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The D2 Dopamine Receptor Gene and Nicotine Dependence Among Bladder Cancer Patients and Controls

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Abstract

Multiple twin, family, and genetic studies have rendered substantial evidence supporting an association between hereditary factors and smoking initiation and maintenance. To investigate further the relationships between the *DRD2* genotypes, cigarette use and nicotine dependence, we examined the prevalence of polymorphisms in the TaqIA (A1 and A2) and the TaqIB (B1 and B2) alleles among a series of 608 non-Hispanic White bladder cancer patients and 608 matched controls. Among ever-smoking controls, A1 and B1 genotypes exhibited a greater smoking intensity and were significantly younger at the age of initiation than A2A2 or B2B2 genotypes (two-sided $P < 0.05$). Among former smoking cases, persons with the A1 genotypes exhibited significantly higher mean pack-years and years of smoking, and were younger at the age of initiation than were persons with the A2A2 genotype (two-sided $P < 0.05$). Additionally, current smokers with the A1 genotypes reported fewer quit attempts than those with the A2A2 genotype (two-sided $P < 0.01$). The present study suggests that the *DRD2* alleles A1 and B1 confer greater vulnerability to tobacco use.

Keywords

DRD2; Bladder cancer; Nicotine dependence

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Introduction

Multiple twin, family, and genetic studies have rendered substantial evidence supporting an association between hereditary factors and smoking initiation and maintenance (Li et al. 2004; Munafo et al. 2004). For example, Carmelli et al. (1992) reported that the concordance rates for smoking and quitting smoking were higher among monozygotic twins than among dizygotic twins, and a study of smoking behavior in adult adoptees and their biological and adoptee families (Osler et al. 2001) suggested that there is a genetic influence on smoking within the same generation. A recent meta-analysis of 14 twin studies estimated that 46–84% of smoking initiation and smoking persistence is heritable (Li et al. 2003).

Extensive evidence confirms that nicotine is the dependence-producing component of tobacco (Services 1988). Nicotine stimulates the release of several neurotransmitters, which are associated with enhanced pleasure, improved task performance and memory, reduced anxiety and tension, and weight control (Benowitz 1992, 1999). Nicotine has been shown to stimulate dopamine release in the nucleus accumbens, possibly through activation of acetylcholine receptors located in mesolimbic dopaminergic pathways (Pomerleau 1992; Wise 1987, 1998; Wise and Rompre 1989). It has been hypothesized that persons with a functional alteration in the dopamine reward pathway might be more prone to drug addiction, including nicotine dependence (Noble et al. 1994).

Polymorphisms of several dopamine receptor genes have been detected and examined as related to addiction, including nicotine dependence (Noble 2000). The human D2 dopamine receptor gene (*DRD2*) encodes a G-protein coupled dopamine receptor, D2, which inhibits adenylyl cyclase activity. In 1989, Grandy and colleagues reported that the *DRD2* gene is located on chromosome 11q (Grandy et al. 1989). Blum et al. further described a restriction fragment length polymorphism (RFLP), TaqIA, in the 3′ untranslated region of *DRD2*, with two alleles A1 and A2 (Blum et al. 1990). Although originally thought to be within a non-coding region of the *DRD2* gene, it has recently been noted that the TaqIA site is actually within an exon of a new gene, *ANKK1*, approximately 10 kilobase pairs (kb) downstream of *DRD2* (Neville et al. 2004). The *ANKK1* is a nonsynonymous change that replaces a glutamate for a lysine. There is evidence that this gene, with regions of homology to ankryn-repeats and tyrosine kinase active sites, is expressed in the central nervous system (Neville et al. 2004).

The A1 allele is reported to be associated with a decrease in *DRD2* receptor density in the striatum (Jonsson et al. 1999; Noble et al. 1993; 1994; Pohjalainen et al. 1998; Thompson et al. 1997), lower mean relative glucose metabolic rate in dopaminergic regions in the human brain, and low receptor density (Munafo et al. 2009). Persons who have a reduced number of dopamine binding sites in the brain have a deficit in their reward system, thus experiencing an enhanced reward when exposed to dopaminergic agents (Noble et al. 1994; Thompson et al. 1997). It is speculated that these persons need to use larger amounts of nicotine to increase synaptic dopamine, thereby becoming more rapidly tolerant and at a higher risk for nicotine addiction (Noble et al. 1994; Thompson et al. 1997).

Investigators have also explored associations between smoking and the Taq1 B alleles (B1 and B2) located closer to the regulatory and structural coding regions of the gene (Smith et al. 1992; Spitz et al. 1998; Wu et al. 2000). Results from a previous study in our laboratory found a significantly higher prevalence of the B1 allele among current and former smokers compared to nonsmokers among non-Hispanic whites (Spitz et al. 1998) and also showed that smokers carrying the B1 allele (i.e. B1B1 or B1B2) were likely to exhibit more severe nicotine withdrawal symptoms when they quit (Robinson et al. 2007). Persons who exhibited genotypes with at least one A1 (A1A1/A1A2) or B1 (B1B1/B1B2) allele were

more likely to have ever smoked, started smoking at an earlier age, and attempted to quit fewer times compared to persons without the polymorphism (Spitz et al. 1998). These same relationships have been duplicated among Mexican–American smokers (Wu et al. 2000) and other investigators (Comings et al. 1996; Noble et al. 1994) have reported that the A1 and B1 alleles are more prevalent among current and former smokers than among never smokers.

The current study examined the prevalence of the *DRD2* genotypes in TaqIA (A1A1, A1A2 and A2A2) and TaqIB (B1B1, B1B2 and B2B2) alleles among bladder cancer patients and control subjects who were matched on age, sex, and ethnicity. Our main hypothesis was based on that of our earlier study, which determined that individuals who are ever smokers (100 cigarettes or more smoked in their lifetime) will be more likely to exhibit the genotypes predictive of tobacco use (A1A1,A1A2 and B1B1,B1B2) than will never smokers (Wu et al. 2000). Similarly, we hypothesized that individuals who exhibit substantial dependence will be more likely to exhibit the at predictive genotypes than will individuals with lower levels of dependence. Lastly, because smoking is significantly associated with bladder cancer risk, we hypothesized that the associations would differ among bladder cancer cases versus healthy controls.

Measures and Methods

Study Population

Patients with newly diagnosed (within the previous year), untreated (no chemotherapy or radiation) bladder cancer were recruited from the University of Texas M. D. Anderson Cancer Center and Baylor College of Medicine as a part of an ongoing study. There were no eligibility restrictions on age, histology, or stage; however, the tumor must have originated from the bladder and be histologically confirmed once entered into the study. Control subjects with no prior diagnosis of any type of cancer, except nonmelanoma skin cancer, were recruited from Kelsey Seybold, the largest private multi-specialty physician group in Houston (Hudmon et al. 1997). These participants were frequency-matched to the cases based on sex, age (±5 years), and ethnicity to evaluate the main effect of the genotype.

After informed consent was administered, study variables were assessed through 45-min structured, face-to-face interviews. The response rates for cases and controls are 92 and 76.7%, respectively. Given the low proportion of subjects who reported being Hispanic (fewer than 5%), Black (fewer than 5%), or other (fewer than 2%), we limited our analysis to include only non-Hispanic Whites. Our final sample consisted of 608 cases and 608 frequency-matched controls. At the conclusion of the interview, a 40 mL blood sample was drawn for immediate DNA isolation and future molecular analysis. Laboratory personnel were blinded to case and control status. The study protocol was approved by the M. D. Anderson and Kelsey Seybold Institutional Review Boards.

Tobacco-Related Phenotypes

Our measures for assessing tobacco use characterized current and past tobacco use as well as nicotine dependence. Ever smoking was defined as cumulative lifetime smoking of one hundred or more cigarettes. Smoking status was defined as current smokers (smoking within the past month), recent quitters (quit within the past year), and former smokers (ever smokers who had quit at least 1 year prior to the interview). Other phenotypes included age at smoking initiation, number of cigarettes smoked per day, pack-years smoked (average packs per day smoked, multiplied by the number of years smoked), age at cessation (for former smokers), and the amount of nicotine and tar consumed (mg/ cigarette). Smoke-years

were defined as the number of years a participant smoked cigarettes, not including the times that he or she might have quit smoking for a month or more.

Nicotine dependence was assessed for both current and former smokers using the Fagerström Test for Nicotine Dependence (FTND) (Heatherton et al. 1991), a 6-item derivation of the original Fagerström Tolerance Questionnaire (FTQ) (Fagerstrom 1978). The wording of this scale, which was designed and validated for use among current smokers, was modified in this study for retrospective use among former smokers. Thus, former smokers' responses to the items reflect the period of time during which they used to smoke regularly. Retrospective use of the FTQ/FTND has been examined and shown to exhibit acceptable psychometric properties (Hudmon 2005). The items (with wordings for former smokers designated in block parentheses), response options (score) were as follows: (a) How soon after you wake up do you [did you] smoke your first cigarette? ≤5 min (3), 6–30 min (2), half hour to an hour (1), and more than 1 h (0); (b) How difficult is [was] it to keep from smoking in places where it is [was] not allowed? difficult (1), somewhat difficult (1), not usually difficult (0), not at all difficult (0), and could smoke everywhere I went (0); (c) Which cigarette would you hate [have hated] to give up the most? the first cigarette in the morning (1), any other cigarette (0); (d) Do [did] you smoke more frequently during the first 2 h after waking up than during the rest of the day? yes (1), no (0); (e) Do [did] you smoke on days that you are [were] so sick you are [were] in bed? yes, always (1), yes, quite often (1), no, not usually (0), no, never (0), and never sick in bed (0); and (f) How many cigarettes do [did] you smoke per day? 10 or less (0), 11–20 (1), 21–30 (2), and 31 or more (3). For both current and former smokers, a scale score was created as the sum of the constituent items, and subjects were classified as follows: no dependence (score of 2 or lower), low to moderate dependence (scores of 3 to 5), or substantial dependence (scores of 6 or higher).

Finally, current smokers were characterized based on their number of prior quit attempts, desire to quit smoking, and their confidence in their ability to quit using the following items: (a) Have you made a serious attempt to quit in the last 12 months and stayed off cigarettes for 24 h or more? (yes, no, don't know); (b) How much, if at all, do you want to quit smoking? (not at all, a little, somewhat, very much, extremely); (c) How confident are you that you could quit smoking for good if you wanted to? (not at all, a little, somewhat, very much, extremely); and (d) How many times have you tried to quit smoking for at least 24 h?

Molecular Analysis

Total genomic DNA was extracted from each coded blood sample, and aliquots were used for polymerase chain reaction (PCR) analysis. The oligonucleotide primers 5′-CC GTCGACCCTTCCTGAGTGTCATCA-3′ and 5′-CCGTC GACGGCTGGCCAAGTTGTCTA-3′ were used to amplify a 317 base-pair (bp) fragment spanning the polymorphic TaqIA site of the *DRD2* gene. The oligonucleotide primers 5′-GATACCCACTTCAGGAAGTC-3′ and 5′-GATGTGT AGGAATTAGCCAGG-3′ were used to amplify a 459-bp fragment spanning the polymorphic TaqIB site of the *DRD2* gene. PCR was performed in 25-μL reaction mixtures containing 1.5 mM $MgCl_2$, 0.2 mM 2′′-deoxynucleotide 5′-trphosphates (dNTPs), 0.5 μM primers, 100 ng of template DNA, 1.5 U of Taq polymerase (Promega, Madison, WI), and 1 × PCR buffer. After and initial denaturation at 94°C for 4 min, the DNA was amplified with 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. Part of the PCR product (15 μL) was digested with 5 U of Taq I for overnight at 65°C for the TaqIA polymorphism and 5 U of Taq I for overnight at 65 °C for the *DRD2* TaqIB polymorphism. Digestion products (15 μL) were then resolved on a 3% agarose gel containing ethidium bromide. There were three *DRD2* TaqIA genotypes: (1) the predominant homozygote A2A2, which exhibits two restriction digestion fragments of 180, 129, and 4 bp; (2) the

heterozygote A1A2, which exhibits three restriction fragments of 309, 180, 129, and 4 bp; (3) the rare homozygote A1A1 which produces 309 and 4 bp fragments. There were also three *DRD2* TaqIB genotypes: (1) the predominant homozygote B2B2, which exhibits two restriction fragments of 169 and 190 bp; (2) the heterozygote B1B2, which exhibits three restriction fragments of 459, 269, and 190 bp; (3) the rare homozygote B1B1, which produces only the uncleaved 459-bp fragment.

Statistical Analysis

Standard summary statistics were used to characterize the study population. Bivariate relationships were examined using Chi-squared tests of independence, Fisher's Exact tests, or *t*-tests, as appropriate. When variables did not approximate normality (as indicated by the Shapiro–Wilk statistic), the Wilcoxon Rank Sum test was used. Except where otherwise indicated, because parametric statistics and nonparametric statistics yielded the same conclusions, the parametric results are presented.

Parallel to our prior study (Spitz et al. 1998), few subjects exhibited the homozygous variant genotypes (A1A1 and B1B1), and data from these participants were combined with data from the heterozygous participants (Table 1). As suggested by Weir (1990), linkage disequilibrium was evaluated to detect whether a statistically significant association existed between the two genotypes.

Analyses were performed using SAS® software, Version 9.1. Linkage disequilibrium analysis was performed using HelixTree® software, Version 4.0.3. For all statistical tests, a two-sided *P*-value of less than 0.05 was considered to be statistically significant.

Results

Study Population

Characteristics of the study population, which included 608 bladder cancer cases and 608 matched controls, are shown by case–control status in Table 1. As summarized in Table 1, the case patients were matched to the controls subjects by age and sex; however, the cases were significantly more likely to have fewer than 12 years of education ($P < 0.0001$). Just over one quarter of cases versus 7.9% of controls were current smokers, 47.7% of cases and 47.4% of controls were former smokers, and 26.2% of cases and 44.7% of controls were never smokers ($P < 0.0001$). Mean pack-years, the number of cigarettes smoked per day, and the number of years smoked were significantly higher among cases than among controls, regardless of whether the participant was a current or former smoker ($P < 0.05$). There were no overall case–control differences in the distribution of the A1 and B1 genotypes.

Current smokers were significantly more likely to be 65 years or under, whereas never and former smokers were more likely to be over the age of 65 years, regardless of case–control status ($P < 0.001$; Table 2). Furthermore, current and former smoking cases and controls were more likely to be male than were their never smoker counterparts ($P < 0.001$), have a significantly higher percentage of participants with fewer than 12 years and 13–16 years of education, and a significantly lower percentage of participants with 17 years or more of education ($P < 0.001$). Significant differences between smoking status, and nicotine and tar content were observed (Table 2). Both former smoking cases and controls consumed a higher content (mg/cigarette) of nicotine than current smoking cases and controls. Likewise, both former smoking cases and controls consumed a higher content (mg/cigarette) of tar than current smoking cases and controls. A significant correlation was observed between nicotine content and tar content ($P < 0.001$). Never smokers and ever smokers did not significantly differ by *DRD2* genotype when stratified by case–control status (Table 2). However, a borderline significant association was observed among control subjects, with B1

genotypes more likely than B2B2 genotypes to be ever smokers versus never smokers (P = 0.10). There was a statistically significant correlation between the presence of the A1 and B1 alleles (r = 0.79 for the cases and r = 0.78 for the controls). Twelve cases (2.2%) and twelve controls (2.2%) were homozygous for both rare genotypes (data not shown).

Linkage disequilibrium was observed among the total sample population (LD correlation, r: 0.8047, D': 0.8996, *P-value*: $2.331e^{-157}$), and among cases (LD correlation, r: 0.8120, D': 0.9189, $P = 2.010e^{-80}$) and controls (LD correlation, r: 0.7978, D': 0.8812, $P = 6.034e^{-79}$) independently. All were statistically significant ($P < 0.01$); thus, the prevalence of a specific A allele predicted the presence of a particular B allele (data not shown).

Tobacco-Related Phenotypes

We observed significant associations between the *DRD2* genotypes and various smoking-related variables (Table 3). Among ever smoking controls, persons with the A1 and B1 genotypes were significantly heavier smokers than were their counterparts. Specifically, persons with the A1 genotypes had a significantly higher mean pack-years smoked (33.99 ± 29.24 vs. 25.03 ± 26.44; P = 0.006), mean years of smoking (25.88 ± 14.17 vs. 21.50 ± 13.82; P = 0.009), and mean number of cigarettes per day (24.51 ± 15.16 vs. 20.64 ± 15.35; P = 0.033), and were significantly younger at the age they started smoking (16.88 ± 3.60 vs. 18.18 ± 4.64; P = 0.007). Likewise among controls, persons with the B1 genotypes had a significantly higher mean pack-years (33.66 ± 30.24 vs. 25.61 ± 26.11; P = 0.017) and mean number of cigarettes smoked per day (24.81 ± 15.45 vs. 20.66 ± 15.18; P = 0.025).

There were no statistically significant associations between genotype and smoking-related variables among cases, although the A1 genotypes exhibited a modest association with mean pack-years (P = 0.142) and mean number of years smoking (P = 0.086; Table 3). Among former smokers, persons with the A1 genotypes exhibited significantly higher mean pack-years and years of smoking and were younger at the age they started smoking than were persons with the A2A2 genotype ($P < 0.05$). No significant associations were found among current smokers or the B1 and B2 genotypes (data not shown).

An overall FTND index score was computed for former and current smokers. In general, there were no differences in the estimated level of dependence (none, moderate, or substantial) by smoking status (data not shown). Sixty-nine (22%) of 321 former smokers and 28 (20%) of 140 current smokers were classified as having substantial addiction (FTND scores of 6 or greater within a range of 0–10). The overall mean index was 3.3 ± 2.4 for former smokers and 4.5 ± 2.3 for current smokers ($P < 0.001$). When the indices were analyzed by case–control status, case subjects who were former smokers had a significantly higher mean score (3.6 ± 2.4) than did their control counterparts (2.9 ± 2.4; P = 0.001). A similar association was seen among current smokers (cases, 4.8 ± 2.3; controls, 3.7 ± 2.2; $P < 0.05$). When controls were analyzed by genotype, current and former smokers with the A1 genotypes had significantly higher mean scores (3.4 ± 2.3) than those with the A2A2 genotype (2.8 ± 2.5, P = 0.01). Although not statistically significant, a similar trend was observed among controls for the B1/B2B2 genotypes (P = 0.085), and among cases for the A1/A2A2 (P = 0.333) and B1/B2B2 genotypes (P = 0.072) (data not shown).

There were no significant differences in genotype for intention to quit and quitting confidence (Table 4). Among currently smoking controls, we observed a statistically significant difference in the mean number of quit attempts between the A1 genotypes and the A2A2 genotype (4.15 ± 5.08 vs. 10.54 ± 11.37; P = 0.019). A similar, modest association was observed among the mean number of quit attempts between the B1 and B2B2 genotypes (P = 0.056). Contrary to our hypothesis, there were no statistically significant associations among currently smoking cases.

Discussion

There exists substantial evidence, through multiple twin, family, and molecular studies, suggesting a genetic influence on smoking initiation, maintenance, and cessation behavior. The current study among bladder cancer patients and controls provides some additional support regarding the hypothesized link between the D2 dopamine receptor gene and selected smoking behaviors among non-Hispanic Whites, particularly among the controls. However, the association appears to be limited to control vs. cancer patient populations. In particular, we observed that among controls the A1 and B1 genotypes exhibited a greater smoking intensity (pack-years, mean years of smoking, and cigarettes smoked per day) and were significantly younger at the age at initiation. Additionally we observed that among the total population and among controls, current smokers with the A1 genotypes reported fewer quit attempts than did current smokers with the A2A2 genotype.

A recent meta-analysis found significant associations between the *DRD2* A1 and smoking behavior among 13 studies investigating smoking initiation (OR = 0.75, 95% CI 0.65–0.85), adopting and persisting with smoking (OR = 0.73, 95% CI 0.63–0.84), persistence only (OR = 0.80, 95% CI 0.72–0.90), and cigarette consumption (OR = 2.53) (Munafo et al. 2004). No significant effects were noted across 12 studies of smoking prevalence (never/former/current) and effects listed above were no longer significant when a random effects model was applied to the data. Two of the larger studies reporting negative data on smoking prevalence and the *DRD2 Taq*IA polymorphism used atypical control groups. The study by Batra et al. (2003) used “nonsmokers and light smokers,” whereas Bierut et al. (2000) used “nonhabitual” smokers. In another meta-analysis, which excluded these two studies (Li et al. 2004), a significantly higher prevalence of the A1 allele was found in 2,182 smokers versus 2,117 non-smokers (OR = 1.47; 95% CI 1.31–1.66). A recent study by Audrain-McGovern et al. (2004a), not included in these meta-analyses, noted that among 9th grade adolescents exposed to smoking, the likelihood of progressing to more regular smoking by the 11th grade increased almost twofold with each additional A1 allele. This effect was more pronounced among those with substantial depressive symptoms. Our data support this association between the uncommon alleles and smoking prevalence, observing among controls that the rare A1 and B1 genotypes of the *DRD2* receptor gene were more common in ever smoking subjects than in never smoking subjects (A1: 37.2 vs. 33.3%, B1: 34.2 vs. 27.8%). Furthermore, the rare genotypes were more common in current smoking controls than former smoking controls (A1: 45.5 vs. 35.8%, B1: 43.2 vs. 32.7%). Similar to other studies, a trend was not observed among the case subjects in our study (Spitz et al. 1998).

In a prior study, we observed that smokers with the variant alleles tended to report smoking fewer cigarettes per day (Spitz et al. 1998). In contrast, our present data concur with Comings et al. (1996) who reported a trend for lower prevalence of the variant alleles in lighter smokers than in heavier smokers. Among the total sample population and among controls, our data indicate significantly greater smoking intensity among the A1 and B1 genotypes than among the A2A2 and B2B2 genotypes, with higher mean pack-years, mean years of smoking, and mean number of cigarettes smoked per day. The opposing finding could be attributed to our earlier sample being comprised of mostly heavier smokers and having less variability in smoking consumption.

Our data are consistent with previous observations of the inverse relationship between the A1 and B1 genotypes and the age at onset of smoking (Comings et al. 1996; Spitz et al. 1998; Wu et al. 2000). Among control subjects, persons with the A1 allele were significantly younger at smoking initiation. This may indicate that, for such individuals, experimentation with smoking at an earlier age is more likely to lead to continued use. Whether or not this is due to a preexisting genetically mediated deficit in the regulation of the smokers that is

offset by nicotine self-administration can not be determined from this data. However, previous studies have indicated that the presence of both the A1 allele and depressive symptoms among adolescents may increase the likelihood of becoming a regular smoker (Audrain-McGovern, et al. 2004b; Lerman et al. 1999).

Current and former smokers with the variants alleles had higher nicotine dependence scores than their A2A2 and B2B2 genotype counterparts; however, as observed in our previous study (Benowitz 1992, 1999), not all differences were statistically significant. However, it is becoming increasingly clear that nicotine dependence represents a multifactorial construct of which the FTND may provide only a limited measure. The lack of association may be related to previous associations being spurious, but may also have to do with “noise” in the measurement and the failure to capture other aspects of this construct accounting for the variance due to genotype.

Individuals who are more vulnerable to addiction have been shown to be less likely to quit smoking (Benowitz 1992, 1999). In our study, among the total sample population, those with the A1 and B1 genotypes had a significantly lower mean number of former quit attempts, and a marginally statistically significant finding for attempting to quit less often. However, there were no differences for intentions to quit and quit confidence. This finding further supports the observation in our previous studies (Benowitz 1992, 1999) that individuals with the uncommon alleles are less willing or able to give up their smoking habit than those who have only the major alleles, and further suggests an enhanced addiction among the uncommon alleles.

We observed the expected relationships between case– control status and smoking intensity (pack-years, cigarettes smoked/day, years smoked), with cases exhibiting a greater intensity than controls; however contrary to our expectations, we did not observe that bladder cancer patients who smoke were more likely to exhibit the genotypes predictive of tobacco use (A1 and B1) than the control subjects. Bladder cancer is a multifactorial disease with many competing risk factors. It may be that factors other than the *DRD2* gene are stronger predictors of bladder cancer risk. Furthermore, one polymorphism or one gene may only have a modest effect on cancer risk (Cardenas et al. 2002). The current study, along with previous findings, emphasizes the need for future comprehensive polygenetic studies that include the *DRD2* gene. Among associations regarding nicotine-related phenotypes, significant findings were lacking among cases, which may be due to reduced variability in smoking behavior among the cases relative to the controls (i.e. a selection bias for less educated, heavier and more dependent smokers among the cases).

This association study has limitations, including the potential for recall bias that should be considered when interpreting our results. The smoking-related phenotypes were assessed retrospectively in study participants and were self-reported without subsequent verification. Secondly, although this study consisted of an adequate sample size, it was somewhat limited in its narrow range of smoking-related phenotypes. Therefore, an emphasis on a more comprehensive approach to the genetic determinants of smoking, including improved measures of dependence, the rate of convergence on regular smoking, biological measures of nicotine pharmacokinetics, receptor activity, and multiple comparisons is vital in future research (Swan et al. 2003).

Although extensive evidence strongly indicates the dopamine reward system’s involvement in the development of nicotine dependence and patterns of tobacco use, the extent at which specific variant alleles contribute is inconsistent. The present study, along with previous research, suggests that the *DRD2* alleles A1 and B1 may confer greater vulnerability to tobacco use in controls. These markers could be used to identify especially vulnerable

individuals for whom it might be possible to design targeted pharmacologic interventions that act on the receptors to decrease withdrawal symptoms, and therefore help people abstain from smoking and prevent relapse. This patient-specific approach to therapy and drug dosage may prove to be more effective and have fewer side effects than conventional therapy.

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Table 1

Distribution of select variables by case–control status

Variable	Case patients (n = 608)[b]	Control subjects (n = 608)[b]	P-value[c]
Sex, n (%)[a]			1.000
Male	479 (78.78)	479 (78.78)	
Female	129 (21.22)	129 (21.22)	
Age, mean ± SD	63.84 ± 10.92	62.98 ± 10.65	0.165
Education, n (%)[a]			<0.001
12 or less	189 (31.09)	118 (19.41)	
13–16	290 (47.70)	331 (54.44)	
17 or more	129 (21.22)	159 (26.15)	
Smoking status, n (%)[a]			<0.001
Never smoker	159 (26.15)	272 (44.74)	
Former smoker	290 (47.70)	288 (47.37)	
Current smoker	159 (26.15)	48 (7.89)	
Pack-years, mean ± SD	43.53 ± 31.50	28.74 ± 27.58	<0.001
Former	38.02 ± 30.87	27.33 ± 27.70	<0.001
Current	53.62 ± 30.23	37.19 ± 25.53	<0.001
Cigarettes/day, mean ± SD	25.77 ± 14.09	21.94 ± 14.95	<0.001
Former	25.65 ± 15.23	22.09 ± 15.48	0.006
Current	25.99 ± 11.76	21.04 ± 11.34	0.011
Years smoking cigarettes, mean ± SD	31.92 ± 14.06	23.69 ± 14.19	<0.001
Former	27.55 ± 13.59	21.88 ± 13.38	<0.001
Current	39.90 ± 11.14	34.56 ± 14.14	0.019
Age started smoking, mean ± SD	17.67 ± 4.17	17.68 ± 4.30	0.992
Former	17.74 ± 3.91	17.69 ± 4.37	0.868
Current	17.54 ± 4.61	17.60 ± 3.85	0.931
Duration of smoking cessation, mean ± SD			
Former	20.47 ± 12.60	24.75 ± 13.06	<0.001
DRD2 genotype, n(%)[a]			
A2A2	357 (65.03)	366 (64.44)	0.963
A1A2	169 (30.78)	179 (31.51)	
A1A1	23 (4.19)	23 (4.05)	
A1A1 + A1A2	192 (34.97)	202 (35.56)	0.836
B2B2	379 (70.06)	382 (68.58)	0.869
B1B2	150 (27.73)	162 (29.08)	
B1B1	12 (2.22)	13 (2.33)	
B1B1 + B1B2	162 (29.94)	175 (31.42)	0.597

[a] Percentages might not sum to 100% because of rounding

[b] Values might not sum to 608 because of missing data

[c] P-value for student's t-test (continuous variables) or χ^2 test (categorical variables)

Table 2

Distribution of selected variables by smoking status (never, former and current smokers) in cases and controls, respectively

Variable	Case patients[c]				Control subjects[c]			
	Never *n* (%)[a]	Former *n* (%)[a]	Current *n* (%)[a]	*P*-value[b]	Never *n* (%)[a]	Former *n* (%)[a]	Current *n* (%)[a]	*P*-value[b]
Age (years)								<0.001
≤65	79 (49.69)	120 (41.38)	113 (71.07)	<0.001	157 (57.72)	141 (48.96)	40 (83.33)	
>65	80 (50.31)	170 (58.62)	46 (25.93)		115 (42.28)	147 (51.04)	8 (16.67)	
Sex				<0.001				<0.001
Male	103 (64.78)	248 (85.52)	128 (80.50)		192 (70.59)	247 (85.76)	40 (83.33)	
Female	56 (35.22)	42 (14.48)	31 (19.50)		80 (29.41)	41 (14.24)	8 (16.67)	
Education				<0.001				0.002
12 or less	38 (23.90)	91 (31.38)	60 (37.74)		50 (18.38)	53 (18.40)	15 (31.25)	
13–16	72 (45.28)	135 (46.55)	83 (52.20)		132 (48.53)	175 (60.76)	24 (50.00)	
17 or more	49 (30.82)	64 (22.09)	16 (10.06)		90 (33.09)	60 (20.83)	9 (18.75)	
Nicotine (mg)				0.005				<0.001
0.9 or less	–	59 (25.00)	46 (34.33)		–	51 (21.70)	21 (47.73)	
1.0–1.2	–	114 (48.31)	71 (52.99)		–	113 (48.09)	20 (45.45)	
1.3 or more	–	63 (26.69)	17 (12.69)		–	71 (30.21)	3 (6.82)	
Tar (mg)				0.018				<0.001
9 or less	–	13 (5.51)	19 (14.07)		–	8 (3.40)	7 (15.91)	
10–14	–	58 (24.58)	32 (23.70)		–	53 (22.55)	14 (31.82)	
15 or more	–	165 (69.92)	84 (62.22)		–	174 (74.04)	23 (52.27)	
DRD2 genotype								
A1A1 + A1A2	52 (36.62)	92 (35.52)	52 (35.62)	0.974	84 (33.33)	93 (35.77)	20 (45.45)	0.297
A2A2	90 (63.38)	167 (64.48)	94 (64.38)		168 (66.67)	167 (64.23)	34 (54.55)	
B1B1 + B1B2	46 (32.39)	77 (29.73)	43 (29.45)	0.826	70 (27.78)	85 (32.69)	19 (43.18)	0.101
B2B2	96 (67.61)	182 (70.27)	103 (70.55)		182 (72.22)	175 (67.31)	25 (56.82)	

[a] Percentages may not sum to 100% due to rounding

[b] *P*-value is for χ^2 test

[c] Due to missing data, there are 547 cases and 556 controls

Table 3

Distribution of selected smoking variables by genotype in ever smoking cases and ever smoking controls, respectively

Variable	A2A2	A1A1 + A1A2	*P*-value[a]	B2B2	B1B1 + B1B2	*P*-value[a]
Pack years, mean ± SD						
Case patients	41.87 ± 30.00	46.83 ± 2.50	0.142	42.48 ± 29.64	46.01 ± 33.88	0.296
Control subjects	25.03 ± 26.44	33.99 ± 29.24	0.006	25.61 ± 26.11	33.66 ± 30.24	0.017
Years smoking cigarettes, mean ± SD						
Case patients	31.29 ± 13.74	33.79 ± 14.61	0.086	31.93 ± 13.99	32.78 ± 14.36	0.582
Control subjects	21.50 ± 13.82	25.88 ± 14.17	0.009	22.16 ± 13.81	24.99 ± 14.49	0.097
Cigarettes/day, mean ± SD						
Case patients	25.50 ± 13.85	26.28 ± 13.69	0.586	25.43 ± 13.79	26.59 ± 13.78	0.440[b]
Control subjects	20.64 ± 15.35	24.51 ± 15.16	0.033	20.66 ± 15.18	24.81 ± 15.45	0.025
Age started smoking, mean ± SD						
Case patients	17.59 ± 4.05	17.61 ± 4.11	0.968	17.66 ± 4.32	17.47 ± 3.41	0.639[b]
Control subjects	18.18 ± 4.64	16.88 ± 3.60	0.007[2]	17.94 ± 4.55	17.22 ± 3.81	0.169
Duration of smoking cessation, mean ± SD						
Case patients	20.32 ± 12.90	19.61 ± 13.27	0.675	19.83 ± 12.88	20.61 ± 13.38	0.661
Control subjects	26.04 ± 12.96	24.24 ± 12.94	0.283	25.86 ± 12.75	24.44 ± 13.40	0.406

[a] *P*-value is for Student's *t*-test

[b] Student's *t*-test with unequal variances was used

Table 4

Quitting characteristics among currently smoking cases and controls, respectively

	A2A2	A1A1 + A1A2	*P*-value[a]	B2B2	B1B1 + B1B2	*P*-value[a]
Cases						
Attempted to quit, *n* (%)			0.230			0.175
Yes	46 (56.79)	23 (46.00)		51 (56.67)	18 (43.90)	
No	35 (43.21)	27 (54.00)		39 (43.33)	23 (56.10)	
Intention to quit, *n* (%)			0.467			0.431
No intention	6 (7.69)	6 (12.50)		8 (9.20)	4 (10.26)	
Moderate intention	17 (21.79)	13 (27.08)		18 (20.69)	12 (30.77)	
Extreme intention	55 (70.51)	29 (60.42)		61 (70.11)	23 (58.97)	
Quit confidence, *n* (%)			0.316			0.431
No intention	5 (6.41)	7 (14.58)		7 (8.05)	5 (12.82)	
Moderate intention	23 (29.49)	13 (27.08)		23 (26.44)	13 (33.33)	
Extreme intention	50 (64.10)	28 (58.33)		57 (65.52)	21 (53.85)	
Quit Attempts, mean ± SD	8.06 ± 9.55	7.24 ± 14.50	0.715	8.29 ± 12.44	6.50 ± 8.83	0.331
Controls						
Attempted to quit, *n* (%)			0.726			1.000
Yes	7 (30.43)	4 (22.22)		6 (25.00)	5 (29.41)	
No	16 (69.57)	14 (77.78)		18 (75.00)	12 (70.59)	
Intention to quit, *n* (%)			0.713			0.563
No intention	1 (4.35)	2 (11.11)		1 (4.17)	2 (11.76)	
Moderate intention	10 (43.48)	8 (44.44)		10 (41.67)	8 (47.06)	
Extreme intention	12 (52.17)	8 (44.44)		13 (54.17)	7 (41.18)	
Quit confidence, *n* (%)			0.532			0.343
No intention	2 (8.70)	2 (11.11)		2 (8.33)	2 (11.76)	
Moderate intention	8 (34.78)	9 (50.00)		8 (33.33)	9 (52.94)	
Extreme intention	13 (56.52)	7 (38.89)		14 (58.33)	6 (35.29)	
Quit Attempts, mean ± SD	10.54 ± 11.37	4.15 ± 5.08	0.019	9.84 ± 11.40	4.74 ± 5.29	0.056

[a] *P*-value for student's *t*-test (continuous variables) or χ^2 test (categorical variables)