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## Gene Variants of Brain Dopamine Pathways and Smoking-Induced Dopamine Release in the Ventral Caudate/Nucleus Accumbens

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### Abstract

**Context**—Preclinical studies demonstrate that nicotine administration leads to dopamine release in the ventral striatum. However, human studies reveal considerable interindividual variability in the extent of smoking-induced dopamine release.

**Objective**—To determine whether common gene variants of the brain dopamine pathway explain this observed phenotypic variability in humans.

**Design**—Blood samples were drawn to determine gene variants of dopamine system components, and positron emission tomography scanning with the radiotracer raclopride labeled with radioactive carbon ( $^{11}\text{C}$ ) was performed to measure smoking-induced dopamine release.

**Setting**—Academic brain imaging center.

**Participants**—Forty-five tobacco-dependent smokers.

**Interventions**—Subjects either smoked a cigarette (n=35) or did not smoke (n=10) during positron emission tomography scanning.

**Main Outcome Measures**—Gene variants of dopamine system components (the dopamine transporter variable nucleotide tandem repeat,  $D_2$  receptor Taq  $A_1/A_2$ ,  $D_4$  receptor variable nucleotide tandem repeat, and catechol-O-methyltransferase Val158Met polymorphisms) and change in [ $^{11}\text{C}$ ]raclopride binding potential in the ventral caudate/nucleus accumbens on positron emission tomography scans.

**Results**—For subjects who smoked during scanning, those with at least one 9 allele of the dopamine transporter variable nucleotide tandem repeat, fewer than 7 repeats of the  $D_4$  variable nucleotide tandem repeat, and the Val/Val catechol-O-methyltransferase genotype had greater decreases in binding potential (an indirect measure of dopamine release) with smoking than those with the alternate genotypes. An overall decrease in ventral caudate/nucleus accumbens binding potential in

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those who smoked compared with those who did not smoke was also found but was smaller in magnitude than previously reported.

**Conclusions**—Smokers with genes associated with low resting dopamine tone have greater smoking-induced (phasic) dopamine release than those with alternate genotypes. These findings suggest that dopamine system genotype variabilities explain a significant proportion of the interindividual variability in smoking-induced dopamine release and indicate that smoking-induced dopamine release has a genetic predisposition.

ACOMMON PATHWAY FOR the reinforcing properties of most, if not all, addictive drugs is the mesolimbic brain dopamine (DA) system.<sup>1-4</sup> In laboratory animal studies, the reinforcing property of nicotine has repeatedly been linked to DA release in the nucleus accumbens (NAc).<sup>5-14</sup> Recent small studies of human smokers have used raclopride labeled with radioactive carbon (<sup>11</sup>C) with positron emission tomography (PET) to assess change in DA concentration in response to smoking. These studies have generally supported the association between smoking and DA release, especially the relief from craving<sup>15</sup> and hedonic responses<sup>16</sup> that smokers experience when smoking. However, considerable unexplained interindividual variability in smoking-induced DA release was reported in these studies.

The high heritability of nicotine dependence<sup>17</sup> and differences in individual sensitivity to the reinforcing properties of smoking implicate the influence of genetic differences on positive reinforcement from smoking.<sup>18</sup> In the primary analysis of the present study, we sought to determine if known genetic variability in components of the brain DA pathway would help explain observed phenotypic variability in smoking-induced DA release found among human smokers. Although others have associated genetic variability with overall D<sub>2</sub> dopamine receptor densities,<sup>19-22</sup> we are aware of no prior studies that examined the link between DA genetic variability and DA release in humans. For this initial study, we restricted our examination to those well-characterized gene variants with reported functional effects while recognizing that many other genes could contribute to variation in smoking-induced DA release.

Within the mesolimbic DA pathway, nicotine stimulates nicotinic acetylcholine receptors on neurons of the ventral tegmental area that project to the NAc and release DA, resulting in positive reinforcement.<sup>9,23,24</sup> Genotypic (and associated phenotypic) variation of any of the components of this pathway could affect the degree of smoking-induced DA release in individual smokers. From a large number of potential genes of interest, specific gene variants were chosen for analysis from well-studied parts of brain DA pathways, namely (1) the DA transporter (DAT),<sup>25</sup> (2) the D<sub>2</sub> dopamine receptor (DRD2) (a presynaptic and postsynaptic receptor<sup>26</sup> that is activated indirectly by even low concentrations of nicotine<sup>27</sup>), (3) the D<sub>4</sub> dopamine receptor (DRD4) (a receptor that is expressed in low levels in the basal ganglia but may modulate excitatory neurotransmission<sup>28</sup>), and (4) the enzyme catechol-O-methyltransferase (COMT).<sup>29</sup>

Four genetic variants were chosen from these DA system components based on substantial evidence of functional consequences of these variants, possible association with risk for nicotine dependence, and/or influence on brain DA system regulation. For DAT, the variable number of tandem repeat (VNTR) polymorphism of the 3' untranslated region (SLC6A3)<sup>30</sup> was selected because of reports linking the 10-repeat allele with risk for conditions associated with smoking behavior, namely attention-deficit/hyperactivity disorder,<sup>31</sup> hyperactivity,<sup>32</sup> and reward dependence.<sup>33</sup> Studies using single photon emission computed tomography have demonstrated associations between the 10-repeat allele and increased,<sup>34</sup> decreased,<sup>35,36</sup> or unaltered<sup>37-39</sup> DAT density (compared to alternate genotypes) with the larger (sample-size) studies demonstrating decreased or unaltered DAT density. If subjects with the 10-repeat allele

indeed have decreased DAT density, we hypothesize that such subjects would have higher tonic levels of intrasynaptic DA and less phasic DA release in response to smoking.

For DRD2, A<sub>1</sub>/A<sub>2</sub> allelic variation of Taq1 was selected because the A<sub>1</sub> allele is associated with low DRD2 density,<sup>19,20,40-42</sup> increased likelihood of smoking behavior overall,<sup>43</sup> progression to higher levels of smoking by adolescents,<sup>44</sup> less positive responsiveness to bupropion hydrochloride<sup>45,46</sup> (a medication with dopaminergic properties) or other treatments<sup>47</sup> in smokers attempting to quit, and greater feelings of food reward following smoking cessation.<sup>48</sup> Because the A<sub>1</sub> allele is associated with decreased DRD2 density and function, and the DRD2 receptor is associated with inhibition of DA release,<sup>49,50</sup> we hypothesized that subjects with the A<sub>1</sub> allele would have greater DA release (due to less inhibition) than those with the alternate genotype.

For DRD4, variation in the 48–base pair (bp) VNTR in exon 3 was chosen based on the association of the 7-repeat allele with a reduced ability of DA to inhibit cAMP formation,<sup>51</sup> reduced effectiveness of DA-releasing medications,<sup>52</sup> and links with smoking behavior.<sup>53</sup> Because DRD4 stimulation results in sensitization to a DA-releasing drug<sup>54</sup> and blockade in the NAc results in diminished DA release,<sup>55</sup> we hypothesized that subjects with the 7-repeat allele of DRD4 (having less DRD4 function) would have less smoking-induced DA release than subjects with the alternate genotype.

For COMT, we chose the 1947A>G (Val158Met) polymorphism because the Val substitution is associated with 3-fold to 4-fold higher enzyme activity<sup>56</sup> as well as substance abuse<sup>57</sup> and the personality traits novelty seeking and reward dependence.<sup>58</sup> Additionally, a recent review of the functional effects of the COMT polymorphism<sup>59</sup> concluded that the Met allele (low enzyme activity) results in increased levels of tonic DA and reciprocal reductions in phasic DA released in subcortical regions. Based on these reports, we hypothesized that subjects with the Met allele would have less smoking-induced DA release than subjects with the alternate genotype.

The 4 gene variants were studied with the recognition that research linking genetic variants with smoking and/or the brain DA system is an evolving field, and only modest links exist in the scientific literature between genotype and smoking.<sup>60,61</sup> Other genotypes (such as  $\alpha_4\beta_2$  nicotinic acetylcholine receptor,<sup>62</sup> dopamine  $\beta$  hydroxylase,<sup>63</sup> and 5-HTT<sup>60</sup> functional polymorphisms) are certainly candidates for associations with smoking-induced DA release; however, we felt that evidence for including these genotypes was not as strong as for the ones selected. We also recognize that some of the DA genotypes chosen here have had negative reports for associations with smoking,<sup>64-67</sup> and some have had conflicting results regarding which allelic variants (if any) are associated with smoking.<sup>68-71</sup>

In addition to the primary analysis of this study (genotype/PET associations), we also tested for evidence of smoking-induced DA release as we have in the past<sup>15</sup> with the present study having a larger sample of smokers and subjects being scanned on a newer PET scanner with improved sensitivity.

## METHODS

### SUBJECTS AND STUDY OVERVIEW

Forty-five otherwise healthy adult (21-65 years old) cigarette smokers (15-40 cigarettes per day) were interviewed with standardized questions; filled out rating scales related to cigarette usage, mood, and personality; had blood drawn for genetic testing; underwent [<sup>11</sup>C]raclopride PET scanning and either smoked a cigarette (n=35) or did not smoke (n=10) during the scan; and underwent structural magnetic resonance imaging (MRI) (to aid in interpretation of the

PET scans). Subjects completed genetic testing, PET scanning, and administration of rating scales on a single afternoon and underwent structural MRI on a separate morning within 1 week of PET scanning. The present study represents a new group of subjects that have no overlap with those in our previous report.<sup>15</sup>

Subjects were screened initially during a telephone interview in which medical, psychiatric, and substance abuse histories were obtained without personal identifiers. All subjects who qualified were assessed in person using screening questions from the Structured Clinical Interview for *DSM-IV* (SCID).<sup>72</sup> Inclusion and exclusion criteria were the same as in our previous report with the central inclusion criterion being *DSM-IV* nicotine dependence and subjects being excluded for any history of an Axis I psychiatric or substance abuse/dependence diagnosis other than nicotine dependence. After a complete description of the study was provided to the subjects, written informed consent was obtained using forms approved by the local institutional review board.

## GENETIC TESTING

Blood samples were collected and genomic DNA was extracted from whole blood with the Qiagen Kit using the manufacturer's protocol (Qiagen, Valencia, Calif).

The DAT1 (SLC6A3) 40-bp repeat polymorphism was genotyped as described elsewhere.<sup>48</sup> The DRD2 TaqA<sub>1</sub> polymorphism and the COMT Val108/158Met polymorphism were genotyped by polymerase chain reaction, restriction digest, and electrophoresis according to published methods.<sup>19,56</sup> The DRD4 48 bp repeat polymorphism was genotyped as follows, based on a modification of published methods. Polymerase chain reaction amplification of 50 ng of genomic DNA was performed in 20  $\mu$ L reactions containing 6pM deoxynucleoside triphosphate, 8 nmol of forward and reverse primers, 0.2  $\mu$ M Tris (8.4 pH), 1  $\mu$ M potassium chloride, 30 pmol of magnesium, 1 $\times$  Q solution, and 1 U of HotStar Taq DNA polymerase (Qiagen). The primer sequences used were DRD4F, 5'-CTA CCC TGC CCG CTC ATG-3'; DRD4R, 5'-CCG GTG ATC TTG GCA CGC-3'. Using the MJ Research Tetrad thermal cycler (Bio-Rad, Hercules, Calif), DNA was denatured at 95°C for 15 minutes, followed by 10 cycles of 95°C for 30 seconds, 66°C for 30 seconds, and 72°C for 90 seconds and 25 cycles of 95°C for 30 seconds, 55°C for 30 seconds, and 72°C for 90 seconds, followed by a final extension at 72°C for 10 minutes. Ten  $\mu$ L of polymerase chain reaction products were electrophoresed in 3.5% Nusieve Agarose (Cambrex, Baltimore, Md) in 1X TBE (Tris/borate/EDTA) buffer for approximately 4 hours at 70 V. The gels were stained with ethidium bromide, and alleles were determined by comparison with molecular weight standards and with control individuals with previously determined genotypes.

## PET (AND MRI) SCANNING AND IMAGE ANALYSIS

Scanning sessions with PET and MRI followed the same protocol as in our previous report<sup>15</sup> with the exception that scans for the current group were performed on a newer PET scanner (described later in this section). Positron emission tomography scanning consisted of a single [<sup>11</sup>C]raclopride bolus-plus-continuous-infusion PET session. Subjects were instructed to smoke per their usual habit on the morning of the PET session and to smoke a cigarette immediately prior to the test session, which began at noon. Between noon and 1:45 PM, subjects were interviewed and they filled out rating scale questionnaires and other measures. The rating scales included screening questions from the Structured Clinical Interview for *DSM-IV*,<sup>72</sup> the Fagerström Test for Nicotine Dependence,<sup>73,74</sup> the Urge to Smoke (UTS) scale,<sup>75</sup> the Spielberger State-Trait Anxiety Index,<sup>76</sup> and the Hamilton Depression<sup>77</sup> and Anxiety<sup>78</sup> rating scales. Exhaled carbon monoxide levels were measured prior to [<sup>11</sup>C]raclopride injection using a MicroSmokerlyzer (Bedfont Scientific Ltd, Kent, United Kingdom) to make a rough estimate of recent smoking level. In addition to these prescanning ratings, cigarette craving (UTS) and

state anxiety (Spielberger State-Trait Anxiety Index) levels were monitored at 3 other points during PET scanning. Only the UTS scores were analyzed with the PET data (as discussed later in this section) while other rating scales were administered to ensure low levels of psychiatric symptoms in the study sample.

At 1:45 PM, subjects had a 20-gauge intravenous catheter placed and were positioned on the PET scanner (for acquisition of planes parallel to the orbital-meatal line) at 2 PM. Scanning was initiated at 2:10 PM with a slow bolus injection of 185 MBq [<sup>11</sup>C]raclopride in 20 mL normal saline over a 60-second period followed by continuous infusion of the tracer (111 MBq/h) for the remainder of the testing session. This bolus-pluscontinuous-infusion method was performed as described in prior studies.<sup>79,80</sup> Brain scans were acquired continuously for the next 50 minutes.

At 3 PM, subjects were taken off the scanner with the infusion still running and had a 10-minute break in an outdoor area adjacent to the PET scanning room. Thirty-five subjects smoked a regular cigarette (either their favorite brand or a standard study cigarette) during this period; the remaining subjects stood in the same outdoor area but did not smoke during scanning as a control condition (assignment to the smoking or no-smoking condition was random with a ratio of 4:1). The 3-hour period of abstinence prior to the break was chosen based on the finding that craving starts to peak at this time in nicotine-dependent smokers.<sup>81</sup> A study investigator (A.L.B. or R.E.O.) monitored subjects continuously during the break. After the break, subjects were quickly repositioned (<1 minute) in the scanner using a preplaced mark on their forehead and a laser light from the scanner. Subjects were then scanned for 30 minutes longer.

Subjects were scanned on a GE Advance NXi scanner (General Electronic Medical Systems, Milwaukee, Wis) with 35 slices in 3-dimensional mode. We prepared [<sup>11</sup>C]raclopride using an established procedure.<sup>82,83</sup> Positron emission tomography scans were acquired as 10 prebreak and 6 postbreak 5-minute frames. Attenuation correction scanning was performed with the germanium rotating rod source built into the GE scanner for 5 minutes following the postbreak scan, and this attenuation correction was applied to both prebreak and postbreak scans.

An MRI scan of the brain was obtained with the following specifications: 3-dimensional Fourier-transform spoiled-gradient-recalled acquisition; repetition time, 30 ms; echo time, ms; 30° angle; 2 acquisitions; 256×192 view matrix. The acquired volume was reconstructed as 90 contiguous 1.5-mm-thick transaxial slices.

Coregistration of PET scans to MRI scans was performed using the automated image registration method<sup>84</sup> within MEDx 3.3 (Sensor Systems Inc, Sterling, Va). The prebreak (0- to 50-minute) and postbreak (60- to 90-minute) scans were coregistered to the MRI separately. Individual 5-minute time frames were then coregistered to MRI scans using the transformation matrix parameters from the coregistration of the summed images.

Regions of interest were drawn on MRI and transferred onto the coregistered PET scan frames. Region-of-interest placement on each frame was verified by visual inspection by the region drawers (D.S. and E.H.) and the principal investigator. For this study, the volume-of-interest (VOI) value used for statistical analysis was the volume-weighted mean of left and right ventral caudate (VCD) regions of interest (including the NAc) (Figure 1), consisting of 3 slices each, resulting in a single VOI value for each 5-minute frame of PET scanning. Volume-weighted means of left and right dorsal caudates and putamen were drawn as additional regions as in our prior report.<sup>15</sup> The entire cerebellum was drawn as a reference region. Binding potential (BP) for the VCD/NAc (and other striatal regions) was calculated using the simplified reference tissue model<sup>85</sup> with  $BP = B'_{max} / K_d = C_{VCD/NAc} / C_{cerebellum} - 1$ , where C is radioligand concentration. Binding potential was determined for each 5-minute PET frame, and mean BP



values were compared between the frames before (40-50 minutes) and after (60-90 minutes corrected for radiotracer physical decay) the break in scanning. These time frames are almost identical to those recommended and used as optimal for maximizing the signal-to-noise ratio in studies of the [<sup>11</sup>C]raclopride bolus-plus-continuousinfusion method.<sup>86,87</sup> This method assumes 40 minutes for the bolus plus infusion to reach a near steady state.<sup>80,86,87</sup> It also assumes that DA concentration will remain elevated throughout the 30-minute postsmoking scan as indicated by animal microdialysis<sup>5,88-90</sup> and imaging<sup>91</sup> studies of nicotine administration. A single primary ventral striatum VOI was used to avoid multiple comparison corrections, which would reduce the power of this analysis.

## STATISTICAL ANALYSES

**General**—Because of the possibility of type I statistical error in a large data set such as this one (which includes gene variant data for many genes, PET data for multiple regions, and rating scale and demographic data), we limited statistical tests to the fewest possible relevant variables based on prior research. For genetic variant data, 4 gene variants were studied, and each was used to divide the study group into 2 subgroups. The 4 genetic variants (described earlier in the article) and the subgroups based on these variants were the DAT VNTR (9/9 and 9/10 vs 10/10), DRD2 Taq1 (A<sub>1</sub>/A<sub>1</sub> and A<sub>1</sub>/A<sub>2</sub> vs A<sub>2</sub>/A<sub>2</sub>), DRD4 VNTR (less than 7 repeats vs 7 or more repeats), and COMT (Met/Met and Met/Val vs Val/Val) polymorphisms. For PET data, the only variable used was percentage change in BP for the average of left and right VCD/NAc VOIs from the 10-minute period before to the 30-minute period after the break. A single VOI BP value was felt to be sufficient based on our prior report that demonstrated nearly identical BP changes for the left and right VCD/NAc<sup>15</sup> and similar changes across all striatal regions. Statistical tests described in the following sections were performed with SPSS version 11.5 (SPSS, Chicago, Ill).

**Associations Between Genetic Variants and Smoking-Induced DA Release**—For the primary analysis here, an overall univariate analysis of variance was performed for the 35 subjects who smoked during scanning with percentage change in VCD/NAc BP as the dependent variable and the 4 genetic variants as between-subject factors without interactions. Each genetic variant subgroup's VCD/NAc BP percentage change was examined to assess which genetic variants had greater and lesser degrees of smoking-induced DA release. To further characterize results of this primary analysis, *t* tests (2-tailed) were performed comparing the subgroups of the genotypes found significant in the preceding analysis to determine if they differed significantly in levels of smoking (cigarettes per day), number of years smoking, or change in craving (UTS score) from before to after smoking.

**Study of Smoking-Induced DA Release**—In our previous study,<sup>15</sup> an unexpectedly large percentage change in ventral striatal BP with smoking was found in a small sample of subjects who smoked during scanning (n=10). Here, we studied a larger group of subjects using a newer PET scanner and attempted to again test the hypothesis that smoking would result in greater reductions in VOI BP than a nonsmoking control. For this analysis, a *t* test (2-tailed) was performed for percentage change in VCD/NAc BP in the group that smoked (n=35) vs the control group that did not smoke (n=10). Correlation coefficients were also determined between percentage change in VCD/NAc BP and change in UTS (craving) score for the group that smoked to seek to again demonstrate an association between change in the indirect measure of DA release (change in VCD/NAc BP) and change in craving (UTS score).<sup>15</sup>

## RESULTS

### GENERAL SUBJECT CHARACTERISTICS

The study population consisted of adults (mean±SD age, 36.6±10.6 years), who smoked a mean±SD of 23.9±6.0 cigarettes per day, were mostly male (32 men, 13 women), and had been smoking for an average±SD of 17.9±10.2 years. Subjects had a mean±SD exhaled carbon monoxide of 19.0±10.9 parts per million after 1 to 2 hours of abstinence, were moderately nicotine dependent (Fagerström Test for Nicotine Dependence mean±SD score, 5.5±2.0), and had minimal depression (mean±SD total Hamilton Depression 17-item score, 1.8±1.9) or anxiety (mean±SD total Hamilton Anxiety score, 2.3±2.5). There were no significant differences between the groups that did and did not smoke during scanning in any of the preceding variables (*t* tests; range of *P* values, .20 to .88).

### GENETIC VARIANTS AND SMOKING-INDUCED DA RELEASE

The overall analysis of variance revealed a significant effect of genotype on percentage change in VCD/NAc BP ( $F_{4,30}=6.5$ ,  $P=.001$ ). In examining the main effects of the 4 genotypes, subgroups based on the DAT ( $F_{1,30}=4.9$ ,  $P=.03$ ) and DRD4 ( $F_{1,30}=4.4$ ,  $P=.04$ ) VNTRs and the COMT ( $F_{1,30}=10.2$ ,  $P=.003$ ) polymorphism differed in VCD/NAc BP percentage change in subjects who smoked during scanning, indicating that these genotypes are linked with the magnitude of smoking-induced DA release. In examining percentage change in VCD/NAc BP (Table and Figure 2), subgroups with the 9/9 or 9/10 genotype of the DAT VNTR, those with fewer than 7 repeats of the DRD4 VNTR (DRD4.7-), and those with the Val/Val COMT genotype (rapid metabolizers) had greater changes in VCD/NAc BP than those with the alternate genotypes, indicating that these genotypes are linked with greater smoking-induced DA release. No differences between subgroups based on the Taq1 polymorphism were found (Table), and no significant differences between subgroups based on any of the 4 genotypes were found in baseline (prebreak) BP values (all *P* values >.25).

In examining the effect of the polymorphisms on clinical variables, subjects who were DRD4.7- smoked significantly fewer cigarettes per day than those who were DRD4.7+ (22.0±4.1 vs 26.6±4.9 cigarettes per day, respectively;  $t=2.8$ ,  $P=.01$ ) (although the 2 subgroups did not have significantly different exhaled carbon monoxide levels at the time of scanning: 21.5±10.7 vs 19.5±11.9, *t* test,  $P=.60$ ). Subjects who were DRD4.7- also had a greater reduction in mean UTS (craving) score from before to after smoking than DRD4.7+ subjects (-3.7±1.6 vs -2.5±2.2, respectively;  $t=2.0$ ,  $P=.05$ ). No significant differences were found between subgroups based on the DRD4 genotype for number of years smoking or in the subgroups based on the other polymorphisms for the 3 clinical variables. Controlling for smoking habits (number of cigarettes per day) did not change the central results of the study with an overall significant analysis of covariance ( $F_{5,29}=4.0$ ,  $P=.007$ ) and 3 genotype main effects (DRD4,  $F_{1,29}=7.9$ ,  $P=.009$ ; DAT,  $F_{1,29}=5.8$ ,  $P=.02$ ; and COMT,  $F_{1,29}=5.0$ ,  $P=.03$ ) still being found.

### STUDY OF SMOKING-INDUCED DA RELEASE

Subjects in this study who smoked during the break in scanning ( $n=35$ ) had a greater reduction in mean VCD/NAc BP than those who did not smoke ( $n=10$ ) (-8.4±13.8% vs +1.8±12.5%,  $t=2.1$ ,  $P=.04$ ), indicating that smoking during scanning results in greater VCD/NAc DA release than taking a break in scanning without smoking. Results were similar for the left (-8.0±14.8% vs +0.7±13.9%, *t* test,  $P=.11$ ) and right (-8.9±14.5% vs +2.9±11.5%, *t* test,  $P=.02$ ) VCD/NAc. The exploratory VOIs had slightly weaker group differences that did not reach significance (-8.7±12.6% vs -2.2±15.7%,  $t=1.4$ ,  $P=.18$ , for the dorsal caudate and -7.9±11.0% vs -1.5±15.9%,  $t=1.5$ ,  $P=.15$  for the dorsal putamen). Presmoking to postsmoking findings were larger than the frame-to-frame variability for the 2 prebreak (2.2%) and postbreak (1.8%) PET

scans. Changes in cerebellar radioactivity from before to after the break were similar for the smoking and nonsmoking groups ( $-3.3\pm 14.0\%$  vs  $-2.3\pm 8.8\%$ , respectively, *t* test,  $P=.83$ ).

There was a positive correlation between percentage change in VCD/NAc BP and change in UTS (craving) scores from before to after the break ( $r=0.33$ ,  $P=.05$ ), indicating that greater smoking-induced DA release was associated with greater alleviation of craving. Correlations between change in VOI BP and change in UTS scores for the exploratory VOIs were not significant ( $r=0.10$ ,  $P=.55$ , for the dorsal caudate and  $r=0.15$ ,  $P=.39$ , for the dorsal putamen).

## COMMENT

In this study, known genetic variants of the DA system were associated with varying levels of smoking-induced DA release in the VCD/NAc of tobacco-dependent smokers. Specifically, smokers with the 9-repeat allele of the DAT 3' VNTR, DRD4.7-, or the Val/Val genotype of the COMT polymorphism had more smoking-induced DA release (measured indirectly) than those with the 10/10 DAT, DRD4.7+, or the Met COMT genotypes. Also, subjects with the DRD4.7- genotype smoked fewer cigarettes per day and had a greater reduction in craving from before to after smoking than DRD4.7+ subjects. As noted earlier in the article, the 10/10 genotype of DAT is potentially linked with decreased DAT density<sup>35,36</sup> while the DRD4.7+ VNTR has been associated with reduced ability of DA to inhibit cAMP formation,<sup>51</sup> reduced effectiveness of DA releasing medications,<sup>52</sup> and smoking behavior<sup>53</sup> and the Met allele of COMT is associated with slow DA catabolism,<sup>56</sup> thereby indicating potential mechanisms for these genotypes to alter smoking-induced DA release.

Results of this study might be best understood from the perspective of the tonic-phasic model of DA function, with a recent study demonstrating that nicotine amplifies DA release during phasic activity in the striatum.<sup>92</sup> The strongest associations observed with our paradigm are consistent with genetic effects on phasic DA regulation. Subjects with greater DAT densities (9 allele) and those with higher COMT activity (Val/Val) may have decreased tonic intrasynaptic DA levels, leading to increased smoking-induced phasic DA release. Autoinhibitory control (through DRD4) may also be important during phasic DA release. A recent literature review supports this theory by suggesting that subjects with the Val/Val genotype of COMT (rapid catabolizers) have lower tonic extraneuronal DA and higher phasic DA subcortically compared with subjects with the Met allele.<sup>59</sup>

In the present study, evidence was again found for human smoking-induced DA release; however, the magnitude of change in VCD/NAc BP was smaller in this larger subject sample ( $n=35$ ) scanned on a newer scanner than in our previous study<sup>15</sup> ( $-8.4\%$  for the new group compared with  $-25.9\%$  to  $-36.6\%$  for the ventral striatal regions of interest in the previous study<sup>15</sup>). While the new data call into question the magnitude of DA release with human smoking, they reaffirm the presence of smoking-induced DA release because 2 separate samples (using methodology blinded to subject condition) have now shown significant group differences between those who did and did not smoke during a 10-minute break in scanning in the hypothesized direction. The association between decreased craving and increased DA release was also found again, and all of these findings were more robust for the VCD/NAc than for dorsal basal ganglia VOIs.

This study should be interpreted in the context of several limitations. A first potential confound is that subjects were removed from the scanner and repositioned after smoking, which could potentially lead to differences in region placement on prebreak and postbreak scans because of imperfect repositioning. Although efforts were made to reposition the subjects precisely and regions were drawn on coregistered MRI images, it is possible that movement or imperfect alignment of scans led to slight differences in regions for the prebreak and postbreak scans. A



second potential confound is that smoking might alter [ $^{11}\text{C}$ ]raclopride BP by effects on blood flow<sup>93,94</sup> rather than DA release. In our previous<sup>15</sup> and present studies, cerebellar activity (a measure of nonspecific binding) showed little change from before to after the break in either the nonsmoking or the smoking groups, indicating that our results were not strongly influenced by blood flow changes from smoking. Third, although subjects were monitored continuously during the break while they were standing in the outdoor area, subtle movements of hands or feet were not systematically examined. Rapid repetitive movements, such as finger tapping<sup>95</sup> and foot extension/flexion<sup>96</sup> (but not vigorous exercise<sup>97</sup>), have been shown to decrease [ $^{11}\text{C}$ ]raclopride BP and may present a confound if those who smoked differed from those who did not smoke in their movements. Studies comparing regular cigarette smoking with smoking of denicotinized cigarettes and/or nicotine administration would help clarify the role of movement in the present findings. And fourth, there is inherent variability in [ $^{11}\text{C}$ ]raclopride PET scanning due to technical issues in scanning and scan analysis<sup>87,98</sup> and subject differences in radiotracer clearance,<sup>80</sup> which may have increased variability for the BP measures found here.

In summary, this study demonstrates that genotypic differences may account for interindividual differences in smoking-induced DA release. In addition, the present study also demonstrates (indirectly) cigarette smoking-induced striatal DA release in humans. An association between decreased craving and increased DA concentration with smoking was also found. These results may have future implications for subtyping smokers based on clinical characteristics and possibly for identifying smokers who would be more likely to respond to dopaminergic pharmacotherapies or those therapies that affect the brain DA system.

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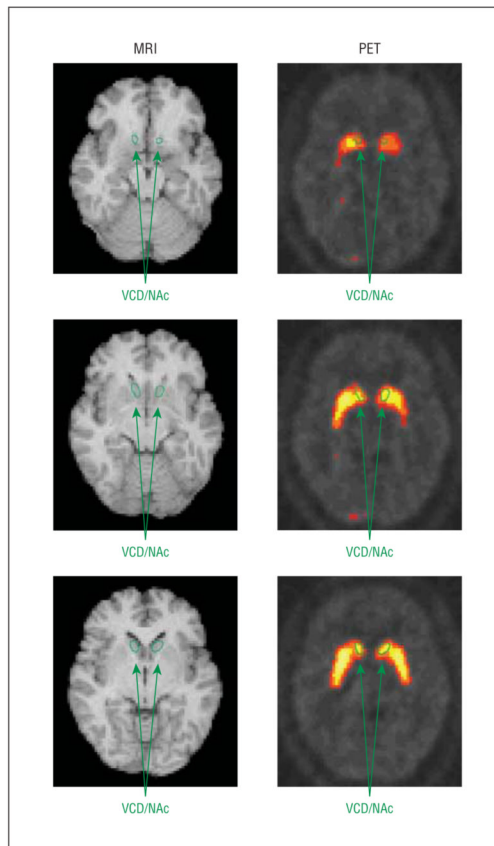
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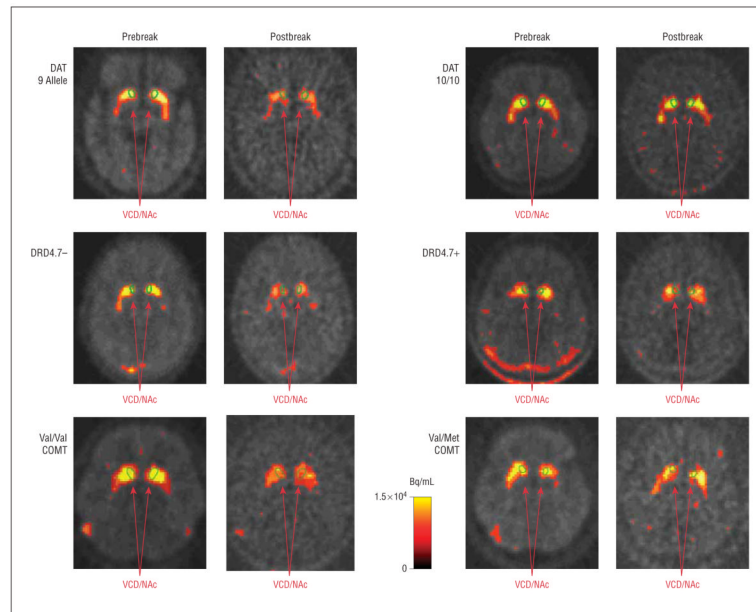
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**Figure 1.** Magnetic resonance image (MRI)/positron emission tomography (PET) coregistration, showing regions of interest drawn on MRI and transferred to a coregistered PET image with raclopride labeled with radioactive carbon ( $^{11}\text{C}$ ). VCD/Nac indicates ventral caudate/nucleus accumbens.



**Figure 2.** Sample images from positron emission tomography with raclopride labeled with radioactive carbon ( $^{11}\text{C}$ ) for genotypes found to differentiate subjects with high vs low dopamine (DA) release. The 2 left columns demonstrate decreases in [ $^{11}\text{C}$ ]raclopride binding (a marker for DA release) with smoking (postbreak scans are decay corrected) for subjects with the 9-allele repeat of the dopamine transporter (DAT) gene, less than 7 repeats (DRD4.7-) for the DRD4 genotype, or the Val/Val genotype of the catechol-O-methyltransferase (COMT) polymorphism. The 2 right columns demonstrate little change in [ $^{11}\text{C}$ ]raclopride binding in subjects with the alternate genotypes. VCD/NAc indicates ventral caudate/nucleus accumbens.

**Table**  
**Smoking-Induced Changes in Ventral Caudate/Nucleus Accumbens Raclopride Labeled With Radioactive Carbon (<sup>11</sup>C) Binding Potential for Subgroups of Smokers Based on Genotype**

Genetic Variant	Change in VCD/NAc BP With Smoking, Mean $\pm$ SD, %	Absolute VCD/NAc BP Change With Smoking, Mean $\pm$ SD	F (ANOVA, <i>df</i> = 1,30)	<i>P</i> Value
DAT				
9 allele (n = 15)	-14.8 $\pm$ 14.7	-0.37 $\pm$ 0.36	4.9	.03
10/10 (n = 20)	-3.6 $\pm$ 11.0	-0.09 $\pm$ 0.26		
DRD2				
A <sub>1</sub> allele (n = 14)	-6.6 $\pm$ 12.1	-0.17 $\pm$ 0.31	0.3	.86
A <sub>2</sub> /A <sub>2</sub> (n = 21)	-9.6 $\pm$ 14.9	-0.23 $\pm$ 0.35		
DRD4				
>7 (n = 24)	-11.3 $\pm$ 13.2	-0.29 $\pm$ 0.33	4.4	.04
$\geq$ 7 (n = 11)	-2.0 $\pm$ 13.3	-0.04 $\pm$ 0.27		
COMT				
Val/Val (n = 8)	-21.7 $\pm$ 15.5	-0.48 $\pm$ 0.35	10.2	.003
Met allele (n = 27)	-4.5 $\pm$ 10.6	-0.13 $\pm$ 0.28		

Abbreviations: ANOVA, analysis of variance; COMT, catechol-O-methyltransferase; DAT, dopamine transporter; DRD2, dopamine 2 receptor; DRD4, dopamine 4 receptor; VCD/NAc BP, ventral caudate/nucleus accumbens binding potential.