New Repeat Polymorphism in the *AKT1* Gene Predicts Striatal Dopamine D2/D3 Receptor Availability and Stimulant-Induced Dopamine Release in the Healthy Human Brain

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The role of the protein kinase Akt1 in dopamine neurotransmission is well recognized and has been implicated in schizophrenia and psychosis. However, the extent to which variants in the *AKT1* gene influence dopamine neurotransmission is not well understood. Here we investigated the effect of a newly characterized variant number tandem repeat (VNTR) polymorphism in *AKT1* [major alleles: L- (eight repeats) and H- (nine repeats)] on striatal dopamine D2/D3 receptor (DRD2) availability and on dopamine release in healthy volunteers. We used PET and [¹¹C]raclopride to assess baseline DRD2 availability in 91 participants. In 54 of these participants, we also measured intravenous methylphenidate-induced dopamine release to measure dopamine release. Dopamine release was quantified as the difference in specific binding of [¹¹C]raclopride (nondisplaceable binding potential) between baseline values and values following methylphenidate injection. There was an effect of *AKT1* genotype on DRD2 availability at baseline for the caudate ($F_{(2,90)} = 8.2, p = 0.001$) and putamen ($F_{(2,90)} = 6.6, p = 0.002$), but not the ventral striatum ($p = 0.3$). For the caudate and putamen, LL showed higher DRD2 availability than HH; HL were in between. There was also a significant effect of *AKT1* genotype on dopamine increases in the ventral striatum ($F_{(2,53)} = 5.3, p = 0.009$), with increases being stronger in HH > HL > LL. However, no dopamine increases were observed in the caudate ($p = 0.1$) or putamen ($p = 0.8$) following methylphenidate injection. Our results provide evidence that the *AKT1* gene modulates both striatal DRD2 availability and dopamine release in the human brain, which could account for its association with schizophrenia and psychosis. The clinical relevance of the newly characterized *AKT1* VNTR merits investigation.

Key words: *AKT1*; dopamine; dopamine receptor; methylphenidate; PET

Significance Statement

The *AKT1* gene has been implicated in schizophrenia and psychosis. This association is likely to reflect modulation of dopamine signaling by Akt1 kinase since striatal dopamine hyperstimulation is associated with psychosis and schizophrenia. Here, using PET with [¹¹C]raclopride, we identified in the *AKT1* gene a new variable number tandem repeat (VNTR) marker associated with baseline striatal dopamine D2/D3 receptor availability and with methylphenidate-induced striatal dopamine increases in healthy volunteers. Our results confirm the involvement of the *AKT1* gene in modulating striatal dopamine signaling in the human brain. Future studies are needed to assess the association of this new VNTR *AKT1* variant in schizophrenia and drug-induced psychoses.

Introduction

Variations in dopamine (DA) signaling have been implicated in neuropsychiatric conditions, such as schizophrenia (SZ) and

substance use disorder (SUD), and in pharmacological responses to drugs. Genes modulating DA signaling in the brain remain

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mostly undetermined (Wong et al., 2000; Gluskin and Mickey, 2016) and few studies have investigated in particular the role of genes involved in downstream DA signaling, though its relevance is evident by findings documenting associations between *DISC1*, *NRG1*, and *AKT1* with SZ (Jönsson et al., 1999; Hall et al., 2004; Gluskin and Mickey, 2016). Furthermore, *AKT1* polymorphisms have been linked to risk for drug-induced psychosis (Di Forti et al., 2012; Morgan et al., 2016).

The involvement of the *AKT1* gene in schizophrenia and psychosis may be mediated by the role of Akt1 kinase in DA receptor signaling through the noncanonical pathway that results in DA receptor internalization and desensitization (Li et al., 2016). Agonist binding to striatal dopamine D2/D3 receptor (DRD2) leads to formation of a multimolecular scaffold comprising β -arrestin 2 and several kinases, including Akt1, protein phosphatase 2A (PP2A), and glycogen synthase kinase-3 (GSK3; for review, see Gainetdinov et al., 2004). Akt1 kinase is a critical downstream effector of this signaling cascade whose activity is counter-regulated by extracellular DA levels such that DA increases reduce its activity (Zheng et al., 2013), whereas DA depletion elevates it (Bychkov et al., 2007; Ohi et al., 2013). DA binding to DRD2 directly induces Akt1 phosphorylation (Jönsson et al., 1999) and is essential for the behavioral manifestation of stimulants and antipsychotics drugs (Jönsson et al., 1999; Beaulieu et al., 2007; Pan et al., 2011). Moreover, cocaine self-administration in rats increased the total and phosphorylated levels of Akt1 in the nucleus accumbens (Hirvonen et al., 2009). Lower Akt1 appears to enhance DRD2 stimulation (Arguello and Gogos, 2008) and *AKT1*^{−/−} mice show enhanced DA neurotransmission (Jönsson et al., 1999) and poorer working-memory performance when challenged with amphetamine (Emamian et al., 2004).

A few studies have investigated the role that variations in the *AKT1* gene have on brain function. In SZ, *AKT1* single nucleotide polymorphism (SNP) rs2494732 was associated with attention deficits and brain morphology (Ohi et al., 2013) and *AKT1* SNP rs1130233 was associated with cingulate response during attentional processing (Blasi et al., 2011). In healthy volunteers, *AKT1* SNP rs1130233 showed involvement in cortical and striatal brain volumes (Tan et al., 2008, 2012b) and in functional responses during working memory (Tan et al., 2012a). To our knowledge no study to date has investigated the association between *AKT1* polymorphisms and striatal density of DRD2 or striatal DA release in humans.

Here, we report on a new variant number tandem repeat (VNTR) polymorphism in the human *AKT1* gene that was associated with striatal DA function. Repeats are potentially good predictors of brain function (Weber and Wong, 1993; Hirvonen et al., 2004) since they represent fast evolving sequences in the human genome (Brinkmann et al., 1998), exhibit elevated mutation rates (Gulcher, 2012), and alter the number of CpG (cytosine–phosphate–guanine) sites amenable to epigenetic regulation. Here we hypothesized that the *AKT1* VNTR would modulate both (1) baseline striatal DRD2 availability and (2) striatal DA release. To test this hypothesis, we used PET with [¹¹C]raclopride

to measure DRD2 availability at baseline and we measured DA release by assessing the changes in the specific binding of [¹¹C]raclopride elicited by the stimulant drug methylphenidate (MP; Volkow et al., 1994).

Materials and Methods

Computational analyses. *In silico* analysis of *AKT1* included assessing transcript variants, promoter genetic elements, interspecies genetic conservation, and reported sequence variations and patterns of alternative splicing, for which we used GenBank (<http://www.ncbi.nlm.nih.gov/genbank>, RRID:SCR_002760), the University of California Santa Cruz (UCSC) Genome Browser (<http://genome.ucsc.edu>, RRID:SCR_005780), and the Evolutionary Conserved Regions Browser (<http://ecrbrowser.dcode.org>, RRID:SCR_001052). Reported variations in the *AKT1* sequence were retrieved from the 1000 Genome Catalog of Human Genetic Variation (http://browser.1000genomes.org/Homo_sapiens/Search/Results?site=ensembl&q=AKT1, RRID:SCR_006828). Evidence-based data on structural variations (copy number variations) in *AKT1* were obtained with the Database of Genomic Variants (<http://dgv.tcag.ca/dgv/>, RRID:SCR_007000; MacDonald et al., 2014). Estimates of repeats variability and their potential suitability as genetic markers were conducted using the Sequence-Based Estimation of Repeat Variability server (<http://www.igs.cnrs-mrs.fr/SERV/>; Legendre and Verstrepen, 2008). Guanine–cytosine (GC) composition of the genetic sequences was assessed with the Genomics %G~C Content Calculator (http://www.sciencebuddies.org/science-fair-projects/project_ideas/Genom_GC_Calculator.shtml).

Participants. For the baseline measures of DRD2 availability, we included 91 unrelated healthy individuals of mixed ancestry who had undergone a baseline PET scan with [¹¹C]raclopride (Table 1). Of these, *N* = 51 (Table 2) also underwent a second PET [¹¹C]raclopride scan after a challenge with MP (0.5 mg/kg, i.v.), which was used to assess DA release (measured as the difference in specific binding of [¹¹C]raclopride between the baseline and the MP scans). All participants agreed to participate in the imaging and genetic studies and provided written informed consent. Participants were excluded if they had a history of substance abuse or dependence (other than nicotine) or a history of psychiatric disorder (as per *Diagnostic and Statistical Manual of Mental Disorders*, fourth edition; RRID:SCR_003682), neurological disease, medical conditions that may alter cerebral function (i.e., cardiovascular, endocrinological, oncological, or autoimmune diseases), current use of prescribed or over-the-counter medications, and/or head trauma with loss of consciousness of >30 min. The Institutional Review Board committee of Stony Brook University approved the studies.

Collection of DNA samples and genotyping. DNA was extracted from venous blood using the Qiagen kit according to manufacturer's instructions (RRID:SCR_008539).

The VNTR region was amplified by PCR using the following primers: forward, ctcacgtctccagagca; reverse, ctggctgctgcaagcaa. The size of the PCR products was established by the QIAxcel system of multicapillary electrophoresis. In the reference genome (UCSC Genome Browser, assembly hg38, RRID:SCR_005780) this simple tandem repeat is represented by a 432 bp sequence. In our population sample we identified primary sequences containing 9- and 7- copies of consensus increments. Herein, we referred to the respective major alleles as L- (eight repeats) and H- (nine repeats). The seven-repeat allele was “rare,” appearing in only 1.5% of the samples. Genotype accuracy was confirmed with an independent PCR analysis of the same samples in multiples (~50% of each sample was tested in duplicates and ~10% in triplicates). All tested samples were successfully genotyped and the PCR results were 100% reproducible. Based on the PCR results, we ascribed the individual genotypes. Allele and genotype frequencies were analyzed with software from the Online Encyclopedia for Genetic Epidemiology Studies (<http://www.oege.org/software/hardy-weinberg.html>; RRID:SCR_001825). We implemented a three-genotype classification, where the carriers of each genotype formed a separate group.

To explore associations between the novel *AKT1* VNTR and the commonly reported *AKT1* polymorphism rs2494732, we genotyped rs2494732 using off-the-shelf Taqman assay for rs2494732 available as a kit

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Table 1. Demographics of *N* = 91 participants who were scanned with PET and [¹¹C]raclopride DRD2 at baseline; separated for *AKT1* genotype

	HH <i>n</i> = 32 (8 female; 25%)		HL <i>n</i> = 43 (7 female; 16%)		LL <i>n</i> = 16 (0 female; 0%)		$\chi^2 = 4.85$	<i>p</i> = 0.089
	Mean	SD	Mean	SD	Mean	SD	$F_{(2,90)}$	<i>P</i> value
Demographics								
Age (years)	33.7	8.1	34.8	8.5	34.0	8.5	0.15	0.86
Education (years)	14.5	2.5	14.0	2.0	13.9	2.1	0.66	0.52
Body mass index	25.5	2.7	26.0	3.0	24.5	3.0	1.41	0.25
Beck depression inventory	3.0	4.2	3.8	6.2	3.1	3.6	0.22	0.80
	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	χ^2	<i>P</i> value
Ethnicity								
African American	16	50	16	37	4	25	12.6	0.049
Caucasian	6	19	18	42	10	63		
Hispanic	5	16	2	5	2	12		
More than one	5	16	7	16	0	0		
	Mean	SD	Mean	SD	Mean	SD	$F_{(2,90)}$	<i>P</i> value*
Baseline D2/3 receptor availability (BP _{ND})								
Caudate	2.4	0.43	2.6	0.58	2.9	0.43	4.78	0.001
Putamen	3.1	0.51	3.3	0.65	3.5	0.49	3.28	0.002
VS	2.9	0.43	2.9	0.50	3.1	0.45	0.67	0.57

*Multivariate test corrected for age, gender, and ethnicity.

Table 2. Demographics of *N* = 54 participants who underwent [¹¹C]raclopride DRD2 baseline and intravenous MP scans; separated for *AKT1* genotype

	HH <i>n</i> = 19 (6 female; 31.6%)		HL <i>n</i> = 27 (7 female; 25.9%)		LL <i>n</i> = 8 (0 female; 0%)		$\chi^2 = 3.17$	<i>p</i> = 0.21
	Mean	SD	Mean	SD	Mean	SD	$F_{(2,53)}$	<i>P</i> value
Demographics								
Age (years)	34.8	7.9	35.3	8.3	38.8	9.8	0.69	0.51
Education (years)	14.9	2.8	14.4	2.1	14.1	1.7	0.42	0.66
Body mass index	25.3	2.3	26.4	2.6	24.4	3.4	2.13	0.13
Beck depression inventory	2.6	3.6	4.4	7.1	3.1	4.2	0.55	0.58
	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	χ^2	<i>P</i> value
Ethnicity								
African American	11	58	11	41	2	25	17.36	0.008
Caucasian	1	5	9	33	6	75		
Hispanic	4	21	1	4	0	0		
More than one	3	16	6	22	0	0		
	Mean	SD	Mean	SD	Mean	SD	$F_{(2,53)}$	<i>P</i> value*
DA release (BP _{ND} placebo–MP)								
Caudate	0.55	0.56	0.36	0.33	0.22	0.16	2.14	0.13
Putamen	0.93	0.66	0.74	0.42	0.49	0.45	1.39	0.26
VS	0.76	0.59	0.35	0.48	0.03	0.72	5.25	0.009

*Multivariate test corrected for age, gender, and ethnicity.

(Thermo Fisher Scientific). We explored its linkage disequilibrium (LD) with the VNTR in our sample using Haploview (Barrett et al., 2005; RRID:SCR_003076).

PET scans. [¹¹C]raclopride scans used in this study have been acquired and reported previously for different study purposes (Volkow et al., 2012b, 2014a,b). All PET scans were performed on a Siemens HR+ scanner following procedures previously reported (Volkow et al., 2014b).

Baseline PET images with [¹¹C]raclopride (*N* = 91) were obtained to measure striatal DRD2 availability. For *N* = 54 of these participants, a second [¹¹C]raclopride was obtained after a challenge with MP, which was used to quantify DA release. The estimates of DA release were calculated as the difference in nondisplaceable binding potential (BP_{ND}) of [¹¹C]raclopride between baseline and MP (Volkow et al., 1994, 2012b). Details on the PET scanning protocol and procedure for MP administration have been previously described (Volkow et al., 2014b). In short, emission scans were started immediately after injection of 4–8 mCi (specific activity 0.5–1.5 Ci/μM at end of bombardment). Twenty dynamic emission scans were obtained from time of injection up to 60 min after

and arterial sampling was used to quantify total carbon-11 and unchanged [¹¹C]raclopride in plasma.

To ensure minimal misplacements upon repositioning, we used a custom-made personalized head holder positioned by a set of two orthogonal lasers aligned to the canthomeatal (CM) plane and the medial sagittal plane for each individual. Motion was minimized by means of the customized head holder, which impeded rotation and lateral movements and by using strap bands to minimize displacements in the *z* planes. Additionally, the dynamic emission scan images were evaluated before analyses to ensure that any motion artifacts or misplacements that would confound the measures were not included in the analyses.

Region of interest analyses. We calculated regional BP_{ND} values for hand-drawn caudate, putamen, and ventral striatum (VS) regions of interest (ROIs) using a procedure previously described (Wang et al., 1999). In short, bilateral caudate, putamen, VS, and cerebellar ROIs were drawn directly in an averaged emission image (summation of images obtained between 10 and 60 min). Striatal ROIs for the caudate, putamen, and VS were obtained from three sequential axial planes where the ROIs were

Figure 1. Striatal ROIs: bilateral caudate, putamen, and VS for PET analyses. ROIs had the same size and shape for every subject. The ratio of the DV in striatal regions was compared to that in the cerebellum to obtain the BP_{ND} , which was used to quantify DRD2 availability.

most visible, and had the same size and shape across subjects: 2.2, 2.2, and 0.8 mm³ respectively (Fig. 1). For the cerebellum, we averaged the values obtained from circular ROIs in the left and right cerebellum (16 mm³) in three contiguous axial planes positioned within 1.0 and 1.7 cm above the CM line. ROI values were computed using the weighted average for the left and right regions from the different slices where the regions were obtained. Time–activity curves in the striatum and cerebellum along with the concentration of the nonmetabolized tracer in plasma were used to obtain the distribution volume (DV) using a reference tissue model (Logan et al., 1990, 1996). The ratio for DV in the striatum to that in the cerebellum corresponds to BP_{ND} and was used to quantify DRD2 availability (Logan et al., 1990).

Statistical analyses on the striatal ROIs were performed with SPSS version 20 (IBM, RRID:SCR_002865). For the baseline DRD2 measures ($N = 91$), we performed multivariate analyses with *AKT1* as the between-group variable and DRD2 (BP_{ND}) in the caudate, putamen, and VS as dependent variables. For MP-induced DA release ($N = 54$), we calculated multivariate analyses using Roy's largest root with *AKT1* as between-group variable and (placebo–MP) DRD2 in caudate, putamen, and VS as dependent variables. *Post hoc* univariate tests (for each striatal region) and *post hoc* Bonferroni's contrasts (for each genotype per striatal region) were performed to investigate directions of effects. Effect sizes are reported as partial η -squared (η^2). Significance threshold was set at $p < 0.05$. Age, gender, and ethnicity were added as covariates. We repeated these analyses for *AKT1* SNP rs2494732, to compare results with the novel *AKT1* VNTR.

Voxelwise analyses. We further analyzed the images on a pixel-by-pixel basis with Statistical Parametric Mapping (SPM8; Wellcome Trust Centre for Neuroimaging, London, UK, RRID:SCR_007037). For this purpose, we normalized the activity in each voxel (average activity, 10–60 min) to that in the cerebellum. We used multiple-regression analyses in SPM8 to assess the effects of *AKT1* genotype on (1) baseline DRD2

availability and (2) MP-induced DA release as computed by the difference images of placebo–MP. Age, gender, and ethnicity were included as covariates. We restricted the SPM analyses to a striatal mask (6228 voxels with 2 mm isotropic resolution) that covered the caudate, putamen, globus pallidus, and VS since the striatum is where our [¹¹C]raclopride measures have sufficient signal-to-noise ratio to measure BP_{ND} in the brain (Wang et al., 2012). Contrast analyses were corrected for familywise errors (FWEs) within the striatal mask with threshold-free cluster enhancement in the Oxford Centre for Functional MRI of the Brain Software Library (RRID:SCR_002823; Smith and Nichols, 2009; Winkler et al., 2014). Contrasts included the following: HH > HL > LL; and the reverse direction: HH < HL < LL. We also report exploratory findings at a liberal threshold of $p < 0.05$ uncorrected $k > 50$.

Results

Identification and characterization of a new VNTR polymorphism in the *AKT1* gene

In the reference genome (assembly hg38), the *AKT1* gene maps to the negative DNA strand of chromosome 14 (14q32.32; chr14: 104,769,350–104,795,743). Along with multiple SNPs (Fig. 2, Common SNPs), *AKT1* encompasses several regions of simple repeats (Fig. 2, Simple Tandem Repeats).

We identified four *AKT1* tandem repeats that exhibited propensity to instability and subsequently tested them in a population sample. One of the analyzed regions was polymorphic. This VNTR resides in intron3, immediately downstream of exon3 (chr14:104779602–104779962), where it is represented by 8.9 imperfect copies (match percentage, 80%) of 42 nucleotide periods positioned in tandem.

AKT1 VNTR alleles and genotype frequencies

For all $N = 91$ participants, the high-VNTR and low-VNTR alleles occurred with similar frequencies (59 and 41%, respectively). Most individuals were heterozygotes (HL, $n = 43$; 47%); followed by homozygous for the “high” allele (HH, $n = 32$; 35%), with the lowest frequency being the homozygous for the “low” allele (LL, $n = 16$; 18%). The genotype frequencies did not deviate from Hardy–Weinberg equilibrium ($\chi^2 = 0.06$, $p = 0.6$). The genotype groups were similar with regards to age ($F_{(2,90)} = 0.15$, $p = 0.9$) and gender ($\chi^2 = 4.85$, $p = 0.09$), but differed in ethnicity ($F_{(2,90)} = 12.6$, $p = 0.049$; Table 1).

In the sample of $N = 54$ individuals who underwent both placebo and MP scans, the frequencies of the *AKT1* VNTR alleles ($n = 19$ HH; $n = 27$ HL; $n = 8$ LL) were also in Hardy–Weinberg equilibrium ($\chi^2 = 0.1$, $p = 0.6$). There were also no group differences in age or gender, but they differed in ethnicity ($F_{(2,53)} = 17.4$, $p = 0.008$; Table 2).

Association of the *AKT1* VNTR with striatal DRD2 availability

For baseline measures, there were no outliers that exceeded 3 SDs for any striatal region. The multivariate ANOVA showed a significant effect of *AKT1* genotype on baseline striatal DRD2 availability ($\Theta = 0.20$, $F_{(3,84)} = 5.47$, $p = 0.002$, $\eta^2 = 0.16$). Effects were significant for the caudate ($F_{(2,90)} = 8.2$, $p = 0.001$, $\eta^2 = 0.16$) and putamen ($F_{(2,90)} = 6.6$, $p = 0.002$, $\eta^2 = 0.13$), but not for the VS ($F_{(2,90)} = 0.5$, $p = 0.6$); all corrected for age, gender, and ethnicity (Fig. 3A). Bonferroni's *post hoc* tests showed that DRD2 availability in the caudate was higher in LL than HH ($p = 0.01$), but there were no statistically significant differences between LL and HL ($p = 0.1$), or between HL and HH ($p = 0.6$). For the putamen, DRD2 availability was also higher in LL compared with HH ($p = 0.04$), but did not differ between HL and LL ($p = 0.3$) or between HL and HH ($p = 0.6$).

Figure 2. Location and genomic features of the human *AKT1* gene. The chromosomal position of the *AKT1* gene indicated with a red bar (chromosomal ideogram). A snapshot of the *AKT1* locus (USCS Genome Browser, hg19) illustrates its principal genomic characteristics, including high CpG density (blue arrow indicates a track of GC percentage of the sequence with full display mode), numerous simple tandem repeats, and multiple alternatively spliced mRNAs (red bracket). Evolutionary conservation track at the bottom illustrates low sequence conservation across the mammalian species, which is constrained to coding elements.

DRD2 availability decreased with age in all three striatal ROIs (caudate $F_{(1,90)} = 63.1$, $\eta^2 = 0.43$; putamen $F_{(1,90)} = 67.3$, $\eta^2 = 0.44$; VS $F_{(1,90)} = 38.8$, $\eta^2 = 0.31$; all p 's < 0.0001), which is consistent with previous findings (Volkow et al., 2000; Ishibashi et al., 2009; Kim et al., 2011). There were no effects of gender or ethnicity on DRD2 availability in any striatal region, and there were no region $\times$ group interactions.

SPM analysis for baseline DRD2 availability showed no significant clusters for either contrast at $p_{\text{FWE}} < 0.05$. Exploratory analyses at $p < 0.05$ uncorrected $k > 50$ showed an effect of *AKT1* VNTR groups on baseline DRD2 availability (HH < HL < LL) in bilateral striatum clusters [right peak (in MNI space): [8, 10, -8], $t = 2.74$, $k = 15$, $p = 0.004$ (uncorrected); left peak: [-12, 10, 2], $t = 2.64$, $k = 6$, $p = 0.005$ uncorrected; covering caudate and anterior putamen]. There were no significant clusters for the reverse contrast of HH > HL > LL.

Association of the *AKT1* VNTR with striatal DA release (placebo–MP)

For DA release measures, there was one >3 SD outlier for the putamen and caudate, but not for VS. *AKT1* genotype had an effect on striatal MP-induced DA release ($\Theta = 0.25$, $F_{(3,47)} = 3.84$, $p = 0.015$, $\eta^2 = 0.20$). Separate univariate ANOVAs showed

that this effect was only significant for the VS ($F_{(2,53)} = 5.3$, $p = 0.009$, $\eta^2 = 0.18$), with Bonferroni's *post hoc* tests showing stronger DA release in HH > HL ($p = 0.05$) and in HH > LL ($p = 0.01$; Fig. 3B). Thus, despite lower DRD2 in *AKT1* genotype group HH < HL < LL, MP-induced DA release shows the opposite effect of an enhanced release in *AKT1* genotype groups HH > HL > LL. An exploratory analysis on raclopride BP_{ND} during intravenous MP alone shows an even stronger effect of *AKT1* genotype (caudate $F = 10.57$, $p < 0.0001$; putamen $F = 10.71$, $p < 0.0001$; VS $F = 2.86$, $p = 0.067$). There was a small nonsignificant age effect on DA release in putamen only ($F_{(2,53)} = 4.5$, $p = 0.038$, $\eta^2 = 0.09$), but no other effects of gender or ethnicity. There were also no region $\times$ group interactions.

There were no significant effects of *AKT1* VNTR groups on striatal DA release at $p_{\text{FWE}} < 0.05$. However, exploratory analyses at $p < 0.005$ uncorrected showed an effect of *AKT1* VNTR groups on striatal DA release (HH > HL > LL) in the bilateral striatum (right peak: [34, 0, -2], $t = 3.41$, $k = 64$, $p = 0.001$ uncorrected; left peak: [-16, 12, -14], $t = 3.38$, $k = 335$, $p = 0.001$ uncorrected). Clusters covered the VS, caudate, and anterior putamen. There were no significant clusters for the reverse contrast HH < HL < LL.

Figure 3. *A*, *AKT1* VNTR genotype modulated baseline DRD2 availability in the caudate ($p = 0.001$) and putamen ($p = 0.002$), but not in the VS ($p = 0.6$); corrected for age, gender, and ethnicity. Bonferroni's *post hoc* tests showed that DRD2 availability in the caudate was higher in LL than HH ($p = 0.01$), but there were no differences between LL and HL ($p = 0.1$) or between HL and HH ($p = 0.6$). *B*, *AKT1* VNTR genotype modulated MP-induced DA release in the VS only ($p = 0.009$); corrected for age, gender, and ethnicity. Bonferroni's *post hoc* tests showed stronger DA release in HH > HL ($p = 0.05$) and in HH > LL ($p = 0.01$). Error bars depict SEM.

Association between *AKT1* SNP rs2494732 and DRD2 and MP-induced DA release

The *AKT1* SNP rs2494732 has been associated with schizophrenia and with cannabis-induced psychoses (Di Forti et al., 2012; Morgan et al., 2016). Thus, we wanted to assess whether the new VNTR polymorphism was in linkage disequilibrium (LD) with rs2494732 and whether this SNP was associated with striatal DRD2 availability and DA release.

We found that there is low LD between the new *AKT1* VNTR and *AKT1* SNP rs2494732 ($d' = 0.524$, $r^2 = 0.167$). *AKT1* rs2494732 genotypes were in Hardy–Weinberg equilibrium (TT = 22; CT = 53; CC = 17; $\chi^2 = 2.89$, $p = 0.52$), and there were no differences in rs2494732 allelic distribution by gender ($\chi^2 = 1.19$, $p = 0.55$), or ethnicity ($\chi^2 = 10.79$, $p = 0.095$). There was, however, a significant association between the allelic distribution of the *AKT1* VNTR (HH/HL/LL) and rs2494732 (CC/CT/TT; $\chi^2 = 20.98$, $p < 0.001$).

Analyses of the association between *AKT1* SNP rs2494732 and DRD2 availability showed a trend-wise effect on baseline DRD2 in the putamen ($F_{(2,89)} = 2.5$, $p = 0.088$, $\eta^2 = 0.06$; Fig. 4*A*). However, there were no significant effects in the caudate ($F_{(2,89)} = 2.1$, $p = 0.12$, $\eta^2 = 0.05$) or VS ($F_{(2,89)} = 0.26$, $p = 0.77$, $\eta^2 = 0.006$).

For DA release, there was an effect of rs2494732 in the putamen only ($F_{(2,53)} = 5.4$, $p = 0.008$, $\eta^2 = 0.17$), with CC showing an enhanced DA response to MP than CT ($p = 0.09$) and TT ($p = 0.008$; Fig. 4*B*). There were no significant effects in the caudate ($F_{(2,53)} = 2.1$, $p = 0.12$, $\eta^2 = 0.05$), or VS ($F_{(2,53)} = 0.26$, $p = 0.77$, $\eta^2 = 0.006$).

Discussion

Here we report the first evidence of an association between a newly identified *AKT1* VNTR polymorphism and striatal baseline DRD2 availability and stimulant-induced striatal DA release.

Our analysis, which represents largest imaging genetics study on DA signaling in humans so far (Gluskin and Mickey, 2016), indicates that the novel *AKT1* VNTR may serve as a predictive maker for striatal dopaminergic neurotransmission.

Association between the *AKT1* VNTR and striatal DRD2 availability

We showed that an *AKT1* VNTR located in intron3, immediately downstream of exon3, was associated with baseline striatal DRD2 availability in the healthy human brain. Specifically, LL had higher DRD2 availability in caudate and putamen ROIs than HH, and heterozygotes (HL) were in between. We only showed a trend effect for *AKT1* rs2494732 and DRD2 availability in the putamen. Exploratory SPM analyses also showed significant clusters for the contrast HH < HL < LL at $p < 0.05$ uncorrected, covering the caudate and anterior putamen, but not for the reverse contrast of HH > HL > LL. These clusters, however, did not meet the cluster-correction criterion at $p_{FWE} < 0.05$. Changes in striatal DRD2 availability have been implicated in SUD (Volkow et al., 2012a; Hou et al., 2014) and SZ (Weinstein et al., 2016), with the latter disorder mainly treated by antipsychotic DRD2 antagonists (Seeman et al., 1976). It is recognized that DRD2 signal through two different pathways: a “traditional” cAMP protein kinase A and phospholipase C pathway (Tranham-Davidson et al., 2004), and a more recently discovered cAMP-independent pathway in which β -arrestin 2 binds PP2A and Akt, activating GSK3 (Beaulieu et al., 2004, 2005). *AKT1* thus regulates G-protein-independent signaling of DRD2 and is involved in DRD2 desensitization and internalization (Oda et al., 2015; Li et al., 2016). Polymorphisms in *AKT1* have been associated with SZ (Ikeda et al., 2004; Norton et al., 2007; Chow et al., 2016), psychosis risk in SZ (van Winkel et al., 2011), psychotic responses to cannabis (Di Forti et al., 2012; Morgan et al., 2016), working-memory brain activations, and cortical and striatal volumes (Tan et al., 2008, 2012a,b; Blokland

Figure 4. *A*, *AKT1* rs2494732 genotype had a trend-wise effect on baseline DRD2 in putamen ($p = 0.088$; corrected for age, gender, and ethnicity), but there were no significant effects for the caudate or VS. *B*, *AKT1* rs2494732 genotype modulated MP-induced DA release in the putamen only ($p = 0.008$), corrected for age, gender, and ethnicity. CC showed an enhanced DA response to MP than CT ($p = 0.031$) and TT ($p = 0.003$). Error bars depict SEM.

et al., 2017). Our study is the first to find that a genetic variant in *AKT1* influences striatal DRD2 availability that might reflect Akt's role in DRD2 internalization and membrane availability.

Reports on the genetic variations that explain individual differences in DRD2 expression in the healthy human brain have mostly focused on the ankyrin repeat and kinase domain containing 1 gene (*ANKK1*) variant rs1800497 (i.e., Taq1A polymorphism; Pohjalainen et al., 1998; Jönsson et al., 1999; Hirvonen et al., 2009; Savitz et al., 2013b; Eisenstein et al., 2016). Overall, these studies have reported lower striatal DRD2 availability for carriers of the minor allele (Lys713) relative to major allele homozygotes (for review, see Gluskin and Mickey, 2016). Brain imaging studies on the *DRD2* gene and striatal DRD2 availability have reported associations with SNP rs1079597 (Jönsson et al., 1999; but see Laruelle et al., 1998), with rs6277 and rs1799732 (Gluskin and Mickey, 2016), and with rs1076560 (Bertolino et al., 2010). We also reported an association between a VNTR polymorphism in the period circadian clock 2 gene *PER2* and striatal DRD2 availability (Shumay et al., 2012).

Association between *AKT1* VNTR and MP-induced DA release in the striatum

Our study also revealed that the *AKT1* VNTR was associated with MP-induced DA release in the VS. Despite lower DRD2 in *AKT1* genotype group HH < HL < LL, MP-induced DA release (i.e., [¹¹C]raclopride BP_{ND} [placebo-MP]) shows the opposite effect of enhanced release in *AKT1* genotype groups HH > HL > LL. SPM analyses did not meet cluster-correction criterion at $p_{FWE} < 0.05$. However, exploratory results at a liberal threshold of $p < 0.05$ uncorrected showed activated clusters for HH > HL > LL covering the caudate and anterior putamen, but not for the reverse contrast HH < HL < LL, which was in line with the ROI analyses. Most imaging studies in healthy controls on the role of genes on striatal DA release have measured responses to a pain stressor and reported associations with *DRD2* rs4274224 (Peciña

et al., 2013); serotonin 5HT2C gene (*HTR2C*) rs6318, Cys23Ser (Mickey et al., 2012); mu opioid receptor gene (*OPRM1*), rs1799971, A118G (Peciña et al., 2015); leptin gene (*LEP*) rs12706832 (Burghardt et al., 2012); and in females only, with oxytocin gene (*OXT*) rs4813625 (Love et al., 2012). The *COMT* Val158Met variant was also associated with DA release in the prefrontal cortex after a psychosocial stressor (Hernaus et al., 2013). Additionally, studies on gambling-induced DA release have reported an association with *DRD3* rs6280 (Savitz et al., 2013a), and with the Taq1A polymorphism (Joutsa et al., 2014). On the other hand, the *COMT* Val158Met variant was not associated with amphetamine-induced DA release in the prefrontal cortex (Narendran et al., 2016).

Thus, to our knowledge, this is the first study that reports on a gene variant that influences stimulant-induced DA release in the healthy human brain. DA stimulation leads to dephosphorylation of Akt and activation of GSK-3-mediated G-protein-independent signaling. *AKT1*^{-/-} mice show enhanced responses to amphetamine and ketamine (Emamian et al., 2004; Featherstone et al., 2013), consistent with the belief that depressed Akt function may cause a DA supersensitive state (Oda et al., 2015). The *AKT1* VNTR may therefore serve as a biomarker for diseases with disrupted DA signaling, including SZ (Laruelle et al., 1996; Abi-Dargham et al., 1998; Kegeles et al., 2010) and SUD (e.g., in cannabis and cocaine abusers: Volkow et al., 2014a,b; van de Giessen et al., 2017).

The HH allele, which showed enhanced DA release in VS may serve as a risk allele for SZ and psychoses. Similarly, the C allele of *AKT1* SNP rs2494732, which has been associated with SZ (van Winkel et al., 2011; Di Forti et al., 2012), and with cannabis-induced psychosis, compared with TT (Morgan et al., 2016), showed enhanced DA responses to MP (Morgan et al., 2016, Fig. 3B). Since large DA increases in the striatum are associated with psychosis (Lieberman et al., 1990), the enhanced striatal DA reactivity of C allele carriers could explain these associations (Di Forti et al., 2012; Morgan et al., 2016). The novel VNTR, how-

ever, is not in strong LD with rs2494732, and it remains to be explored whether it shows any involvement in SZ and drug-induced psychosis.

New VNTR polymorphism in the human *AKT1* gene

The new *AKT1* VNTR is not imputable from other markers because it resides outside *AKT1* haplotype blocks (HapMap), and is not in strong LD with *AKT1* SNPs. The VNTR showed stronger effects on DRD2 than SNP rs2494732, suggesting that it might be a more sensitive marker of striatal dopaminergic signaling. However, future studies are necessary to examine the effects of the novel VNTR in animal models.

The *AKT1* gene has high GC content over the entire gene body (the CG density track; Fig. 2, blue arrow), making it amenable to DNA methylation, which impacts gene transcription. Evolutionary analyses of CpG-rich regions (Cohen et al., 2011) showed that enrichment in GC content of *AKT1* are evolutionally recent and that one of the Weizmann evolutionary CpG islands (CGI: 3.1) encompasses the VNTR under study. The *AKT1* VNTR alters the number of potential DNA methylation sites since each repeated period contains four CpG sites. Another epigenetic mechanism is nucleosome positioning (Kaplan et al., 2009; Tillo and Hughes, 2009; Levo and Segal, 2014) and the *AKT1* VNTR localizes within the nucleosome exclusion region, featuring the highest nucleosome exclusion score within the *AKT1* boundaries. This suggests that the polymorphic sequence has open chromatin structure, further strengthening the notion that the VNTR region might contain regulatory elements. The VNTR may therefore modulate the epigenetic potential of *AKT1*, and, given its proximity to exon 3, its length may affect splicing fidelity.

Study limitations

Our study had at least four limitations. First, our sample size was of mixed ethnicity and, despite correcting for ethnicity, effects may be partially due to genetic admixture. Future studies investigating the effect of *AKT1* on DRD2 and on DA release should control for population stratification, especially since we found an effect of ethnicity of the *AKT1* VNTR. Second, while the *AKT1* SNP rs2494732 has been associated with psychosis and SZ (van Winkel et al., 2011; Di Forti et al., 2012; Morgan et al., 2016) and was hence relevant to our PET DA outcome measures, the role of the novel *AKT1* VNTR in Akt, in DRD2 protein expression, in SZ, or in psychosis remains to be determined. In addition, the current study was limited to two markers on the *AKT1* gene and future studies should investigate other *AKT1* markers, including *AKT1* rs1130233, which has been associated with working-memory processing (Tan et al., 2012a). Future studies might also study interactions of genetic variants that have been associated with DRD2 or DA release, such as Taq1A, Taq1B, and *DRD2* SNPs rs1079597, rs1076560, rs6277, rs1799732, rs12364283, rs2283265 (Zhang et al., 2007; Bertolino et al., 2009a,b; Gluskin and Mickey, 2016). Third, [¹¹C]raclopride binds to both D2 and D3 receptors and while in dorsal striatum its binding reflects mostly D2, since D3 receptors are very low. In VS the levels are similar and thus we cannot differentiate the relative contribution of D2 versus D3 on the association between *AKT1* and DA release in VS. Future studies with higher affinity DRD2 tracers (e.g., [¹⁸F]fallypride) are needed to test whether *AKT1* modulates DRD2 availability in extrastriatal regions. Last, MRI images were not available for all subjects, which limited our capacity to coregister images and determine striatal ROIs. However, we and others have shown that MRI coregistration does not improve quantification of [¹¹C]raclopride binding (Wang et al., 1997; Kuhn et al., 2014). This might explain why the

ROI findings were stronger than the voxelwise statistics, which did not reach significance at p_{FWE} for the effects of *AKT1* on baseline DRD2 availability and MP-induced DA release, but only showed significance at a very liberal threshold.

In sum, our findings confirm an involvement of *AKT1* in regulating dopaminergic striatal signaling both as it relates to DRD2 availability and stimulant-induced DA release.

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