

# **DRD2 genetic variation in relation to smoking and obesity in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial**

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**Objectives** Cigarette smoking is the leading cause of morbidity and mortality worldwide. We investigated the association between smoking behavior and genetic variations in the D2 dopamine receptor (*DRD2*), which mediates nicotine dependence. To assess the specificity of genetic effects, we also investigated other reward-motivated characteristics (obesity, alcohol consumption).

**Methods** Four single nucleotide polymorphisms in *DRD2* were genotyped in 2374 participants selected randomly from the screening arm of the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial after stratifying by sex, age, and smoking status. Smoking, obesity, and alcohol consumption were assessed by questionnaire. Single nucleotide polymorphism and haplotype associations were estimated using odds ratios (ORs) and 95% confidence intervals derived from conditional logistic regression models, adjusted for race/ethnicity.

**Results** *DRD2* polymorphisms were associated with the risk of remaining a current smoker and obesity. Current smokers were more likely than former smokers to possess the variant TaqIA allele (rs#1800497) in a dose-dependent model (OR<sub>CT</sub>=1.2, OR<sub>TT</sub>=1.5, *P* for linear trend=0.007). The *DRD2* haplotype T-C-T-A [TaqIA(C/T) – 957(T/C) – IVS6-83(G/T) – 50977(A/G)] was more common among current than former smokers (OR=1.3, *P*=0.006), particularly among heavy smokers (21+ cigarettes per day; OR=1.6, *P*=0.006), and was more common among obese than normal weight individuals (OR=1.4, *P*=0.02).

## **Introduction**

Cigarette smoking is a major risk factor for coronary heart disease, stroke, and cancer – three leading causes of death worldwide – and thus remains the largest preventable risk factor for morbidity and mortality in the developed world. Former smokers possess substantially decreased risk for developing these diseases compared with current smokers, and risk diminishes the longer one has quit [1,2]. Fewer than 15% of smokers who attempt to quit each year are, however, able to maintain abstinence for greater than 3 months [1]. Understanding

**Conclusions** Genetic variation in *DRD2* is a modifier of the reward-motivated characteristics, smoking and obesity. As fewer than 15% of smokers who attempt to quit are able to maintain abstinence for greater than 3 months, our results support that *DRD2* is an appropriate molecular target for smoking cessation treatments. Our results further support evaluation of *DRD2* antagonists for obesity therapies.

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the environmental and genetic determinants of cigarette smoking behavior is therefore critical for developing effective tobacco cessation therapies and reducing the incidence and mortality from tobacco-related cancers.

The psychopharmacological mechanism of nicotine dependence is mediated, in part, through regulation of the dopaminergic neurotransmitter system. The dopamine reward pathway is a key element of the complex neurocircuitry in the brain that governs addiction [3,4]. Drugs such as nicotine and alcohol are addicting because

the drug-induced increase in dopamine within the brain is much greater than with natural rewards, such as food, and because drug-associated environmental and behavioral cues become effective stimulants themselves [5–7]. Both animal studies and human efficacy trials have demonstrated that bupropion, a relatively selective reuptake inhibitor of dopamine, can alter the reinforcing properties of nicotine and food [8–10] and contribute to smoking cessation [11], thereby supporting the role of dopamine in reward-motivated behaviors.

Although addictive behaviors are complex traits and strong secular trends with environmental, social, and economic components are undoubtedly related to patterns of cigarette smoking, alcohol dependence, and obesity, twin and other genetic studies have provided strong evidence for heritability of reward-motivated behaviors [6,12–17]. Specifically, in models that incorporate both genetic and environmental factors, twin studies consistently estimate that genetic influences account for more than half of the variability in smoking behavior [14], alcohol dependence [12], and body mass index (BMI) [13].

To further understand the genetic contribution to cigarette smoking, common genetic polymorphisms in the dopamine pathway have been investigated in relation to smoking behavior. A meta-analysis of 13 candidate gene studies suggested that the *DRD2*-related TaqIA polymorphism (rs#1800497) is related to smoking behavior. Significant interstudy heterogeneity was, however, observed in the meta-analysis, only two studies reported data on intensity of cigarette smoking and were thus able to control for smoking intensity in analyses of cessation, few studies separated nonsmokers from former smokers, and haplotypes within *DRD2* were not investigated [18].

We therefore evaluated possible associations between smoking behavior and four single nucleotide polymorphisms (SNPs) in *DRD2* among 2374 participants in the Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial, the largest such evaluation to date. We further investigated potential associations between *DRD2* genotypes and haplotypes and other reward-motivated characteristics (obesity, alcohol intake) as secondary hypotheses to assess the specificity of any genetic effects. We hypothesized that individuals with alleles corresponding to decreased *DRD2* functionality would exhibit increased dependence on external stimulation of the dopaminergic system, such as that provided by smoking, alcohol consumption, and food intake.

## Methods

### Study population

The PLCO Cancer Screening Trial study population has been described previously [19]. Briefly, over 150 000

individuals aged 55–74 years from 10 US study centers were randomized during 1992–2001 to undergo a specific cancer screening regimen or receive routine medical care to evaluate the effects of screening on disease-specific mortality. At baseline, participants provided written, informed consent and completed a demographic and risk factor questionnaire. Participants in the screening arm were also asked to provide separate written, informed consent and a blood sample for use in etiologic studies [20].

Data on smoking behavior were self-reported during the baseline questionnaire. Nonsmokers were defined as individuals who had never smoked cigarettes regularly (at least one cigarette per day, on average) for at least 6 months. Individuals who had smoked regularly for at least 6 months answered additional questions regarding the age they began smoking regularly, the usual number of cigarettes they smoked per day, whether they smoked cigarettes regularly at the time of the baseline questionnaire, and the age when they last smoked regularly. Additional information on race/ethnicity, highest level of schooling completed, marital status, adult height, and weight (current and at ages 20 and 50 years) was also obtained from the baseline questionnaire. Data on usual consumption of beer, wine, and liquor were obtained from a food frequency questionnaire.

The study population for this analysis was drawn from the 77 469 participants in the screening arm. A sample of 2406 participants (216 nonsmokers, 1086 current smokers, and 1104 former smokers) was selected randomly after stratifying by sex, age (55–59, 60–64, 65–69, 70–74 years), smoking status (nonsmoker, current smoker, former smoker), and quantity of cigarettes smoked for current and former smokers (1–10, 11–20, 21+ cigarettes per day). Blood samples with sufficient DNA for genotyping were available for 2379 (99%) of these individuals. This study was conducted according to a protocol approved by the Institutional Review Boards of the National Cancer Institute and the ten screening centers.

### Genotyping

We selected for genotyping four SNPs in *DRD2* based on biologic plausibility, previous research [21–24], availability of functional data, and potential linkage disequilibrium with other functional SNPs (Table 1). SNPs are designated by the base pair position relative to the A of the ATG initiation codon if in the 5' untranslated region, by relative position if in an intron, or by relative position and amino acid if within an exon [25], with the exception of the TaqIA polymorphism in *DRD2*, which has been described extensively in the literature [24].

**Table 1 Polymorphisms at the dopamine receptor D2 locus evaluated in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial**

Gene	Name	Chromosomal location	SNP rs#	Polymorphism description (literature annotation)	References
DRD2	Dopamine receptor D2	11q23	rs1800497	Ex8-313G>A, E713K (TaqIA) <sup>a</sup>	[24]
			rs6277	Ex7+147C>T, P319P (957C>T)	[23]
			rs1076560	IVS6-83G>T	[22]
			rs1799978	-50977A>G (-241A>G)	[21]

<sup>a</sup>Note that this single nucleotide polymorphism is in a gene flanking *DRD2* called *ANKK1*.

DNA was extracted from whole blood using the Genra Autopure (Genra Systems, Minneapolis, Minnesota, USA) at the BBI Biotech Repository (Gaithersburg, Maryland, USA). Genotyping was conducted at the National Cancer Institute's Core Genotyping Facility using TaqMan and Sequenom MassARRAY platforms (Applied Biosystems, Foster City, California, USA, and Sequenom, Inc., San Diego, California, USA, respectively). Sequence data and assay conditions are available at: <http://snp500cancer.nci.nih.gov> [25]. Genotyping was completed successfully in  $\geq 98\%$  of DNA samples; no significant deviation from Hardy-Weinberg equilibrium was observed for any SNP. A total of 120 identical quality control samples were interspersed in the study population samples ( $\geq 97\%$  concordance). In addition, genotype-specific quality control samples (composed of four homozygote wild-type, four heterozygote, four homozygote variant, and four DNA negative controls) were included in each plate of 368 samples genotyped. Finally, each individual was genetically profiled using the AmpFLSTR Identifiler PCR Amplification Kit (Applied Biosystems, Foster City, California, USA). Five (0.2%) individuals whose chromosomal sex based on the Identifiler was discordant from that reported on the baseline questionnaire were excluded from this analysis, yielding a final analytic population of 2374 participants (213 nonsmokers, 1068 current smokers, and 1093 former smokers).

Among non-Hispanic whites, haplotypes were visualized using Haploview, version 3.11 [26] based on measures of pairwise linkage disequilibrium between SNPs. SAS/Genetics, version 8.2 (SAS Institute, Inc., Cary, North Carolina, USA), was used to generate maximum likelihood estimates of haplotype frequencies and to assign haplotype pairs (diplotypes) for each individual. Individuals with missing values for one or more genotypes were excluded from haplotype analyses ( $N=43$ ). The probability of the assigned haplotype pair was greater than 95% for 1908/2074 (92%) individuals. The sample size for black individuals ( $< 5\%$ ) was insufficient for haplotype analysis.

### Statistical analysis

Comparison groups for analyses of behavioral characteristics were derived from baseline questionnaire data on

cigarette smoking, alcohol consumption, and height and weight, and assessed using the Pearson  $\chi^2$  statistic. Analyses included smoking history (current versus former smokers; total, and stratified by intensity of cigarette smoking), alcohol consumption (5–14, 15–29, 30–44, 45+ versus  $< 5$  g per day), and obesity at baseline and ages 20 and 50 years (obese, overweight, or underweight versus normal weight individuals). Analyses of alcohol consumption and obesity included all individuals (current, former, and nonsmokers). Intensity of cigarette smoking was categorized as light, medium, or heavy (1–10, 11–20, or 21+ cigarettes per day, respectively). BMI ( $\text{kg}/\text{m}^2$ ) was computed to characterize individuals at baseline and ages 20 and 50 years as underweight, normal weight, overweight, or obese ( $< 18.5$ , 18.5–24.9, 25.0–29.9, or 30+  $\text{kg}/\text{m}^2$ , respectively). Usual consumption of alcohol (g per day) was computed by summing participants' self-reported usual consumption of beer, wine, and liquor from the food frequency questionnaire.

Differences between comparison groups in the distribution of *DRD2* gene variants were quantified using odds ratios (ORs) and 95% confidence intervals (CIs) derived from multivariable conditional logistic regression models, conditioned on the variables used to construct the stratified sample (sex, age, smoking status, and quantity of cigarettes smoked for current and former smokers). The reference group was defined as the homozygote of the most common allele for SNP analyses and as the most common haplotype or haplotype pair, which was also the haplotype or haplotype pair containing the most common allele for each loci, for haplotype and diplotype analyses. For SNP analyses, we also computed a *P*-value for the linear trend based on a three-level variable for each genotype (0 = homozygote wild-type, 1 = heterozygote, 2 = homozygote variant). For haplotype analyses using conditional logistic regression, we considered the most probable haplotype assigned within SAS Genetics. We repeated our haplotype analyses using Haplo Stats, version 2.0.1 (<http://mayoresearch.mayo.edu/mayo/research/biostat/schaid.cfm>), which accounts for phase ambiguity but does not allow for conditional logistic regression modeling. *P*-values for each haplotype, derived from unconditional logistic regression models adjusted for the variables used to construct the stratified sample, are therefore presented in the text.

All models were adjusted for race/ethnicity (white, black, other), and models of smoking behavior and alcohol consumption were also adjusted for BMI (<18.5, 18.5–24.9, 25.0–29.9, 30+ kg/m<sup>2</sup>). Inclusion of additional demographic factors, including education ( $\leq 12$  years, some college, college graduate) and marital status (married, other), in the models did not materially alter the genotype, haplotype, and diplotype parameter estimates ( $\pm 10\%$ ); thus, we excluded these factors from our final analyses and present results from the most parsimonious model. Individuals with missing genotype data were excluded from analyses for that SNP. The SAS System, version 9.1 (SAS Institute, Inc.), was used to conduct statistical analyses. *P*-values are presented with one significant digit. Statistical tests were two-sided with an  $\alpha$ -level of 0.05.

## Results

### Study population

Table 2 presents selected characteristics for our study population of 2374 participants (213 nonsmokers, 1068 current smokers, and 1093 former smokers). Nonsmokers were more likely than current and former smokers to be white ( $P = 0.0003$ ), college graduates ( $P < 0.0001$ ), and married ( $P < 0.0001$ ). BMI at baseline tended to be highest among former smokers, intermediate among nonsmokers, and lowest among current smokers ( $P < 0.0001$ ), whereas BMI at age 20 years did not differ significantly by smoking history. Compared with former smokers, current smokers began smoking at a significantly younger age ( $P < 0.0001$ ) and smoked for a significantly longer period of time ( $P < 0.0001$ ), resulting in higher cumulative lifetime exposure to cigarette smoking (pack-years;  $P < 0.0001$ ).

### Smoking history

Smoking history was significantly associated with polymorphisms in *DRD2* (Table 3). Current smokers were more likely than former smokers to possess the TaqIA and IVS6-83 variant alleles in dose-dependent models (TaqIA: CC is referent;  $OR_{CT} = 1.2$ ,  $OR_{TT} = 1.5$ ,  $P_{trend} = 0.007$ ; IVS6-83: GG is referent;  $OR_{GT} = 1.2$ ,  $OR_{TT} = 1.7$ ,  $P_{trend} = 0.008$ ). The effects of these *DRD2* polymorphisms on risk of remaining a current smoker were most pronounced for heavy smokers (21+ cigarettes per day). Compared with former heavy smokers, current heavy smokers were approximately 1.5 and 2 times as likely to possess the heterozygote and homozygote variant genotypes, respectively, in dose-dependent models for both SNPs (TaqIA: CC is referent;  $OR_{CT} = 1.6$ ,  $OR_{TT} = 2.4$ ,  $P_{trend} = 0.002$ ; IVS6-83: GG is referent;  $OR_{GT} = 1.4$ ,  $OR_{TT} = 1.9$ ,  $P_{trend} = 0.03$ ). Our results were similar when we restricted our analyses to non-Hispanic whites (all current smokers: TaqIA  $P_{trend} = 0.03$ , IVS6-83  $P_{trend} = 0.02$ ; current heavy smokers: TaqIA  $P_{trend} = 0.003$ , IVS6-83  $P_{trend} = 0.05$ ).

Consistent with our analyses of single polymorphisms, current heavy smokers were more likely than former heavy smokers to possess the *DRD2* haplotype [TaqIA (C/T)–957(T/C)–IVS6-83(G/T)–50977(A/G)] that included the variant allele for TaqIA only (T-T-G-A; variant alleles are underlined) and the haplotype that included the variant alleles for both TaqIA and IVS6-83 (T-C-T-A) (C-T-G-A is referent;  $OR_{T-T-G-A} = 2.5$ , 95% CI 1.3–4.9,  $P = 0.006$ ;  $OR_{T-C-T-A} = 1.6$ , 95% CI 1.2–2.3,  $P = 0.006$ ; respectively). These haplotypes remained statistically significant after accounting for phase ambiguity ( $P = 0.008$  and  $P = 0.01$ , respectively). Diplotype analyses for *DRD2* were consistent with the haplotype analyses (data not shown).

### Body mass index

Polymorphisms in *DRD2* also were significantly associated with high BMI (Table 4). Specifically, individuals who were obese (BMI 30+ kg/m<sup>2</sup>) at the time of the baseline questionnaire were more likely than individuals with normal BMI (18.5–24.9 kg/m<sup>2</sup>) to possess the TaqIA and IVS6-83 variant alleles (TaqIA: CC is referent;  $OR_{CT} = 1.4$ ,  $OR_{TT} = 1.3$ ,  $P_{trend} = 0.02$ ; IVS6-83: GG is referent;  $OR_{GT} = 1.3$ ,  $OR_{TT} = 1.7$ ,  $P_{trend} = 0.02$ ). These findings were strikingly similar for individuals who were obese at age 50 years, and for individuals who were overweight/obese (BMI 25+ kg/m<sup>2</sup>) at age 20 years (Table 4). Our results were similar when we restricted our analyses to non-Hispanic whites (BMI at baseline: TaqIA  $P_{trend} = 0.05$ , IVS6-83  $P_{trend} = 0.04$ ).

Consistent with our analyses of single polymorphisms, obese individuals were significantly more likely than individuals with normal BMI to possess the *DRD2* haplotype [TaqIA(C/T)–957(T/C)–IVS6-83(G/T)–50977(A/G)] that included the variant alleles for both TaqIA and IVS6-83 (T-C-T-A) (C-T-G-A is referent; obese at baseline:  $OR_{T-C-T-A} = 1.4$ , 95% CI 1.0–1.8,  $P = 0.02$ ; obese at age 50 years:  $OR_{T-C-T-A} = 1.4$ , 95% CI 1.1–2.0,  $P = 0.02$ ; overweight/obese at age 20 years:  $OR_{T-C-T-A} = 1.3$ , 95% CI 0.98–1.7,  $P = 0.08$ ). Our findings were consistent after accounting for phase ambiguity (baseline:  $P = 0.08$ ; age 50 years:  $P = 0.04$ ; age 20 years:  $P = 0.02$ ). Diplotype analyses were consistent with the haplotype analyses: at all ages, obese individuals were 1.7–2.5 times as likely as individuals with normal BMI to possess two copies of this haplotype (C-T-G-A/C-T-G-A is referent; obese at baseline:  $OR_{T-C-T-A/T-C-T-A} = 1.7$ , 95% CI 0.7–3.7,  $P = 0.2$ ; obese at age 50 years:  $OR_{T-C-T-A/T-C-T-A} = 2.5$ , 95% CI 1.1–5.9,  $P = 0.04$ ; overweight/obese at age 20 years:  $OR_{T-C-T-A/T-C-T-A} = 2.4$ , 95% CI 1.2–4.9,  $P = 0.02$ ) (data not shown).

### Alcohol consumption

We did not observe any notable associations with *DRD2* polymorphisms and consumption of alcohol (Supplemental Table).

**Table 2 Selected characteristics of 2374 participants in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial**

	Nonsmoker <sup>a</sup>			Current smoker <sup>a</sup>			Ex-smoker <sup>a</sup>			<i>P</i> <sup>b</sup>
	N=213			N=1068			N=1093			
	N (%)			N (%)			N (%)			
Sex <sup>a</sup>										
Female	108	(50.7)		529	(49.5)		547	(50.0)		0.9
Male	105	(49.3)		539	(50.5)		546	(50.0)		
Race/ethnicity										
White	197	(92.5)		926	(86.7)		994	(90.9)		0.0003
Black	2	(0.9)		73	(6.8)		40	(3.7)		
Other/unknown	14	(6.6)		69	(6.5)		59	(5.4)		
Age (years) <sup>a</sup>										
55–59	54	(25.4)		273	(25.6)		273	(25.0)		0.09
60–64	54	(25.4)		272	(25.5)		274	(25.1)		
65–69	52	(24.4)		310	(29.0)		271	(24.8)		
70–74	53	(24.9)		213	(19.9)		275	(25.2)		
Education										
≤ 12 years	52	(24.4)		380	(35.6)		325	(29.7)		<0.0001
Some college	68	(31.9)		414	(38.8)		389	(35.6)		
College graduate	92	(43.2)		272	(25.5)		377	(34.5)		
Missing	1	(0.5)		2	(0.2)		2	(0.2)		
Marital status										
Married	175	(82.2)		667	(62.5)		822	(75.2)		<0.0001
Other	37	(17.4)		401	(37.6)		269	(24.6)		
Missing	1	(0.5)		0	(0.0)		2	(0.2)		
Body mass index at age 20 years (kg/m <sup>2</sup> )										
Underweight	16	(7.5)		91	(8.5)		89	(8.1)		0.9
Normal weight	165	(77.5)		811	(75.9)		849	(77.7)		
Overweight/obese	30	(14.1)		148	(13.9)		148	(13.5)		
Missing	2	(0.9)		18	(1.7)		7	(0.6)		
Body mass index at baseline (kg/m <sup>2</sup> )										
Underweight	3	(1.4)		19	(1.8)		4	(0.4)		<0.0001
Normal weight	81	(38.0)		461	(43.2)		356	(32.6)		
Overweight	84	(39.4)		434	(40.6)		471	(43.1)		
Obese	43	(20.2)		146	(13.7)		254	(23.2)		
Missing	2	(0.9)		8	(0.8)		8	(0.7)		
Number of cigarettes smoked per day <sup>a</sup>										
1–10				360	(33.7)		364	(33.3)		0.9
11–20				363	(34.0)		364	(33.3)		
21+				345	(32.3)		365	(33.4)		
Age at initiation of smoking (years)										
< 15				106	(9.9)		81	(7.4)		<0.0001
15–18				478	(44.8)		543	(49.7)		
19–29				400	(37.5)		430	(39.3)		
30+				79	(7.4)		35	(3.2)		
Missing				5	(0.5)		4	(0.4)		
Duration of smoking (years)										
1–24				33	(3.1)		550	(50.3)		<0.0001
25–34				60	(5.6)		266	(24.3)		
35–44				423	(39.6)		206	(18.8)		
45+				545	(51.0)		56	(5.1)		
Missing				7	(0.7)		15	(1.4)		
Pack-years of smoking										
1–10				112	(10.5)		429	(39.2)		<0.0001
11–25				282	(26.4)		268	(24.5)		
26–40				286	(26.8)		188	(17.2)		
41+				388	(36.3)		208	(19.0)		

<sup>a</sup>Sex, age, smoking status, and quantity of cigarettes smoked for current/former smokers were used as design variables to select the study population.  
<sup>b</sup>*P*-value derived from the Pearson  $\chi^2$  statistic. Individuals with missing values were excluded from the Pearson  $\chi^2$  statistic for that variable.

### Discussion

Our results support the role of common genetic polymorphisms in *DRD2* as modifiers of smoking behavior. *DRD2* appears to play a role in the ability of smokers, particularly heavy smokers, to quit smoking. *DRD2* also appears to be associated with obesity, suggesting that *DRD2* may not be specific to smoking behavior, but may be more globally related to reward-motivated behaviors. Strengths of our study include

detailed information on smoking history that enabled us to account for smoking intensity in analyses of smoking history, a large sample size from a multicenter national study, and the simultaneous consideration of additional reward-motivated characteristics (alcohol consumption and obesity) with key relations to morbidity and mortality. Additionally, we evaluated multiple polymorphisms within *DRD2* and present the first analyses relating *DRD2* haplotypes with smoking.

Table 3 Distributions of *DRD2* polymorphisms by current smoking status and intensity of cigarette smoking

SNP	Total					By intensity of cigarette smoking														
						Light (1–10 cigs/day)					Medium (11–20 cigs/day)					Heavy (21+ cigs/day)				
	Former (N)	Current (N)	OR <sup>a</sup>	(95% CI)	P	Former (N)	Current (N)	OR <sup>b</sup>	(95% CI)	P	Former (N)	Current (N)	OR <sup>b</sup>	(95% CI)	P	Former (N)	Current (N)	OR <sup>b</sup>	(95% CI)	P
TaqIA (rs1800497)																				
CC	711	643	1.00	(reference)		225	205	1.00	(reference)		231	229	1.00	(reference)		255	209	1.00	(reference)	
CT	331	360	1.24	(1.03, 1.50)	0.02	113	127	1.21	(0.88, 1.67)	0.2	119	116	1.02	(0.74, 1.40)	0.9	99	117	1.59	(1.13, 2.23)	0.008
TT	42	56	1.49	(0.98, 2.28)	0.06	22	22	1.07	(0.57, 2.00)	0.8	10	16	1.65	(0.73, 3.75)	0.2	10	18	2.36	(1.03, 5.42)	0.04
P for trend	P=0.007					P=0.4					P=0.4					P=0.002				
CT/TT	373	416	1.25	(1.04, 1.50)	0.02	135	149	1.14	(0.83, 1.55)	0.4	129	132	1.06	(0.78, 1.45)	0.7	109	135	1.68	(1.21, 2.34)	0.002
957 (rs6277)																				
TT	310	275	1.00	(reference)		104	84	1.00	(reference)		107	99	1.00	(reference)		99	92	1.00	(reference)	
TC	501	471	1.07	(0.87, 1.32)	0.5	155	142	1.19	(0.82, 1.72)	0.4	168	171	1.09	(0.77, 1.55)	0.6	178	158	0.92	(0.63, 1.33)	0.6
CC	260	298	1.32	(1.04, 1.67)	0.02	96	120	1.58	(1.06, 2.36)	0.02	81	85	1.17	(0.78, 1.78)	0.4	83	93	1.17	(0.76, 1.79)	0.5
P for trend	P=0.02					P=0.026					P=0.4					P=0.5				
TC/CC	761	769	1.09	(0.90, 1.33)	0.4	251	262	1.22	(0.86, 1.74)	0.3	249	256	1.06	(0.76, 1.48)	0.7	261	251	0.99	(0.70, 1.41)	1.0
IVS6-83 (rs1076560)																				
GG	788	722	1.00	(reference)		250	240	1.00	(reference)		265	250	1.00	(reference)		273	232	1.00	(reference)	
GT	267	292	1.21	(1.00, 1.48)	0.06	96	98	1.06	(0.76, 1.48)	0.7	88	96	1.21	(0.86, 1.70)	0.3	83	98	1.41	(0.99, 2.01)	0.06
TT	27	41	1.72	(1.04, 2.84)	0.04	13	16	1.22	(0.57, 2.62)	0.6	6	13	2.54	(0.95, 6.84)	0.07	8	12	1.93	(0.74, 5.02)	0.2
P for trend	P=0.008					P=0.6					P=0.06					P=0.03				
GT/TT	294	333	1.30	(1.07, 1.58)	0.008	109	114	1.11	(0.80, 1.55)	0.5	94	109	1.34	(0.96, 1.86)	0.09	91	110	1.49	(1.05, 2.11)	0.03
-50977 (rs1799978)																				
AA	960	933	1.00	(reference)		318	309	1.00	(reference)		323	322	1.00	(reference)		319	302	1.00	(reference)	
AG	118	119	1.01	(0.77, 1.33)	0.9	40	42	1.05	(0.66, 1.67)	0.8	34	37	1.11	(0.67, 1.81)	0.7	44	40	0.90	(0.56, 1.46)	0.7
GG	5	4	0.80	(0.21, 3.08)	0.7	2	2	1.05	(0.14, 7.75)	1.0	3	1	0.33	(0.03, 3.26)	0.3	0	1	<sup>d</sup>		
P for trend	P=1.0					P=0.8					P=0.9									
AG/GG	123	123	0.94	(0.72, 1.24)	0.7	42	44	0.96	(0.60, 1.53)	0.9	37	38	0.95	(0.58, 1.57)	0.9	44	41	0.92	(0.57, 1.49)	0.7
<i>DRD2</i> haplotype <sup>c</sup>																				
C-T-G-A	984	885	1.00	(reference)		313	262	1.00	(reference)		323	322	1.00	(reference)		348	301	1.00	(reference)	
C-T-G-G	16	17	1.27	(0.63, 2.57)	0.5	7	5	0.83	(0.25, 2.82)	0.8	4	8	2.26	(0.67, 7.67)	0.2	5	4	1.08	(0.28, 4.21)	0.9
C-C-G-A	520	462	1.00	(0.85, 1.17)	1.0	152	140	1.13	(0.85, 1.50)	0.4	173	155	0.90	(0.69, 1.18)	0.5	195	167	0.99	(0.75, 1.29)	0.9
C-C-G-G	66	61	1.04	(0.72, 1.50)	0.9	21	18	0.99	(0.52, 1.91)	1.0	19	16	0.92	(0.46, 1.83)	0.8	26	27	1.18	(0.65, 2.12)	0.6
T-T-G-A	74	70	1.02	(0.72, 1.45)	0.9	21	23	1.17	(0.63, 2.18)	0.7	37	18	0.46	(0.26, 0.84)	0.01	16	29	2.54	(1.31, 4.93)	0.006
T-C-T-A	254	287	1.32	(1.08, 1.60)	0.006	93	86	1.10	(0.78, 1.55)	0.6	83	100	1.28	(0.92, 1.79)	0.1	78	101	1.64	(1.15, 2.32)	0.006

OR, odds ratio; CI, confidence interval; BMI, body mass index.

<sup>a</sup>OR estimated using conditional logistic regression, conditioning on age, sex, and number of cigarettes smoked; model also adjusted for race/ethnicity and BMI.<sup>b</sup>OR estimated using conditional logistic regression, conditioning on age and sex; model also adjusted for race/ethnicity and BMI.<sup>c</sup>Haplotype analyses were conducted among non-Hispanic whites only.<sup>d</sup>OR could not be computed because of at least one zero count cell.

**Table 4 Distributions of DRD2 polymorphisms by body mass index**

SNP	Current BMI					BMI at age 50 years					BMI at age 20 years				
	Normal		Obese			Normal		Obese			Normal		Overweight/Obese		
	N	N	OR <sup>a</sup>	(95% CI)	P	N	N	OR <sup>a</sup>	(95% CI)	P	N	N	OR <sup>a</sup>	(95% CI)	P
<b>TaqIA (rs1800497)</b>															
CC	591	259	1.00	(reference)		783	145	1.00	(reference)		1166	196	1.00	(reference)	
CT	266	162	1.40	(1.08, 1.82)	0.01	372	90	1.34	(0.98, 1.83)	0.07	583	109	1.08	(0.83, 1.41)	0.6
TT	40	21	1.28	(0.71, 2.31)	0.4	52	15	1.60	(0.84, 3.06)	0.2	74	21	1.55	(0.91, 2.64)	0.1
P for trend			P=0.02					P=0.04					P=0.2		
CT/TT	306	183	1.39	(1.08, 1.78)	0.01	424	105	1.37	(1.01, 1.85)	0.04	657	130	1.14	(0.88, 1.46)	0.3
<b>957 (rs6277)</b>															
TT	246	110	1.00	(reference)		343	56	1.00	(reference)		501	78	1.00	(reference)	
TC	420	202	0.95	(0.71, 1.28)	0.7	556	118	1.14	(0.79, 1.64)	0.5	836	155	1.12	(0.82, 1.52)	0.5
CC	222	126	1.24	(0.87, 1.76)	0.2	297	75	1.25	(0.81, 1.91)	0.3	463	89	1.04	(0.73, 1.50)	0.8
P for trend			P=0.3					P=0.3					P=0.8		
TC/CC	642	328	1.03	(0.78, 1.37)	0.8	853	193	1.17	(0.83, 1.66)	0.4	1299	244	1.09	(0.82, 1.46)	0.5
<b>IVS6-83 (rs1076560)</b>															
GG	647	295	1.00	(reference)		861	166	1.00	(reference)		1285	225	1.00	(reference)	
GT	223	132	1.31	(1.00, 1.73)	0.05	307	72	1.22	(0.87, 1.70)	0.2	480	85	0.97	(0.73, 1.29)	0.8
TT	24	15	1.68	(0.83, 3.42)	0.2	36	11	1.85	(0.87, 3.90)	0.1	53	15	1.52	(0.82, 2.83)	0.2
P for trend			P=0.02					P=0.08					P=0.5		
GT/TT	247	147	1.35	(1.03, 1.75)	0.03	343	83	1.28	(0.93, 1.76)	0.1	533	100	1.02	(0.78, 1.34)	0.9
<b>-50977 (rs1799978)</b>															
AA	787	399	1.00	(reference)		1047	233	1.00	(reference)		1621	295	1.00	(reference)	
AG	105	40	0.72	(0.48, 1.09)	0.1	153	16	0.41	(0.23, 0.72)	0.002	193	28	0.78	(0.51, 1.21)	0.3
GG	5	3	1.20	(0.26, 5.56)	0.8	5	1	0.63	(0.06, 6.87)	0.7	7	1	0.63	(0.07, 5.53)	0.7
P for trend			P=0.2					P=0.003					P=0.2		
AG/GG	110	43	0.74	(0.50, 1.11)	0.1	158	17	0.41	(0.24, 0.72)	0.002	200	29	0.78	(0.51, 1.19)	0.3
<b>DRD2 haplotype<sup>b</sup></b>															
C-T-G-A	810	372	1.00	(reference)		1101	202	1.00	(reference)		1621	264	1.00	(reference)	
C-T-G-G	10	5	1.02	(0.31, 3.35)	1.0	21	1	0.31	(0.04, 2.49)	0.3	25	3	0.76	(0.21, 2.67)	0.7
C-C-G-A	405	206	1.04	(0.83, 1.30)	0.7	543	115	1.09	(0.83, 1.43)	0.5	866	141	0.98	(0.78, 1.23)	0.8
C-C-G-G	54	25	1.00	(0.59, 1.69)	1.0	78	10	0.63	(0.31, 1.29)	0.2	101	20	1.26	(0.75, 2.12)	0.4
T-T-G-A	57	25	1.11	(0.66, 1.86)	0.7	82	12	1.05	(0.54, 2.06)	0.9	119	20	1.10	(0.66, 1.85)	0.7
T-C-T-A	210	137	1.36	(1.04, 1.77)	0.02	293	81	1.44	(1.05, 1.97)	0.02	457	99	1.27	(0.98, 1.66)	0.08

OR, odds ratio; CI, confidence interval; BMI, body mass index.

<sup>a</sup>OR estimated using conditional logistic regression, conditioning on age, sex, smoking status, and number of cigarettes smoked; model also adjusted for race/ethnicity.

<sup>b</sup>Haplotype analyses were conducted among non-Hispanic whites only.

Supplemental Table Distribution of *DRD2* polymorphisms by usual level of alcohol consumption (grams per day)

SNP	<5		5-14			15-29			30-44			45+					
	N	N OR <sup>a</sup>	(95% CI)	P	N	OR <sup>a</sup>	(95% CI)	P	N	OR <sup>a</sup>	(95% CI)	P	N	OR <sup>a</sup>	(95% CI)	P	
<b>TaqIA (rs1800497)</b>																	
CC	812	215	1.00	(reference)	165	1.00	(reference)		88	1.00	(reference)		109	1.00	(reference)		
CT	409	117	1.07	(0.82, 1.39)	0.6	78	1.01	(0.75, 1.38)	0.9	32	0.67	(0.43, 1.03)	0.07	56	1.01	(0.70, 1.46)	1.0
TT	62	12	0.66	(0.34, 1.27)	0.2	10	0.88	(0.44, 1.78)	0.7	5	0.57	(0.21, 1.53)	0.3	8	0.80	(0.36, 1.80)	0.6
<i>P</i> for trend				<i>P</i> =0.7				<i>P</i> =0.9				<i>P</i> =0.05				<i>P</i> =0.8	
CT/TT	471	129	1.02	(0.79, 1.32)	0.9	88	1.03	(0.77, 1.38)	0.9	37	0.68	(0.45, 1.04)	0.08	64	1.00	(0.70, 1.43)	1.0
<b>957 (rs6277)</b>																	
TT	350	104	1.00	(reference)	69	1.00	(reference)		36	1.00	(reference)		38	1.00	(reference)		
TC	583	156	0.95	(0.71, 1.27)	0.7	126	1.12	(0.80, 1.56)	0.5	66	1.07	(0.69, 1.66)	0.8	82	1.28	(0.83, 1.98)	0.3
CC	334	79	0.80	(0.57, 1.12)	0.2	58	0.96	(0.65, 1.41)	0.8	20	0.51	(0.28, 0.92)	0.02	50	1.38	(0.86, 2.24)	0.2
<i>P</i> for trend				<i>P</i> =0.2				<i>P</i> =0.8				<i>P</i> =0.04				<i>P</i> =0.2	
TC/CC	917	235	0.89	(0.67, 1.17)	0.4	184	1.11	(0.81, 1.52)	0.5	86	0.91	(0.59, 1.39)	0.7	132	1.37	(0.91, 2.06)	0.1
<b>IVS6-83 (rs1076560)</b>																	
GG	899	244	1.00	(reference)	176	1.00	(reference)		94	1.00	(reference)		120	1.00	(reference)		
GT	340	93	0.96	(0.73, 1.27)	0.8	68	1.07	(0.78, 1.47)	0.7	27	0.74	(0.47, 1.18)	0.2	46	0.97	(0.66, 1.42)	0.9
TT	43	5	0.40	(0.15, 1.04)	0.06	9	1.33	(0.62, 2.85)	0.5	3	0.45	(0.13, 1.60)	0.2	7	0.99	(0.41, 2.41)	1.0
<i>P</i> for trend				<i>P</i> =0.2				<i>P</i> =0.5				<i>P</i> =0.09				<i>P</i> =0.9	
GT/TT	383	98	0.92	(0.70, 1.21)	0.5	77	1.14	(0.84, 1.55)	0.4	30	0.76	(0.48, 1.19)	0.2	53	1.00	(0.68, 1.45)	1.0
<b>-50977 (rs1799978)</b>																	
AA	1128	302	1.00	(reference)	231	1.00	(reference)		114	1.00	(reference)		155	1.00	(reference)		
AG	142	42	1.11	(0.76, 1.62)	0.6	23	0.74	(0.46, 1.19)	0.2	11	0.78	(0.41, 1.51)	0.5	18	0.88	(0.51, 1.53)	0.6
GG	8	0 <sup>c</sup>			0 <sup>c</sup>				0 <sup>c</sup>				0 <sup>c</sup>				
<i>P</i> for trend																	
AG/GG	150	42	1.02	(0.70, 1.50)	0.9	23	0.73	(0.45, 1.18)	0.2	11	0.77	(0.40, 1.49)	0.4	18	0.85	(0.49, 1.48)	0.6
<b><i>DRD2</i> haplotype<sup>b</sup></b>																	
C-T-G-A	1138	303	1.00	(reference)	239	1.00	(reference)		125	1.00	(reference)		142	1.00	(reference)		
C-T-G-G	17	8	1.82	(0.75, 4.41)	0.2	6	1.65	(0.63, 4.34)	0.3	2	1.13	(0.24, 5.30)	0.9	3	1.19	(0.32, 4.46)	0.8
C-C-G-A	600	149	0.97	(0.77, 1.21)	0.8	126	1.01	(0.79, 1.30)	0.9	59	0.84	(0.60, 1.18)	0.3	97	1.31	(0.97, 1.76)	0.07
C-C-G-G	85	19	0.91	(0.54, 1.54)	0.7	11	0.63	(0.33, 1.21)	0.2	5	0.53	(0.21, 1.36)	0.2	11	1.03	(0.52, 2.04)	0.9
T-T-G-A	86	29	1.21	(0.77, 1.91)	0.4	11	0.59	(0.31, 1.15)	0.1	6	0.49	(0.21, 1.16)	0.1	11	1.00	(0.50, 2.00)	1.0
T-C-T-A	327	89	1.01	(0.77, 1.33)	1.0	71	1.12	(0.82, 1.51)	0.5	28	0.65	(0.41, 1.03)	0.06	46	1.02	(0.70, 1.49)	0.9

<sup>a</sup>OR estimated using conditional logistic regression, conditioning on age, sex, smoking status, and number of cigarettes smoked; model also adjusted for race/ethnicity and BMI.

<sup>b</sup>Haplotype analyses were conducted among non-Hispanic Whites only.

Our study extends the findings of a previous meta-analysis of 13 studies that suggested that the *DRD2* TaqIA polymorphism is related to smoking behavior [18]. Importantly, we evaluated haplotypes and other reward-motivated behaviors in a large (>2000 individuals) population-based sample. We find that the *DRD2* haplotype [TaqIA(C/T)–957(T/C)–IVS6-83(G/T)–50977(A/G)] that included the variant alleles for all three SNPs we evaluated in the 3' region of the gene, T-C-T-A, was more common among current than former smokers and more common among obese than normal weight individuals, suggesting increased dependence on external stimulation of the dopaminergic system, which is consistent with *in vivo* findings in obese individuals [6,27]. Importantly, our findings are consistent with some previous research on the functional alterations of the dopamine reward pathway associated with these variants: both the TaqIA-T and 957C alleles have been associated with decreased *DRD2* receptor density and striatal binding potential *in vivo* [28–30]. The 957T allele has also, however, been associated with decreased mRNA stability [23], suggesting that the functionality of this SNP is not firmly established. The strong linkage disequilibrium between these SNPs and other coding regions of *DRD2* (<http://www.hapmap.org>), suggests that the *DRD2* TaqIA polymorphism, which codes for a nonsynonymous substitution in a 3'-gene [31], functions as a marker in linkage disequilibrium for variation within *DRD2*, and not as the functional polymorphism responsible for variation in D2 receptor function. Our *DRD2* haplotype analysis of smoking and obesity phenotypes is therefore an important strength of the present study that directly implicates *DRD2*.

We did not observe associations between *DRD2* SNPs and alcohol consumption [32], although our ability to detect such an association was limited because of the relatively low levels of alcohol consumption in our study population and the imprecision of using usual adult consumption of alcohol to measure alcohol dependence. Additional limitations of our study included a lack of data on level of nicotine addiction (e.g. Fagerstrom Tolerance Questionnaire) and on interindividual variation in nicotine metabolism [33]. We also could not distinguish between current smokers who had and those who had not ever made a quit attempt, although there is evidence that a substantial proportion of current smokers attempt to quit smoking each year [34], suggesting that any potential misclassification would have been small.

In conclusion, our results provide strong epidemiologic support to previous data derived from animal models, *in vivo* and *in vitro* functional studies, and smaller human genetic studies that have suggested that genetic variation in the dopamine reward pathway plays an important role in reward-motivated characteristics, specifically cigarette

smoking and obesity. Our individual SNP and haplotype associations provide further insight into understanding the genetic components of these behaviors, which is critical for the development of more effective treatments [35–38]. Our results support that *DRD2* is an appropriate molecular target for smoking cessation treatments, and suggest the exploration of *DRD2* antagonists for obesity therapies. Investigations of the complex reward system, including other neurotransmitter and nicotine metabolism pathways with broad genotype and phenotype coverage, are warranted to fully understand the genetics underlying these complex traits.

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