D_2 Dopamine Receptor Gene Polymorphisms among African-Americans and Mexican-Americans: A Lung Cancer Case-Control Study[1]

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Abstract

Recent research suggests that variant alleles (*A1* and *B1*) of the *DRD2* gene play a role in determining smoking status. However, no studies have evaluated these variant alleles in African-Americans and Mexican-Americans. The primary objective of this study, therefore, was to test the hypothesis that ever smokers in these ethnic groups are more likely than never smokers to have the *DRD2* alleles associated with tobacco use (*A1* and *B1*). Furthermore, because of a predicted higher prevalence of smokers in a family because of the patterns of inheritance of the genotypes associated with tobacco use, we also anticipated that individuals with these at-risk *DRD2* alleles would be more likely to have a family history of smoking-related cancers. Because other inherited genetic variants may interact with smoking on cancer risk, we also hypothesized that this association might differ between cancer patients and control subjects.

PCR was used to perform genotyping on peripheral WBC DNA from 140 lung cancer patients (43 Mexican-Americans and 97 African-Americans) and 222 age-, sex-, and ethnicity-matched controls (111 Mexican-Americans and 111 African-Americans). A personal family history was obtained from each participant. There were no statistically significant differences in the distribution of the *DRD2* genotypes between cases and controls, although the frequency of the *B1* genotype significantly differed by ethnicity ($P = 0.002$ for controls and $P = 0.001$ for cases). The *DRD2* genotypes and smoking status showed a correlation among Mexican-American controls, although not among African-American controls. The cigarette pack-years in control subjects for the two ethnic groups combined were 30.8, 21.9, and 18.6 for the *A1A1*, *A1A2*, and *A2A2* genotypes and 36.5, 20.8, and 18.5 for the *B1B1*, *B1B2*, and *B2B2* genotypes, respectively. Similar trends were found for the number of cigarettes smoked per day among control subjects. From the standpoint of polymorphisms, however, there was a borderline significantly increased (3.6 times greater) frequency of smoking-related cancers among the first-degree relatives of case subjects with an *A1* allele than among those without an *A1* allele. There was also an elevated (1.8 times greater) frequency of smoking-related cancer among first-degree relatives of case subjects with a *B1* allele compared with patients without a *B1* allele, but this finding was not statistically significant. This phenomenon was not observed among control subjects. We noted a trend toward interaction of *DRD2 A1* genotypes and case status for increased risk of smoking-related cancer among first-degree relatives. These findings suggest that the variant *DRD2* genotypes are associated with a greater likelihood to smoke and a greater smoking intensity, as well as with a familial aggregation of smoking-related cancers. However, a large study is needed to confirm this finding.

Introduction

African-Americans in the United States are at higher risk than are Caucasians of developing and dying from lung cancer (1). At the same time, although African-American smokers are more likely to try to quit, they have a lower success rate than do Caucasian smokers (2). Recent family, twin, and molecular genetic studies provide compelling evidence of a role for genetic factors governing such smoking behavior (3–11). For example, it has been reported that the concordance rates for smoking, not smoking, and quitting are higher for monozygotic twins than for dizygotic twins. Specifically, the concordance rate for smoking in 82 pairs of identical twins reared apart was 79% (12). In addition, a meta-analysis of data from eight studies revealed an estimated heritability rate of 60% for smoking (12).

There is substantial evidence that nicotine is the dependence-producing component of tobacco (13). Once delivered to the brain, nicotine exerts psychoactive effects that affect mood and cognitive function. Specifically, nicotine intake is associated with enhanced pleasure, improved task performance and memory, reduced anxiety and tension, and weight control. It also relieves the effects of nicotine withdrawal (14). Nicotine acts through the dopamine reward pathway for its reinforcing effect. It has been hypothesized that persons with a functional deficit in the dopamine reward pathway may be more prone to drug addiction, including nicotine dependence (15).

There are two primary families of dopamine receptors, D_1 and D_2, which are differentiated by their ligand specificity, central nervous system distribution, physiological actions, and their stimulatory (D_1) or inhibitory (D_2) effects on G-protein

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second-messenger systems (16). In 1989, Grandy *et al.* (17) reported that the human D_2 dopamine receptor gene is located on chromosome 11q. Since then, several polymorphisms on this gene have been identified. In particular, studies conducted in the early 1990s examined associations between a polymorphism in the *Taq*I A allele (*A1* and *A2*) and substance abuse, including tobacco use (15, 18). More recently, investigators have also explored associations between the *Taq*I B allele (*B1* and *B2*) and smoking (9). D_2 dopamine receptor polymorphisms have been associated with obesity (19), neuropsychiatric disorders (20), and substance abuse, including alcoholism (16, 21–23), cocaine use (24), polysubstance use (18), and tobacco use (9, 15, 25).

Several studies have shown that the *A1* allele is associated with a reduced number of dopamine binding sites in the brain (16, 26–28). It has been hypothesized that such persons who have a reduced dopamine D_2 receptor density have a deficit in their reward system and experience an enhanced reward when exposed to dopaminergic agents, thereby making them more prone to nicotine addiction (15). Results from a previous study in our laboratory support this hypothesis, in that persons who exhibited certain D_2 polymorphisms (*A1* or *B1*) were more likely to have ever smoked, started smoking at an earlier age, and attempted to quit fewer times compared with persons without the polymorphism (9). Other investigators have reported that the *A1* and *B1* alleles are more prevalent among current and former smokers than among never smokers (15).

No studies have evaluated these polymorphisms in minority populations. Because African-Americans suffer disproportionately higher incidences of lung cancer and cardiovascular disease, it is important that these issues be explored. The primary objective of this study of African-Americans and Mexican-Americans was to test the hypothesis that ever smokers in ethnic groups other than Caucasians are more likely than never smokers in these ethnic groups to exhibit the *DRD2*[3] alleles associated with tobacco use (*A1* and *B1*). Furthermore, because of a predicted higher prevalence of smokers in a family attributable to the patterns of inheritance of the genotypes associated with tobacco use, we also anticipated that individuals with these at-risk *DRD2* alleles would be more likely to report a family history of smoking-related cancers. Because other inherited genetic variants may interact with smoking on cancer risk, we also explored whether this association differs between cancer patients and control subjects.

Materials and Methods

Subjects. The study population consisted of a subset derived from a case-control study of lung cancer among minority populations. The case subjects included 140 African-American or Mexican-American lung cancer patients who had newly diagnosed, histologically confirmed lung cancer. There were no age or cancer stage restrictions for inclusion. Subjects were identified through The University of Texas M. D. Anderson Cancer Center and community and Veterans Affairs hospitals in Houston and San Antonio, Texas, during 1990 to 1994.

Controls. The control group was a convenience sample accrued from community centers, churches, cancer-screening programs, and employee groups. The group consisted of 222 healthy volunteers who were frequency-matched to the cases by age ($\pm$5 years), sex, and ethnicity (2:1 for Mexican-Americans and 1:1 for African-Americans). Because we recruited Mexican-American cases from Houston, as well as from Galveston and San Antonio, we matched only very loosely on residence, *i.e.,* (Houston/Galveston *versus* San Antonio). The controls were recruited from 37 community locations, of which two were prostate cancer screening programs.

Data Collection. Epidemiological data were collected by personal interview. After informed consent was obtained, a structured interview lasting ~45 min was conducted by trained bilingual interviewers. Data were collected on sociodemographic characteristics, recent and prior tobacco use, other lifestyle habits, and family history of cancer. At the completion of the interview, blood was drawn into heparinized tubes for cytogenetic and molecular genetic analyses.

Polymorphism Analyses. The subjects were genotyped for the *Taq*I A and *Taq*I B sites of the *DRD2* gene using methods described previously (9). Genomic DNA was extracted from the blood sample and used as a template for the PCR. Primers 5′-CCG TCG ACC CTT CCT GAG TGT CAT CA-3′ and 5′-CCG TCG ACG GCT GGC CAA GTT GTC TA-3′ were used to amplify a 310-bp fragment spanning the polymorphic *Taq*I A site of the *DRD2* gene. Primers 5′-GAT ACC CAC TTC AGG AAG TC-3′ and 5′-GAT GTG TAG GAA TTA GCC AGG-3′ were used to amplify a 459-bp fragment spanning the polymorphic *Taq*I B site of the *DRD2* gene. PCR was performed in 30-μl reaction mixtures containing 1.5 mM $MgCl_2$, 0.2 mM deoxynucleotide triphosphates, 0.5 μM primers, 1 μg of template DNA, and 1.5 units of Taq polymerase with a PCR buffer consisting of 20 mM Tris-HCl (pH 8.4) and 50 mM KCl (Boehringer Mannheim, Indianapolis, IN). After an initial denaturation at 94°C for 4 min, the DNA was amplified during 35 cycles of 30 s at 94°C, 30 s at 58°C, and 30 s at 72°C, followed by a final extension step of 5 min at 72°C. A portion of the PCR product (20 μl) was digested with 5 units of *Taq*I for 22 h at 65°C to reveal the *DRD2 Taq*I A polymorphism and digested with 5 units of *Taq*I for 5 h at 65°C to reveal the *DRD2 Taq*I B polymorphism. Twenty μl of the PCR digestion products were then resolved on a 3% agarose gel (5 V/cm) containing ethidium bromide. There were three *DRD2 Taq*I *A* genotypes: (*a*) the predominant homozygote, *A2A2*, indicated by two fragments, 180 and 130 bp; (*b*) the heterozygote, *A1A2*, revealed by three fragments, 310, 180, and 130 bp; and (*c*) the rare homozygote, *A1A1*, shown by the uncleaved 310-bp fragment. There were also three *DRD2 Taq*I *B* genotypes: (*a*) the predominant homozygote, *B2B2*, indicated by two fragments, 267 and 192 bp; (*b*) the heterozygote, *B1B2*, revealed by three fragments, 459, 267, and 192 bp; and (*c*) the rare homozygote, *B1B1*, shown by the uncleaved 459-bp fragment.

Measures and Statistical Analysis. The *DRD2* genotype data were merged with the interview data. Ever smokers were defined as individuals who had smoked >100 cigarettes in their lifetime. Former smokers were defined as those who had quit at least 1 year before the interview. Latency was defined as the length of time that had elapsed since the cessation of smoking. Pack-years were calculated using the average number of cigarettes smoked/day and the number of years smoked. Subjects who reported one or more cases of lung, oral cavity, pharyngeal, esophageal, pleural, cervical, urinary bladder, kidney, or head and neck cancers among first-degree relatives were coded as having a positive family history of smoking-related cancers.

Bivariate relationships were assessed using χ^2 tests of independence. Fisher's exact tests were used to test independence when cell sizes were small. To adjust for relevant covariates, the relationship between genotype and a family history of

[3] The abbreviations used are: *DRD2,* D_2 dopamine receptor gene; CI, confidence interval.

Table 1 Study participant characteristics

Characteristics	Mexican-American			African-American		
	Cases $n = 43$	Controls $n = 111$	*P*	Cases $n = 97$	Controls $n = 111$	*P*
Sex						
Male	34 (79.1)	83 (74.8)		68 (70.1)	73 (65.8)	
Female	9 (20.9)	28 (25.2)	0.58	29 (29.9)	38 (34.2)	0.50
Mean age in years (SD)	65.6 (9.8)	63.77 (12.4)	0.40	59.04 (9.7)	60.07 (12.5)	0.53
Smoking status						
Never	5 (11.6)	49 (44.1)		3 (3.1)	39 (35.1)	
Former	19 (44.2)	26 (23.4)		31 (32.0)	35 (31.5)	
Current	19 (44.2)	36 (32.4)	0.001	63 (65.9)	37 (33.3)	<0.001
Mean pack-years (SD)	47.31 (36.3)	11.06 (18.1)	<0.001	48.77 (32.9)	14.24 (18.3)	<0.001
Mean no. of cigarettes/day (SD)	22.58 (13.2)	6.65 (10.2)	<0.001	25.40 (14.6)	9.52 (10.9)	<0.001
Mean no. of years smoked (SD)	36.09 (18.9)	18.13 (21.1)	<0.001	36.34 (13.7)	18.34 (18.4)	<0.001

Table 2 Distribution of DRD2 *Taq*I *A* and *Taq*I *B* genotypes by ethnic group among case and control subjects

Study group	*Taq*I *A*					*Taq*I *B*				
	A1A1	*A1A2*	*A2A2*	*P*	*A1* frequency	*B1B1*	*B1B2*	*B2B2*	*P*	*B1* frequency
Control subjects										
Mexican-American	15 (14.0)[a]	60 (56.1)	32 (29.9)		0.42	14 (14.4)	42 (43.3)	41 (42.3)		0.36
African-American	13 (11.7)	56 (50.5)	42 (37.8)	0.46	0.37	4 (4.3)	28 (29.8)	62 (66.1)	0.002	0.19
Case subjects										
Mexican-American	6 (14.0)	20 (46.5)	17 (39.5)		0.37	6 (15.8)	17 (44.7)	15 (39.5)		0.38
African-American	10 (10.4)	52 (54.2)	34 (35.4)	0.67	0.38	1 (1.3)	23 (29.1)	55 (69.6)	<0.001	0.16

[a] No. of subjects (%).

cancer among first-degree relatives was computed using standard logistic regression procedures.

Results

Study Population. Our study population was composed of 43 Mexican-American cases, 97 African-American cases, 111 Mexican-American controls, and 111 African-American controls. Study population characteristics are presented in Table 1 by ethnicity and case-control status. The two groups did not differ significantly by sex or age. Among case subjects, 88% of Mexican-Americans and 97% of African-Americans were ever smokers; of control subjects, 56% of Mexican-Americans and 65% of African-Americans were smokers (both *P*s, <0.001). The mean pack-years, number of cigarettes smoked/day, and years smoked were significantly higher in cases than in controls for both ethnic groups (all *P*s, <0.001).

***DRD2* Genotypes and Ethnicity.** Table 2 summarizes the distribution of the *DRD2* genotypes by ethnicity and case-control status. The *DRD2 Taq*I *B* genotype distribution was strongly associated with ethnicity in both cases and controls. Specifically, the common genotype (*B2B2*) was significantly less prevalent among Mexican-American control subjects than among African-American control subjects (42.3% compared with 66.1%). The *B1* allele frequencies were 0.36 for Mexican-American control subjects and 0.19 for African-American control subjects; a similar pattern was evident in the case subjects. The *Taq*I *A* genotypes did not, however, differ significantly by ethnicity. There also were no statistically significant differences in the distribution of the *Taq*I *A* or *Taq*I *B DRD2* genotypes between cases and controls of either ethnic group (all *P*s, >0.4; data not shown). Among controls, no deviations from the Hardy-Weinberg equilibium were detected for either the *Taq*I *A* or *Taq*I *B* genotypes.

***DRD2* Genotypes and Smoking Variables.** We observed significant correlations between the *DRD2* genotype and various smoking-related variables among control subjects (Table 3). Specifically, among Mexican-American controls with the *A1A1* genotype, 20.0% were never smokers and 60.0% were current smokers, compared with 53.1% and 28.1%, respectively, for those with the *A2A2* genotype. A similar trend was observed for Mexican-American control subjects with the *B1B1* genotype (21.4%, never smokers; 64.3%, current smokers). In contrast, among Mexican-American control subjects with the *B2B2* genotype, 53.7% were never smokers and 19.5% were current smokers. In Mexican-Americans, the A1 frequencies for never smokers, former smokers, and current smokers were 0.35, 0.44, and 0.50. A χ^2 test for linear trend found increasing A1 frequency for these three groups, respectively ($P = 0.06$). The B1 frequencies for never smokers, former smokers, and current smokers were 0.28, 0.30, and 0.52. The χ^2 test for linear trend showed a strong trend ($P = 0.005$) in increasing B1 frequency for these three groups. These patterns were not observed among African-American subjects (data not shown).

As Table 3 also shows, persons with the *A1A1* genotype were heavier smokers (30.8 pack-years and 21.9 cigarettes per day) than were subjects with the *A2A2* genotype (18.6 pack-years and 12.6 cigarettes/day). Because the pattern for amount of smoking was similar for Mexican-American and African-American controls, we present the data for the two ethnic groups combined. There was also evidence of a gene-dose effect, *i.e.*, as the number of *A1* allele copies increased, the number of pack-years and cigarettes/day reported increased correspondingly. In addition, individuals with the *A1A1* genotype had started smoking at earlier mean ages (15.6 ± 5.0 years) than subjects with the *A2A2* genotype (18.8 ± 6.9 years). Subjects with the *B1* genotypes also showed a greater smoking

Table 3 Cigarette smoking characteristic by DRD2 *Taq*1 *A* and *Taq*1 *B* genotypes among controls

Characteristics	*Taq*1 A					*Taq*1 B				
	A1A1	*A1A2*	*A2A2*	*A1* frequency	*P*	*B1B1*	*B1B2*	*B2B2*	*B1* frequency	*P*
Smoking status (Mexican-American)										
Never	3 (20.0)	28 (46.67)	17 (53.1)	0.35		3 (21.4)	19 (45.24)	22 (53.7)	0.28	
Former	3 (20.0)	15 (25.00)	6 (18.8)	0.44		2 (14.3)	9 (21.43)	11 (26.8)	0.30	
Current	9 (60.0)	17 (28.33)	9 (28.1)[a]	0.50	0.16	9 (64.3)	14 (33.33)	8 (19.5)	0.52	0.06
Smoking status (African-American)										
Never	8 (61.5)	16 (28.57)	15 (35.7)	0.41		3 (75.0)	11 (39.29)	22 (35.5)	0.24	
Former	3 (23.1)	19 (33.93)	13 (31.0)	0.36		0 (0.0)	9 (32.14)	20 (32.3)	0.16	
Current	2 (15.4)	21 (37.50)	14 (33.3)	0.34	0.31	1 (25.0)	8 (28.57)	20 (32.3)	0.17	0.69
Ever smokers[b]		Mean (SD)								
Pack-years smoked	30.8 (27.4)	21.9 (18.4)	18.6 (16.3)		0.24[c]	36.5 (28.4)	20.8 (20.4)	18.5 (16.1)		0.07[d]
Cigarettes/day	19.0 (14.5)	13.7 (9.9)	12.6 (9.8)		0.15[c]	20.1 (16.3)	13.7 (11.8)	12.6 (9.3)		0.22[d]
Years smoked	34.3 (21.1)	31.4 (16.1)	29.5 (14.7)		0.34[c]	37.1 (21.6)	29.6 (17.5)	30.1 (14.7)		0.18[d]
Age started smoking	15.6 (5.0)	19.7 (8.0)	18.8 (6.9)		0.12[c]	16.6 (8.6)	20.4 (9.9)	18.6 (5.7)		0.07[d]
Latency	21.7 (18.3)	27.1 (11.9)	28.3 (16.6)		0.41[c]	31.0 (14.1)	24.2 (12.9)	27.7 (14.6)		0.72[d]

[a] $P = 0.048$ between current smokers and never smokers.
[b] Data for the two ethnic groups were combined because no differences in these variables were identified between the two groups.
[c] Wilcoxon Rank Sum test between *A2A2* and *A1A1* genotype.
[d] Wilcoxon Rank Sum test between *B2B2* and *B1B1* genotype.

Table 4 Associations between smoking-related cancer in first-degree relatives and *DRD2* genotypes

FDR[a] cancer status	*A1A1* and *A1A2*	*A2A2*	Crude OR (95% CI)	Adjusted OR[b] (95% CI)	*B1B1* and *B1B2*	*B2B2*	Crude OR (95% CI)	Adjusted OR[b] (95% CI)
Case subjects								
Cancer	16 (84.2)	3 (15.8)			7 (46.7)	8 (53.3)		
No cancer	69 (59.0)	48 (41.0)	3.7 (1.0–13.4)[c]	3.6 (1.0–13.2)[d]	38 (38.0)	62 (62.0)	1.4 (0.5–4.3)	1.8 (0.5–5.7)
Control subjects								
Cancer	29 (64.4)	16 (35.6)			16 (40.0)	24 (60.0)		
No cancer	113 (66.1)	58 (33.9)	0.9 (0.5–1.9)	1.0 (0.5–2.1)	72 (48.0)	78 (52.0)	0.7 (0.4–1.5)	0.7 (0.3–1.5)

[a] FDR, first-degree relatives. OR, odds ratio; CI, confidence interval.
[b] Adjusted for age, sex, ethnicity, smoking status, and number of first-degree relatives.
[c] $P < 0.05$.
[d] $P = 0.05$.

intensity than those with the *B2* genotype. There were no statistically significant associations between genotype and smoking-related phenotypes among case subjects, although we did observe a modest association ($P < 0.10$) between the *A1* genotype and the mean number of cigarettes smoked/day (data not shown).

***DRD2* Genotypes and Cancer Risk.** Table 4 shows the associations between self-reported smoking-related cancers among first-degree relatives and the *DRD2* genotypes as a function of case-control status for the two ethnic groups combined. For Mexican-Americans, there were an average of 9.0 first-degree relatives/case and 8.0 first-degree relatives/control. For African-Americans, there were an average of 8.7 first-degree relatives/case and 9.5 first-degree relatives/control. After adjusting for age, sex, ethnicity, smoking status, and the number of first-degree relatives, case subjects with an *A1* allele (*A1A1* or *A1A2* genotypes) proved to be 3.6 times (CI, 1.0–13.2) more likely to report a family history of smoking-related cancers among first-degree relatives than were case subjects without an *A1* allele (*A2A2* genotype). Specifically, of the case subjects with an *A1* allele, 84.2% had a family history of smoking-related cancer in a first-degree relative compared with 15.8% of the individuals with the *A2A2* genotype. In addition, case subjects with a *B1* allele (*B1B1* or *B1B2*) were 1.8 times (CI, 0.5–5.7) as likely to have a family history of smoking-related cancer than were case subjects without a *B1* allele (*B2B2*), although the difference in the frequency was not statistically significant. Among control subjects, there was no such relationship between *A1* or *B1* genotypes and a family history of smoking-related cancers. We also noted a nearly significant interaction between *DRD2 A1* genotypes and case/control status for increased risk of smoking-related cancer among first-degree relatives. Case individuals with the *DRD2 A1A1* or *A1A2* genotypes were 3.7 (CI, 0.8–16.4) times more likely to have an affected first-degree relative than other groups. In addition, case individuals with the *DRD2 B1B1* or *B1B2* genotypes were 2.3 (CI, 0.6–8.7) times more likely to have an affected first-degree relative than other groups (data not shown).

Discussion

There is a growing body of evidence implicating genetic factors as correlates of smoking behavior and of lung cancer risk, and the investigation reported here provides support for the hypothesized link between the dopamine receptor genes and smoking status in a minority population. In particular, we observed a difference in the frequency of the *B1* risk allele by ethnicity for both case and control subjects, *i.e.*, it was significantly less common among African-American controls than among Mexican-American controls; a relationship between the smoking status and genotype status for Mexican-American control sub-

jects; relationships between the smoking intensity (pack-years and cigarettes smoked/day) and genotype status among controls (ethnic groups combined); and evidence that lung cancer patients of both ethnic groups with the *A1* allele exhibited familial aggregation of smoking-related cancers.

To date, studies of the *DRD2* genotypes and smoking primarily have focused on Caucasians. The study reported here is perhaps the first to provide insight into ethnic group-related differences in the *DRD2* genotypes. Among Mexican-Americans, we observed a significant relationship between smoking status and the *Taq*I *B* genotypes; a similar, but nonsignificant, trend was apparent for the *Taq*I *A* genotypes. These findings confirm our previous observations in a study in a Caucasian population (9). In both instances, never smokers were less likely to have the risk alleles (*A1* or *B1*) than were current smokers. Among African-Americans, however, we observed no relationship between smoking status and the *Taq*I *A* or *Taq*I *B* genotypes. The discordant findings by smoking status in the two ethnic groups could be attributed to the relatively greater importance of contributing factors such as psychosocial, environmental, or socioeconomic determining the complex behavior of smoking. In addition, there may be ethnic differences in nicotine pharmacokinetics and metabolism. It is plausible that such factors may be more important in determining the ever-smoking status among African-Americans than are the *DRD2* genotypes. Furthermore, the *B1* allele seems more predictive than the *A1* allele in smoking status. The frequency of *B1* allele for African-Americans was less than half that for Mexican-Americans.

We also observed that among Mexican-American controls, the *A1* allele frequency increased from 0.35 in never smokers to 0.44 for former smokers, and 0.50 for current smokers with a χ^2 test for linear trend *P* of 0.06. The *B1* allele frequencies were 0.28 for never smokers, 0.30 for former smokers, and 0.52 for current smokers with a χ^2 test for linear trend *P* of 0.005. In African-American controls, the *A1* allele frequency was 0.41 for never smokers, 0.36 for former smokers, and 0.34 for current smokers, and the *B1* frequency was 0.24 for never smokers, 0.16 for former smokers, and 0.17 for current smokers. In a previously published study (9), we identified *A1* allele frequencies of 0.16 for never smokers, 0.22 for former smokers, and 0.23 for current smokers among Caucasian controls. The *B1* frequencies in these Caucasian controls were 0.03, 0.15, and 0.17, respectively. The *B1* allelic frequencies that we observed in the current study were two times higher among Mexican-Americans (0.38 for cases and 0.36 for controls) than among African-Americans (0.16 for cases and 0.19 for controls). This point suggests a need to determine how, and the extent to which, these allelic differences translate into ethnic differences in the effects of nicotine, both behaviorally and biologically.

It was a somewhat unexpected finding that the relationships between *DRD2* and several of the smoking phenotypes were observed among controls but not among lung cancer cases. For example, smoking status was strongly related to *Taq*I *B1* allele frequencies among Mexican-American controls but not among Mexican-American cases. It is possible that factors other than *DRD2* are stronger contributors to tobacco use as well as development of lung cancer. For example, the *CYP2A6* gene, which has been shown to be related to nicotine metabolism and lung cancer susceptibility (29), may be a more influential factor for tobacco use among lung cancer patients.

There is a pressing demand for studies that elucidate the functional effects of genetic polymorphisms. For example, although there is some evidence that the presence of an *A1* allele is associated with a decreased number of D_2 dopamine receptors in the brain (16), the mechanism by which the *DRD2 A1* or *B1* alleles increase susceptibility to tobacco use are not known. One possible mechanism is that this decreased density of receptors results in a deficiently functioning dopamine reward system, such that persons with an *A1* allele experience an enhanced reward when exposed to dopaminergic agents, rendering them more vulnerable to nicotine dependence. However, until researchers can delineate the mechanisms by which genetic variants alter the biological and psychological effects of nicotine, we cannot fully understand the observed relationships between genes and smoking behavior.

This study is the first to examine the relationship between the *DRD2* genotypes and a family cancer history. Specifically, we tested the hypothesis that the *A1* and *B1* alleles are risk factors for smoking and that if these polymorphisms segregate in families, the rates of smoking-related cancers among the first-degree relatives of probands with these genotypes would be elevated. However, our results supported this hypothesis only for cases with the *A1* allele. In particular, the odds of a person with an *A1* allele (*A1A1* or *A1A2* genotypes) having a positive family history was more than three times greater than that for persons with the *A2A2* genotype. Although the risk estimate is large, its confidence intervals are wide. This hypothesis therefore needs to be examined further in larger studies. We also observed a similar but smaller trend for the *B1* risk allele, but the difference seen for the *B1* and *B2* alleles was not statistically significant, suggesting that the *A1* allele is a more powerful risk factor for this phenotype than the *B1* allele is. Regardless, a relationship between the *DRD2* alleles and a family history of tobacco-related cancers was found only among case subjects, suggesting that a genetic susceptibility to tobacco exposure was also associated (or aggregated) with a predisposition to smoking. Among control subjects, on the other hand, persons with the dopamine receptor genes appear to be at an elevated risk for smoking, but this elevated smoking risk does not appear to translate into a familial risk of tobacco-related cancers. We also noted a nearly significant interaction between *DRD2 A2* genotypes and case/control status for increased risk of smoking-related cancer among first-degree relatives. A large study is needed to confirm this finding.

The present study was limited in that it examined a narrow range of phenotypes. If we are to gain a better understanding of a genetic predisposition to tobacco use and/or nicotine dependence, investigators must examine a more comprehensive range of phenotypes, such as measures of dependence, the rate of convergence on regular smoking, biological measures of nicotine pharmacokinetics, and receptor activity. Further drawbacks in our study were that the smoking-related phenotypes were assessed retrospectively in study participants, and all data were self-reported without subsequent verification. In addition, it is possible that the validity of the self-reported data may vary between cases and controls. The potential for recall bias regarding first-degree relatives with a tobacco-related cancer should be considered when interpreting the results of our study.

There is little debate that the dopamine reward system is involved in the development of nicotine addiction and patterns of tobacco use, although other neurotransmitters and factors such as nicotine metabolism also appear to play a role. The extent to which genetic makeup modulates susceptibility to tobacco use and the development of nicotine dependence is not entirely clear. Although there does not appear to be a single gene that can serve as a predictor of smoking behavior, it is possible that if the candidate genes are examined in combination, in the form of a genetic risk profile, researchers may be

able to gain a clearer picture of the extent to which genetics plays a role in tobacco use.

Even if genetics does play an important role in tobacco use and nicotine addiction, it is not likely that knowledge of genetic risk alone could lead to the development of effective tobacco prevention strategies. Smoking is a complex behavior that also results from a host of environmental and psychological factors. Future research efforts should therefore focus on an integrative approach that incorporates psychological, social, cultural, behavioral, pharmacological, and genetic influences, as well as the interactions among these factors (30).

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