. *Pharmacogenetics*, *6*, 223–234.

Comings, D. E., Comings, B. G., Muhleman, D., Dietz, G., Shahbahrami, B., Tast, D., ... Kovacs, B. W. (1991). The dopamine D2 receptor locus as a modifying gene in neuropsychiatric disorders. *Jama*, *266*, 1793–1800.

Davis, C., Levitan, R. D., Yilmaz, Z., Kaplan, A. S., Carter, J. C., & Kennedy, J. L. (2012). Binge eating disorder and the dopamine D2 receptor: Genotypes and sub-phenotypes. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *38*, 328–335.

de Weijer, B. A., van de Giessen, E., van Amelsvoort, T. A., Boot, E., Braak, B., Janssen, I. M., ... Booij, J. (2011). Lower striatal dopamine D2/3 receptor availability in obese compared with non-obese subjects. *EJNMMI Research*, *1*, 37.

Duan, J., Wainwright, M. S., Comeron, J. M., Saitou, N., Sanders, A. R., Gelernter, J., & Gejman, P. V. (2003). Synonymous mutations in the human dopamine receptor D2 (DRD2) affect mRNA stability and synthesis of the receptor. *Human Molecular Genetics*, *12*, 205–216.

Dubertret, C., Bardel, C., Ramoz, N., Martin, P. M., Deybach, J. C., Ades, J., ... Gouya, L. (2010). A genetic schizophrenia-susceptibility region located between the ANKK1 and DRD2 genes. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *34*, 492–499.

Duran-Gonzalez, J., Ortiz, I., Gonzales, E., Ruiz, N., Ortiz, M., Gonzalez, A., ... Nair, S. (2011). Association study of candidate gene polymorphisms and obesity in a young Mexican-American population from South Texas. *Archives of Medical Research*, *42*, 523–531.

Eisenstein, S. A., Gredysa, D. M., Antenor-Dorsey, J. A., Green, L., Arbelaez, A. M., Koller, J. M., ... Hershey, T. (2015a). Insulin, central dopamine D2 receptors, and monetary reward discounting in obesity. *PLoS One*, *10*, e0133621.

Eisenstein, S. A., Koller, J. M., Piccirillo, M., Kim, A., Antenor-Dorsey, J. A., Videen, T. O., ... Hershey, T. (2012). Characterization of extrastriatal D2 in vivo specific binding of [(1)(8)F](N-methyl)benperidol using PET. *Synapse*, *66*, 770–780.

Eisenstein, S. A., Antenor-Dorsey, J. A., Gredysa, D. M., Koller, J. M., Bihun, E. C., Ranck, S. A., ... Hershey, T. (2013). A comparison of D2 receptor specific binding in obese and normal-weight individuals using PET with (N-[(11)C]methyl)benperidol. *Synapse*, *67*, 748–756.

Eisenstein, S. A., Bischoff, A. N., Gredysa, D. M., Antenor-Dorsey, J. A., Koller, J. M., Al-Lozi, A., ... Hershey, T. (2015b). Emotional eating phenotype is associated with central dopamine D2 receptor binding independent of body mass index. *Science Report*, *5*, 11283.

Fehr, C., Yakushev, I., Hohmann, N., Buchholz, H. G., Landvogt, C., Deckers, H., ... Schreckenberger, M. (2008). Association of low striatal dopamine d2 receptor availability with nicotine dependence similar to that seen with other drugs of abuse. *American Journal of Psychiatry*, *165*, 507–514.

First Mb, S. R., Gibbon, M., & Williams, J. B. W. (2002).) *Structured clinical interview for DSM-IV-TR axis I disorders, research version, patient edition with psychotic screen (SCID-I/P W/PSY Screen)*. New York: Biometrics Research, New York State Psychiatric Institute.

Fischl, B., Salat, D. H., Busa, E., Albert, M., Dieterich, M., Haselgrove, C., ... Dale, A. M. (2002). Whole brain segmentation: Automated labeling of neuroanatomical structures in the human brain. *Neuron*, *33*, 341–355.

Gluskin, B. S., & Mickey, B. J. (2016). Genetic variation and dopamine D2 receptor availability: A systematic review and meta-analysis of human in vivo molecular imaging studies. *Translational Psychiatry*, *6*, e747.

Grandy, D. K., Litt, M., Allen, L., Bunzow, J. R., Marchionni, M., Makam, H., ... Civelli, O. (1989). The human dopamine D2 receptor gene is located on chromosome 11 at q22-q23 and identifies a TaqI RFLP. *American Journal of Human Genetics*, *45*, 778–785.

Haltia, L. T., Rinne, J. O., Merisaari, H., Maguire, R. P., Savontaus, E., Helin, S., ... Kaasinen, V. (2007). Effects of intravenous glucose on dopaminergic function in the human brain in vivo. *Synapse*, *61*, 748–756.

Hirvonen, M., Laakso, A., Nagren, K., Rinne, J. O., Pohjalainen, T., & Hietala, J. (2004). C957T polymorphism of the dopamine D2 receptor (DRD2) gene affects striatal DRD2 availability in vivo. *Molecular Psychiatry*, *9*, 1060–1061.

Hirvonen, M. M., Laakso, A., Nagren, K., Rinne, J. O., Pohjalainen, T., & Hietala, J. (2009a). C957T polymorphism of dopamine D2 receptor gene affects striatal DRD2 in vivo availability by changing the receptor affinity. *Synapse*, *63*, 907–912.

Hirvonen, M. M., Lumme, V., Hirvonen, J., Pesonen, U., Nagren, K., Vahlberg, T., ... Hietala, J. (2009b). C957T polymorphism of the human dopamine D2 receptor gene predicts extrastriatal dopamine receptor availability in vivo. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *33*, 630–636.

Hoenicka, J., Quinones-Lombrana, A., Espana-Serrano, L., Alvira-Botero, X., Kremer, L., Perez-Gonzalez, R., ... Palomo, T. (2010). The ANKK1 gene associated with addictions is expressed in astroglial cells and upregulated by apomorphine. *Biological Psychiatry*, *67*, 3–11.

Howes, O. D., Kambeitz, J., Kim, E., Stahl, D., Slifstein, M., Abi-Dargham, A., & Kapur, S. (2012). The nature of dopamine dysfunction in schizophrenia and what this means for treatment. *Archives of General Psychiatry*, *69*, 776–786.

Jonsson, E. G., Nothen, M. M., Grunhage, F., Farde, L., Nakashima, Y., Propping, P., & Sedvall, G. C. (1999). Polymorphisms in the dopamine D2 receptor gene and their relationships to striatal dopamine receptor density of healthy volunteers. *Molecular Psychiatry*, *4*, 290–296.

Kambeitz, J., Abi-Dargham, A., Kapur, S., & Howes, O. D. (2014). Alterations in cortical and extrastriatal subcortical dopamine function in schizophrenia: Systematic review and meta-analysis of imaging studies. *British Journal of Psychiatry*, *204*, 420–429.

Karimi, M., Moerlein, S. M., Videen, T. O., Luedtke, R. R., Taylor, M., Mach, R. H., & Perlmutter, J. S. (2011). Decreased striatal dopamine receptor binding in primary focal dystonia: A D2 or D3 defect? *Movement Disorders*, *26*, 100–106.

Laakso, A., Pohjalainen, T., Bergman, J., Kajander, J., Haaparanta, M., Solin, O., ... Hietala, J. (2005). The A1 allele of the human D2 dopamine receptor gene is associated with increased activity of striatal L-amino acid decarboxylase in healthy subjects. *Pharmacogenetics Genomics*, *15*, 387–391.

Laruelle, M. (1998). Imaging dopamine transmission in schizophrenia. A review and meta-analysis. *Quarterly Journal of Nuclear Medicine 42*, 211–221.

Laruelle, M., Gelernter, J., & Innis, R. B. (1998). D2 receptors binding potential is not affected by Taq1 polymorphism at the D2 receptor gene. *Molecular Psychiatry*, *3*, 261–265.

Lawford, B. R., Young, R. M., Noble, E. P., Sargent, J., Rowell, J., Shadforth, S., ... Ritchie, T. (2000). The D(2) dopamine receptor a(1) allele and opioid dependence: Association with heroin use and response to methadone treatment. *American Journal of Medical Genetics*, *96*, 592–598.

Martinez, D., Broft, A., Foltin, R. W., Slifstein, M., Hwang, D. R., Huang, Y., ... Laruelle, M. (2004). Cocaine dependence and d2 receptor availability in the functional subdivisions of the striatum: Relationship with cocaine-seeking behavior. *Neuropsychopharmacology*, *29*, 1190–1202.

Martinez, D., Gil, R., Slifstein, M., Hwang, D. R., Huang, Y., Perez, A., ... Abi-Dargham, A. (2005). Alcohol dependence is associated with blunted dopamine transmission in the ventral striatum. *Biological Psychiatry*, *58*, 779–786.

Messas, G., Meira-Lima, I., Turchi, M., Franco, O., Guindalini, C., Castelo, A., ... Vallada, H. (2005). Association study of dopamine D2 and D3 receptor gene polymorphisms with cocaine dependence. *Psychiatric Genetics*, *15*, 171–174.

Moerlein, S. M., Perlmutter, J. S., & Welch, M. J. (2004). Radiosynthesis of (N-[C-11]methyl)benperidol for PET investigation of D2 receptor binding. *Radiochimica Acta*, *92*, 333–339.

Moerlein, S. M., Perlmutter, J. S., Markham, J., & Welch, M. J. (1997). In vivo kinetics of [18F](N-methyl)benperidol: A novel PET tracer for assessment of dopaminergic D2-like receptor binding. *Journal of Cerebral Blood Flow and Metabolism*, *17*, 833–845.

Moerlein, S. M., LaVenture, J., Gaehle, G. G., Robben, J., Perlmutter, J. S., & Mach, R. H. (2010). Automated production of N-([C-11]methyl) benperidol ([C-11]NMB) for clinical application. *European Journal of Nuclear Medicine*, *37*, S366–S366.

Noble, E. P., Blum, K., Ritchie, T., Montgomery, A., & Sheridan, P. J. (1991). Allelic association of the D2 dopamine receptor gene with receptor-binding characteristics in alcoholism. *Archives of General Psychiatry*, *48*, 648–654.

Noble, E. P., Noble, R. E., Ritchie, T., Syndulko, K., Bohlman, M. C., Noble, L. A., ... Grandy, D. K. (1994). D2 dopamine receptor gene and obesity. *International Journal of Eating Disorders*, *15*, 205–217.

Noble, E. P., Blum, K., Khalsa, M. E., Ritchie, T., Montgomery, A., Wood, R. C., ... Anglin, M. D. (1993). Allelic association of the D2 dopamine receptor gene with cocaine dependence. *Drug and Alcohol Dependence*, *33*, 271–285.

O'Hara, B. F., Smith, S. S., Bird, G., Persico, A. M., Suarez, B. K., Cutting, G. R., & Uhl, G. R. (1993). Dopamine D2 receptor RFLPs, haplotypes and their association with substance use in black and Caucasian research volunteers. *Human Heredity*, *43*, 209–218.

Parsons, M. J., Mata, I., Beperet, M., Iribarren-Iriso, F., Arroyo, B., Sainz, R., ... Kerwin, R. (2007). A dopamine D2 receptor gene-related polymorphism is associated with schizophrenia in a Spanish population isolate. *Psychiatric Genetics*, *17*, 159–163.

Persico, A. M., Bird, G., Gabbay, F. H., & Uhl, G. R. (1996). D2 dopamine receptor gene TaqI A1 and B1 restriction fragment length polymorphisms: Enhanced frequencies in psychostimulant-preferring polysubstance abusers. *Biological Psychiatry*, *40*, 776–784.

Pohjalainen, T., Rinne, J. O., Nagren, K., Lehikoinen, P., Anttila, K., Syvalahti, E. K., & Hietala, J. (1998). The A1 allele of the human D2 dopamine receptor gene predicts low D2 receptor availability in healthy volunteers. *Molecular Psychiatry*, *3*, 256–260.

Ritchie, T., & Noble, E. P. (2003). Association of seven polymorphisms of the D2 dopamine receptor gene with brain receptor-binding characteristics. *Neurochemical Research*, *28*, 73–82.

Savitz, J., Hodgkinson, C. A., Martin-Soelch, C., Shen, P. H., Szczepanik, J., Nugent, A. C., ... Drevets, W. C. (2013). DRD2/ANKK1 Taq1A polymorphism (rs1800497) has opposing effects on D2/3 receptor binding in healthy controls and patients with major depressive disorder. *International Journal of Neuropsychopharmacology*, *16*, 2095–2101.

Silvestri, S., Seeman, M. V., Negrete, J. C., Houle, S., Shammi, C. M., Remington, G. J., ... Seeman, P. (2000). Increased dopamine D2 receptor binding after long-term treatment with antipsychotics in humans: A clinical PET study. *Psychopharmacology (Berlin)*, *152*, 174–180.

Spitz, M. R. D. M., Pillow, P., Hu, Y., Amos, C. I., Hong, W. K., & Wu, X. (2000). Variant alleles of the D2 dopamine receptor gene and obesity. *Nutrition Research*, *20*, 371–380.

Stice, E., Spoor, S., Bohon, C., & Small, D. M. (2008). Relation between obesity and blunted striatal response to food is moderated by TaqIA A1 allele. *Science*, *322*, 449–452.

Thomas, G. N., Critchley, J. A., Tomlinson, B., Cockram, C. S., & Chan, J. C. (2001). Relationships between the taqI polymorphism of the dopamine D2 receptor and blood pressure in hyperglycaemic and normoglycaemic Chinese subjects. *Clinical Endocrinology (Oxford)*, *55*, 605–611.

Thompson, J., Thomas, N., Singleton, A., Piggott, M., Lloyd, S., Perry, E. K., ... Court, J. A. (1997). D2 dopamine receptor gene (DRD2) Taq1 a polymorphism: Reduced dopamine D2 receptor binding in the human striatum associated with the A1 allele. *Pharmacogenetics 7*, 479–484.

Volkow, N. D., Wang, G. J., Fowler, J. S., Logan, J., Hitzemann, R., Ding, Y. S., ... Piscani, K. (1996). Decreases in dopamine receptors but not in dopamine transporters in alcoholics. *Alcoholism, Clinical, and Experimental Research*, *20*, 1594–1598.

Volkow, N. D., Fowler, J. S., Wolf, A. P., Schlyer, D., Shiue, C. Y., Alpert, R., ... Christman, D. (1990). Effects of chronic cocaine abuse on postsynaptic dopamine receptors. *American Journal of Psychiatry*, *147*, 719–724.

Volkow, N. D., Chang, L., Wang, G. J., Fowler, J. S., Ding, Y. S., Sedler, M., ... Pappas, N. (2001). Low level of brain dopamine D2 receptors in methamphetamine abusers: Association with metabolism in the orbitofrontal cortex. *American Journal of Psychiatry*, *158*, 2015–2021.

Wagner, A. K., Scanlon, J. M., Becker, C. R., Ritter, A. C., Niyonkuru, C., Dixon, C. E., ... Price, J. C. (2014). The influence of genetic variants on striatal dopamine transporter and D2 receptor binding after TBI. *Journal of Cerebral Blood Flow and Metabolism*, *34*, 1328–1339.

Wang, G. J., Volkow, N. D., Logan, J., Pappas, N. R., Wong, C. T., Zhu, W., ... Fowler, J. S. (2001). Brain dopamine and obesity. *Lancet*, *357*, 354–357.

Zhang, Y., Bertolino, A., Fazio, L., Blasi, G., Rampino, A., Romano, R., ... Sadee, W. (2007). Polymorphisms in human dopamine D2 receptor gene affect gene expression, splicing, and neuronal activity during working memory. *Proceedings of the National Academy of Science United States of America*, *104*, 20552–20557.