NIH Public Access
Author Manuscript
Am J Med Genet B Neuropsychiatr Genet. Author manuscript; available in PMC 2010 March 5.

Published in final edited form as:
Am J Med Genet B Neuropsychiatr Genet. 2009 March 5; 150B(2): 254–261. doi:10.1002/ajmg.b.30801.

The impact of genetic variation in DRD2 and SLC6A3 on smoking cessation in cohort of participants one year after enrollment in a lung cancer screening study

Mindi A. Styn, PhD,
University of Pittsburgh School of Nursing, Department of Health and Community Systems

Tomoko Nukui, PhD,
University of Pittsburgh School of Medicine, Department of Medicine

Marjorie Romkes, PhD,
University of Pittsburgh School of Medicine, Department of Medicine

Kenneth Perkins, PhD,
University of Pittsburgh School of Medicine, Department of Psychiatry

Stephanie R. Land, PhD, and
University of Pittsburgh Graduate School of Public Health, Department of Biostatistics

Joel L. Weissfeld, MD, MPH
University of Pittsburgh Graduate School of Public Health, Department of Epidemiology

Abstract

Smoking cessation strategies continue to have disappointing results. By determining the interindividual genetic differences that influence smoking behaviors, we may be able to develop tailored strategies that increase the likelihood of successful cessation. This study attempts to determine genetic influences on the relationship between the dopamine pathway and smoking cessation by examining associations with a variable number tandem repeat variation in *SLC6A3* and the *DRD2* variants *TaqIA (A2* vs. *A1), TaqIB (B2* vs. *B1), C957T (C* vs. *T)*, and *-141C Ins/Del (C* vs. *Del)*. Baseline smokers in the Pittsburgh Lung Screening Study who provided information on smoking status one year later were evaluated. We frequency-matched those who were not abstinent at one year to those who were abstinent at one year by gender, decade of age, and time of enrollment (three month intervals) in a three to one ratio (N=881). Logistic regression was used to identify the effect of genotype on abstinence at one year. In a model containing the matching variables and other genotypes, *DRD2 TaqIA* was significantly associated with being abstinent at one year (p=0.01). Compared to participants who were homozygous *TaqIA* major allele (*A2A2*), participants who carried at least one minor allele (*A1*) were less likely to quit (Odds Ratio: 0.47, 95% CI: 0.24–0.94). The other dopamine receptor genotypes and the *SLC6A3* genotype were not associated with smoking status at one-year. The association between *DRD2 TaqIA* and smoking cessation supports the hypothesis that genetic variation in the dopamine pathway influences smoking cessation.

Keywords

tobacco use cessation; genotype; case-control study; dopamine

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

INTRODUCTION

Mortality rates for lung cancer, the leading cause of cancer death, are increased more than 10-fold for current smokers[American Cancer Society, 2003]. Although the prevalence of cigarette smoking has decreased over the past few decades in reaction to public health campaigns, many smokers are still unable to quit. According to the National Health Interview Survey, 70% of smokers indicate that they want to quit; however, only 4.7% could abstain from smoking for at least three months [CDC, 2002]. The vast majority (over 90%) of individuals who quit successfully do so without any assistance [American Cancer Society, 2003]. The variability in quitting success in the population may be due to interindividual differences in the strength of addiction. These interindividual differences may be partially influenced by genetics. Therefore, identification of and consideration of genetic influences on smoking behavior in the success of quitting regimens may improve cessation rates.

The results of numerous twin studies conducted since the mid-twentieth century in various populations support a hypothesis that genotype is a major factor in all phases of smoking behavior, from initiation to dependence and persistence [Arinami et al., 2000; Rossing, 1998]. The reported heritability estimates are typically over 50% (range of 28 to 84%) [Arinami et al., 2000; Kendler et al., 1999; Rossing, 1998; True et al., 1999]. Although twin studies have been valuable in establishing a genetic link to smoking behavior, they are unable to provide insight into which specific genes influence behavior.

The dopamine pathway is a major reward pathway implicated in addiction to nicotine and other drugs of abuse. The effectiveness and efficacy of bupropion, an anti-depressant that acts on the dopamine pathway [Swan et al., 2005] supports the hypothesis that genetic variation along the dopamine pathway may cause differences in smoking behaviors including cessation. Even stronger evidence for a role of the dopamine pathway in smoking addiction is provided by positron emission tomography imaging studies, which demonstrate that nicotine induces dopamine release [Brody et al., 2006; Brody et al., 2004]. It has been hypothesized that differences in the density of receptors may explain the interindividual differences in response to the dopamine release that occurs in smokers. However, other factors, such as the amount of dopamine available, may also explain these differences.

The dopamine receptor 2 gene (*DRD2*) has been the subject of much addiction research. Several functional *DRD2* variants have been identified. Many studies have reported a link between *DRD2* genetic variation and variation in smoking behaviors [Comings et al., 1996; Noble et al., 1994; Robinson et al., 2007; Spitz et al., 1998; Wu et al., 2000] including smoking cessation [Lerman et al., 2006]. However, most of these have focused on a single allele and in most studies this allele is *DRD2 TaqIA*. The current study examines *DRD2 TaqIA, DRD2 TaqIB, C957T* and *-141Ins/Del*.

Dopamine transporter's (DAT) role in removing dopamine from the synapse has led to research on the *SLC6A3* gene (also called *DAT1*) and various dopamine-related disorders. Variations in a 40-base pair repeat in the 3' untranslated region have been linked to variations in the density of transporter molecules on the surface of neurons, in dopamine transporter binding, and in protein availability {Heinz, 2000 #382}{Jacobsen, 2000 #211}{van Dyck, 2005 #357}. The 10-repeat allele (major allele) and the 9-repeat allele are most frequently observed. Laboratory studies suggest that variations in the number of repeats influence variations in gene transcription and expression [Fuke et al., 2001; Jacobsen et al., 2000; Michelhaugh et al., 2001; van Dyck et al., 2005]. Studies investigating a link between this gene and smoking behavior have reported mixed results [Jorm et al., 2000; Lerman et al., 1999; Lerman et al., 2003; Sabol et al., 1999; Vandenbergh et al., 2002].

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

The purpose of this study was to determine the relationship between genetic variation on the dopamine pathway and smoking cessation. We conducted a frequency-matched case-control study of participants who were baseline smokers at the time of enrollment in a lung screening study, comparing those who were not smoking at the one-year follow-up to those who were smoking at the one-year follow-up. We evaluated whether *DRD2* and *SLC6A3* genotypes differed between the two groups of participants.

MATERIALS AND METHODS

Population

The current study focused on participants in the Pittsburgh Lung Screening Study (PLuSS), a research-based low-dose computed tomography (CT) screening program conducted as part of the University of Pittsburgh Lung Cancer SPORE program. PLuSS participants, recruited between January, 2002 and April, 2005, were current and former cigarette smokers between the ages of 50 and 79 years who smoked at least one-half pack per day for at least 25 years. At baseline, participants completed a questionnaire and assessment that included a CT scan and pulmonary function test. At approximately one year after their baseline CT scan, participants completed the annual study update that included a repeat CT scan and a brief telephone questionnaire to determine if any changes in vital status, cancer status, or smoking status had occurred. This research protocol was reviewed and approved by the University of Pittsburgh Institutional Review Board. All participants provided written informed consent prior to enrollment in the study.

Expired air carbon monoxide measures were used to validate self-reported smoking abstinence in a subset of 228 PLuSS participants who returned for a follow-up visit between August, 2005 and January, 2006. Participants were asked their smoking status prior to taking the test, and the readings were compared the self-reported smoking status. Using the recommended cutoff of 8 parts per million or higher as a positive test for smoking,[SRNT Subcommittee on Biochemical Verification, 2002] we found that 13 (12%) of the 108 participants who reported not smoking tested positive for smoking.

Genotyping analyses were conducted in a subset of PLuSS participants who were smoking at baseline and had provided information regarding their smoking status during the annual study update telephone interview at the time of subset selection. Participants who had lung cancer diagnosed in the one-year follow-up interval were excluded. Because the PLuSS population was over 90% Caucasian, the genotyping subset was limited to Caucasians.

Participants were classified as Abstinent versus Not Abstinent at one year based on their response to the annual study update question, “Are you currently smoking cigarettes?” The Abstinent at one year group was further restricted to those participants who remained abstinent for more than 30 days prior to the follow-up telephone questionnaire. For this case-control analysis, participants who were in the Not Abstinent group served as the control group and the Abstinent group served as the case group. Subset selection occurred at two time points based on the status of the PLuSS database: October, 2004 and July, 2005. Most (90%) of the participants who were smoking at baseline were also smoking at one year; thus, all individuals in the Abstinent group were included in the subset. Those in the Not Abstinent group were frequency-matched to those in the Abstinent group by gender, decade of age, and date of enrollment (in three month intervals). To increase statistical power, three controls were matched to each case. Within each stratum (cross of gender, decade of age, and date of enrollment), three individuals who were not abstinent at one year were selected for each individual in the Abstinent group. In cases where a stratum did not contain enough Not Abstinent participants, Not Abstinent participants were selected from an adjacent date of enrollment or decade of age stratum. Of the 900 participants who were selected (225 Abstinent

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

at one year, 675 Not Abstinent at one year), 881 had blood specimens available and were successfully genotyped for *DRD2 TaqIA, DRD2 TaqIB*, and *SLC6A3* (219 Abstinent at One Year, 662 smokers). *DRD2 C957T* results could not be obtained for 13 additional participants, and *DRD2 -141C Ins/Del* results could not be obtained for one additional participant. In the final data set, no significant case-control group differences in gender, decade of age, or date of enrollment between individuals who were abstinent at one year and smokers was observed. Moreover, the 881 participants who were genotyped and the 1394 participants who were smoking at baseline but not genotyped were similar in gender composition, age at baseline, educational status, and age at smoking initiation.

Genotyping Procedures

The *DRD2* (*TaqIA:* rs1800497, *TaqIB:* rs1079597, *C957T:* rs6277, *-141C Ins/Del:* rs1799732) polymorphisms were genotyped by TaqMan allele discrimination assays using the Applied Biosystems 7700 system (Applied Biosystems, Foster City, CA). These assays were designed using the Applied Biosystems Primer express software, version 1.5. All reactions were performed using 1X Universal Master mix, 200nM VIC or FAM labeled probes and 20ng of genomic DNA. For *DRD2 TaqIA* and *TaqIB* site screening, 600 nM PCR primer concentrations were used. For the *C957T* and *-141C Ins/Del* polymorphisms, 900 nM PCR primer concentrations were used. Thermal cycling was initiated with a pre-PCR step, 2 min incubation at 50° C, followed by 10 min at 95° C, and then by 49 cycles of 15 sec at 95° C and 1 min at 67° C (*TaqIA*), 59° C (*TaqIB*), 64° C (*C957T*) or 55° C (*-141C Ins/Del*). Positive and negative PCR controls were included with each amplification reaction.

The *SLC6A3* 40 bp VNTR in the 3'-untranslated region was determined by a PCR-based assay adapted from Kang, et al with minor modification [Kang et al., 1999]. Briefly, each 25 μl reaction contained 1X PCR Optimized Buffer B (InVitrogen, Carlsbad, CA), 5% DMSO, 200 μM of dNTP, 900 μM of primers, 1.2 units of AmpliTaq Gold (Applied Biosystems, Foster City, CA) and 20ng of genomic DNA. Thermal cycling was initiated with 94° C activation for 10 minutes, followed by 40 cycles of 1 minute at 94° C and 3 minutes at 72° C ending with a 72° C extension for 10 min. The PCR products were electrophoresed on 8% polyacrylamide gels using the 50bp gel marker (Fisher Science) to determine PCR product length. Each gel also included two known heterozygote genotype samples as positive controls (8/9 repeats and 9/10 repeats) and one negative PCR control sample.

All genotyping assays were repeated in 10% of samples to verify the reproducibility of the assay. All results were interpreted independently by two laboratory personnel who were blinded to the case-control status of participants. In the event of a discrepancy, the genotyping assay was repeated until concordance was reached.

Data Analysis

Frequencies and Hardy-Weinberg equilibrium were assessed for each variant. The genetic variables were examined by genotype and by presence or absence of the minor allele. The relationships between genetic variables and one-year smoking abstinence were initially assessed using univariate analysis techniques (chi-squared tests, t-tests, and Fisher's exact tests as appropriate). Crude odds ratios were calculated to evaluate the contribution of the genetic variables to smoking cessation. We used logistic regression to calculate adjusted odds ratios for each genetic variable while controlling for matching variables (gender, age, date of enrollment). Logistic regression was used to evaluate a full model that included all of the genetic variables.

Exploratory analysis of *DRD2* haplotypes was conducted by first constructing haplotypes using the PHASE program (Version 2.1). The case-control permutation test in PHASE was used to

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

determine if significant differences existed in the haplotype frequencies between the Abstinent at one year group and the continuing smokers. Chi-square tests were then used to evaluate the association of each of the five most frequent haplotypes with quitting. The odds of quitting given the presence of each of the five most frequent haplotypes were also calculated.

RESULTS

The allele frequencies for each gene are displayed in table I and are consistent with frequencies reported in the literature for Caucasians. Aside from the most common 9- and 10-repeat *SLC6A3* alleles other *SLC6A3* alleles in our sample included 6-, 7-, 8-, 11-, and 12-repeat alleles. *DRD2 C957T* and *DRD2 TaqIB* were in Hardy-Weinberg equilibrium. *DRD2 TaqIA* was not Hardy-Weinberg equilibrium ($p<.01$); the *A1* allele appeared to be over represented. The *DRD2-141C Ins/Del* genotype was not in Hardy-Weinberg equilibrium ($p<.01$); the minor appeared to be under represented. *SLC6A3* was also not in Hardy-Weinberg equilibrium ($p<.01$); the heterozygote genotype was over represented. Results of the univariate analysis for selected variables are displayed in table II. *DRD2 TaqIA* genotype ($p=.001$) and presence of *DRD TaqIA* minor allele ($p=0.029$) were the only predictor variables that were significantly associated with smoking behavior at one year.

Table III shows the odds ratios for each variant. *DRD2 TaqIA* had a statistically significant relationship with smoking behavior in a model that contained matching variables and the other *DRD2* variants ($p=0.03$). Participants carrying a minor allele were less likely than participants with the *A2A2* genotype (homozygous for the major allele) to be abstinent at one year. No other SNPs were significant when either the crude or adjusted odds ratios were calculated. Associations between *SLC6A3* and smoking at one year were not statistically significant and no significant interaction between *SLC6A3* and *DRD2 TaqIA* was found.

A global test of the association between *DRD2* haplotype and smoking at one year was not statistically significant ($p=0.24$). Of the 16 potential haplotypes, 12 were included in the best pairs summary for the 867 participants with complete genotyping data. The level of certainty for the best pairs was greater than 75% for 830 (95.7%) of the participants. Of the 12 haplotypes, 5 were present with a frequency of at least 5%. These haplotypes are listed in table IV. The remaining haplotypes had a combined frequency of 2%. All five haplotypes were evaluated as dichotomous variables (presence vs. absence of the haplotype). Haplotype number 4 was further evaluated as a three level variable based on the number of copies of the haplotype that were present (0 vs. 1 vs. 2). Chi square analyses were significant only for haplotype 4 coded as a three level variable. The odds ratios for this variable in relation to smoking cessation are shown in table V. Having two copies of the haplotype significantly increases the odds of quitting.

DISCUSSION

Evidence suggests that the dopamine pathway is involved in smoking behaviors and specifically in smoking cessation. This study followed baseline smokers for one year to determine the effect of genetic variation in the dopamine pathway on smoking cessation. The findings from this study further support the role of the dopamine pathway in smoking cessation by replicating previous reports that link *DRD2 TaqIA* to quitting and by suggesting that differences in *DRD2* haplotypes influence smoking.

Many of the variants were not in Hardy Weinberg equilibrium in the cases, controls or both; however, considering that neither the cases nor the controls were selected from the general population and that both groups were selected specifically with regard to their smoking status this is not surprising. The significant findings suggest that the variants may be related to

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

smoking behaviors. Although we only found that *DRD2 TaqIA* was significantly related to smoking cessation, the other variants may be related to other characteristics such as smoking dose or age at initiation, which were not examined.

Although the DRD2 TaqIA variant is most likely not on the DRD2 gene, but on an adjacent gene, *ANKK1*, it appears to act on DRD2 and result in reduced dopamine receptor density [Neville et al., 2004]. Studies investigating the relationship between the *DRD2 TaqIA* variant and smoking behaviors have had mixed results. Most studies have found increased odds of smoking with the *A1*(minor) allele; however, few have reached statistical significance [Comings et al., 1996; Noble et al., 1994; Wu et al., 2000]. A recent cross-sectional study conducted on 2374 samples from the Prostate, Lung, Colorectal, and Ovarian (PLCO) cancer screening trial found a significant association between *DRD2 TaqIA* and smoking. Participants carrying the *A1* allele were more likely to be current smokers, rather than former smokers, at baseline [Morton et al., 2006]. Consistent with previous reports, we found that participants carrying the *A1* allele were less likely to be abstinent at one year versus those who were homozygous for the major allele. This association was apparent regardless of whether crude odds ratios were calculated or other genetic and non-genetic variables were included in the model.

Unlike *DRD2 TaqIA*, no association between receptor density and *DRD2 TaqIB* has been reported; however, the *TaqIB* has been the subject of increased study because it is closer to the coding regions of *DRD2*. In two reports that investigated the relationship between this variant and smoking, the *DRD2 TaqIB* minor allele (*B1*) appeared to be positively related to smoking; however, this only reached statistical significance in specific subject groups [Spitz et al., 1998; Wu et al., 2000]. A recent report looking at the relationship between this variant and smoking withdrawal found that participants homozygous for the *B2* allele experienced improvement in their withdrawal symptoms overtime while those with the *B1* allele did not [Robinson et al., 2007].

DRD2 C957T alters protein synthesis such that the *T* allele results in decreased synthesis [Duan et al., 2003]. Lerman et al. investigated potential associations between this SNP and smoking cessation pharmacotherapy in two clinical trials. The first compared bupropion therapy to placebo. The second compared transdermal nicotine and nicotine spray. In both, participants with the *CC* or *CT* genotypes were less likely than those with the *TT* genotype to be abstinent [Lerman et al., 2006].

Our lack of statistically significant associations between *DRD2 TaqIB* and *C957T* and smoking cessation may be due to sample size limitations. In comparison to many other studies our sample size of 881 is large; however, it still may not be large enough to expose statistically significant differences in smoking behaviors related to genotype. The PLCO study reported statistically significant results for these variants although the crude odds ratios are nearly identical to ours [Morton et al., 2006].

The *DRD2 -141C Insertion/Deletion (-141C Ins/Del)* variant is a functional polymorphism that has been shown to influence promoter activity [Blomqvist et al., 2000]. We could find only three reports that investigated the relationship between this variant and smoking behaviors. Our findings of no relationship between this variant and smoking status agree with those of Yoshida and Morton [Morton et al., 2006; Yoshida et al., 2001]. Lerman et al. examined potential associations between this SNP and pharmacotherapy as described above for *C957T*. An interaction between treatment group and *-141C Ins/Del* genotype was observed in both pharmacotherapy trials, and, regardless of treatment group, at the end of treatment participants with any deletion were more likely to be abstinent [Lerman et al., 2006].

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Three studies have found an association between reduced smoking behaviors and presence of the 9-repeat allele of *SLC6A3*. All three found that the 9-repeat allele had a protective effect against smoking [Lerman et al., 1999; Sabol et al., 1999; Timberlake et al., 2006]. In contrast, Vandenberg, et al., reported no relationship between *SLC6A3* and smoking behavior among nonsmokers (< 100 lifetime cigarettes), former smokers, and current smokers. However, contrary to the previously reported associations, when never-smokers (0 lifetime cigarettes) and non-smokers (>0 but <100 lifetime cigarettes) were analyzed as separate groups, the 10-repeat allele was more frequent among never smokers [Vandenbergh et al., 2002]. Jorm et al. found no association between the *SLC6A3* gene and either smoking initiation or cessation [Jorm et al., 2000]. Our findings for the dopamine transporter are consistent with those of Jorm et al. in that we found no direct association between smoking and variation in the *SLC6A3* gene.

Lerman et al. found that *DRD2* may modified the effects of *SLC6A3* such that the risk of smoking is further reduced in individuals with both the *SLC6A3-9* genotype and the *DRD2 TaqI A A2A2* genotype [Lerman et al., 1999]. In a randomized trial of bupropion for smoking cessation, *SLC6A3* was not significant as a main effect overall, but was significant among participants with the *DRD2 TaqIA A2A2* genotype. In the multivariate model in the entire sample, the *DRD2*SLC6A3* interaction term was significant [Lerman et al., 2003]. Recently, Timberlake et al. reported no significant interaction between *DRD2* and *SLC6A3* in relation to smoking behavior [Timberlake et al., 2006]. Our findings do not support the hypothesis that a *DRD2 TaqIA* by *SLC6A3* gene-gene interaction exists with smoking cessation.

In the haplotype analysis we found that individuals with the *A2 B2 T C* haplotype were more likely to quit smoking than those with other haplotypes, and that this relationship increased with the number of copies of the haplotype. When individual allele frequencies are examined in relationship to smoking abstinence, only slight differences are seen in allele frequencies between continuing smokers and those who were abstinent at one year (data not shown), but the combination of alleles (haplotypes) results in a strong association. This suggests that multiple variations along the dopamine pathway may influence smoking cessation and that association studies need to consider haplotypes rather than individual alleles.

The disparate findings related to dopamine genes and smoking cessation makes it difficult to draw any conclusions regarding the relationship of these genes to smoking behavior. The reason for contradicting results may be related to variation in the measurement of smoking cessation, differences in comparison groups, and information regarding quit strategies.

The primary limitation of our study was the lack of available information on quit strategies used by participants. Since the PLuSS study was not designed to examine smoking cessation limited data were available regarding the number of quit attempts, the duration of each quit attempt and the quit strategy used. Also, since participants were selected for genotyping after they completed their annual follow-up, their smoking status could not be biochemically verified. Our findings in a different subset of PLuSS participants allowed us to estimate that approximately 12% of smokers may have been misclassified as nonsmokers. Moreover, the participants likely spanned a broad range of readiness to quit. Although most probably had some smoking-related health concerns that motivated them to enroll in PLuSS, some may have used negative findings as reassurance that their smoking was not damaging their health.

A primary strength of this study is that data regarding smoking status were collected prospectively and the successful quit attempt was captured in the one-year follow up time period. Therefore, participants were similar at baseline in regard to smoking status and having had extensive smoking histories. This study is fairly large for a longitudinal study; most studies of this size are cross-sectional. Although PLuSS offered voluntary group behavioral counseling

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

for smoking cessation participants were free to use any quit strategy available during the follow-up year. Thus, because participants were not required to use one specific quit strategy and because PLuSS recruited from the general population, our findings are more generalizable than they would be if a specific strategy were prescribed. The number of *DRD2*-related SNPs that we examined is a further strength of the study. The trend in gene association studies is moving away from single SNP studies and to a deeper examination of genes by conducting haplotype analyses.

ACKNOWLEDGEMENTS

This study was funded by the University of Pittsburgh Cancer Institute's Specialized Program of Research Excellence in Lung Cancer (NCI P50 CA90440) pilot project program.

REFERENCES

American Cancer Society. Cancer Facts and Figures 2003. 2003.

Arinami T, Ishiguro H, Onaivi E. Polymorphisms in genes involved in neurotransmission in relation to smoking. European Journal of Pharmacology 2000;410(2–3):215–226. [PubMed: 11134671]

Blomqvist O, Gelernter J, Kranzler H. Family-based study of DRD2 alleles in alcohol and drug dependence. American Journal of Medical Genetics 2000;96(5):659–664. [PubMed: 11054774]

Brody A, Mandelkern M, Olmstead RE, scheibal D, Hahn E, Shiraga S, Zamora-Paja E, Farahi J, Saxena S, London E, et al. Gene variants of brain dopmaine pathways and smoking-induced dopamine release in the ventral caudate/nucleus accumbens. Archives of General Psychiatry 2006;63:808–816. [PubMed: 16818870]

Brody A, Olmstead R, London E, Farahi J, Meyer J, Grossman P, Lee G, Huang J, Hahn E, Mandelkern M. Smoking-induced ventral striatum dopamine release. American Journal of Psychiatry 2004;161:1211–1218. [PubMed: 15229053]

CDC. Cigarette smoking among adults - United States, 2000. MMWR 2002;51(29):642–645. [PubMed: 12186222]

Comings D, Ferry L, Bradshaw-Robinson S, Burchette R, Chiu C, Muhleman D. The dopamine D2 receptor (DRD2) gene: a genetic risk factor in smoking. Pharmacogenetics 1996;6(1):73–79. [PubMed: 8845863]

Duan J, Wainwright MS, Comeron JM, Saitou N, Sanders AR, Gelernter J, Gejman PV. Synonymous mutations in the human dopamine receptor D2 (DRD2) affect mRNA stability and synthesis of the receptor. Human Molecular Genetics 2003;12(3):205–216. [PubMed: 12554675]

Fuke S, Suo S, Takahashi N, Koike H, Sasagawa N, Ishiura S. The VNTR polymorphism of the human dopamine transproter (DAT1) gene affects gene expression. The Pharmacogenomics Journal 2001;1:152–156. [PubMed: 11911442]

Jacobsen LK, Staley JK, Zoghbi SS, Seibyl JP, Kosten TR, Innis RB, Gelernter J. Prediction of dopamine transporter binding availability by genotype: a preliminary report. American Journal of Psychiatry 2000;157(10):1700–1703. [PubMed: 11007732]

Jorm AF, Henderson AS, Jacomb PA, Christensen H, Korten AE, Rodgers B, Tan X, Easteal S. Association of smoking and personality with a polymorphism of the dopamine transporter gene: Results from a community survey. American Journal of Medical Genetics 2000;96:331–334. [PubMed: 10898910]

Kang AM, Palmatier MA, Kidd KK. Global variation of a 40-bp VNTR in the 3'-untranslated region of the dopamine transporter gene (SLC6A3). Biological Psychiatry 1999;46(2):151–160. [PubMed: 10418689]

Kendler KS, Neale MC, Sullivan P, Corey LA, Gardner CO, Prescott CA. A population-based twin study in women of smoking initiation and nicotine dependence. Psychological Medicine 1999;29(2):299–308. [PubMed: 10218922]

Lerman C, Caporaso NE, Audrain J, Main D, Bowman ED, Lockshin B, Boyd NR, Shields PG. Evidence suggesting the role of specific genetic factors in cigarette smoking. Health Psychology 1999;18(1):14–20. [PubMed: 9925041]

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

Lerman C, Jepson C, Wileyto EP, Epstein LH, Rukstalis M, Patterson F, Kaufmann V, Restine S, Hawk L, Niaura R, et al. Role of Functional Genetic Variation in the Dopamine D2 Receptor (*DRD2*) in Response to Bupropion and Nicotine Replacement Therapy for Tobacco Dependence: Results of Two Randomized Clinical Trials. Neuropsychopharmacology 2006;31:231–242. [PubMed: 16123753]

Lerman C, Shields PG, Wileyto EP, Audrain J, Hawk LH Jr, Pinto A, Kucharski S, Krishnan S, Niaura R, Epstein LH. Effects of dopamine transporter and receptor polymorphisms on smoking cessation in a bupropion clinical trial. Health Psychology 2003;22(5):541–548. [PubMed: 14570538]

Michelhaugh SK, Fiskerstrand C, Lovejoy E, Bannon MJ, Quinn JP. The dopamine transporter gene (SLC6A3) variable number of tandem repeats domain enhances transcription in dopamine neurons. Journal of Neurochemistry 2001;79(5):1033–1038. [PubMed: 11739616]

Morton L, Wang S, Bergen A, Chatterjee N, Kvale P, Welch R, Yeager M, Hayes R, Chanock S, Caporaso N. DRD2 genetic variation in relation to smoking and obesity in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial. Pharmacogenetics and Genomics 2006;16:901–910. [PubMed: 17108814]

Neville MJ, Johnstone EC, Walton RT. Identification and characterization of ANKK1: a novel kinase gene closelt linked to DRD2 on chromosome band 11q23.1. Human Mutation 2004;23(6):540–545. [PubMed: 15146457]

Noble EP, St Jeor ST, Ritchie T, Syndulko K, St Jeor SC, Fitch RJ, Brunner RL, Sparkes RS. D2 dopamine receptor gene and cigarette smoking: a reward gene? Medical Hypotheses 1994;42(4):257–260. [PubMed: 8072432]

Robinson JD, Lam CY, Minnix JA, Wetter DW, Tomlinson GE, Minna JD, Chen TTL, Cinciripini PM. The DRD2 TaqIB polymorphism and its relationship to smoking abstinence and withdrawal symptoms. Pharmacogenomics Journal 2007;7:266–274. [PubMed: 17189962]

Rossing MA. Genetic influences on smoking: candidate genes. Environmental Health Perspectives 1998;106(5):231–238. [PubMed: 9647893]

Sabol SZ, Nelson ML, Fisher C, Gunzerath L, Brody CL, Hu S, Sirota LA, Marcus SE, Greenberg BD, Lucas FRt, et al. A genetic association for cigarette smoking behavior. Health Psychology 1999;18 (1):7–13. [PubMed: 9925040]

Spitz MR, Shi H, Yang F, Hudmon KS, Jiang H, Chamberlain RM, Amos CI, Wan Y, Cinciripini P, Hong WK, et al. Case-control study of the D2 dopamine receptor gene and smoking status in lung cancer patients. Journal of the National Cancer Institute 1998;90(5):358–363. [PubMed: 9498485]

SRNT Subcommittee on Biochemical Verification. Biochemical verification of tobacco use and cessation. Nicotine & Tobacco Research 2002;4(2):149–159. [PubMed: 12028847]

Swan GE, Valdes AM, Ring HZ, Khroyan TV, Jack LM, Ton CC, Curry SJ, McAfee T. Dopamine receptor DRD2 genotype and smoking cessation outcomes following treatment with bupropion SR. The Pharmacogenomics Journal 2005;5:21–29. [PubMed: 15492764]

Timberlake D, Haberstick B, Lessem J, Smolen A, Ehringer M, Hewitt J, Hopfer C. An association between the DAT1 polymorphism and smoking behavior in young adults from the national longitudinal study of adolescent health. Health Psychology 2006;25(2)190-1907

True WR, Xian H, Scherrer JF, Madden PA, Bucholz KK, Heath AC, Eisen SA, Lyons MJ, Goldberg J, Tsuang M. Common genetic vulnerability for nicotine and alcohol dependence in men. Archives of General Psychiatry 1999;56(7):655–661. [PubMed: 10401514]

van Dyck CH, Malison RT, Jacobsen LK, Seibyl JP, Staley JK, Laruelle M, Baldwin RM, Innis RB. Increased dopamine transporter availability associated with the 9-repeat allele of the SLC6A3 gene. Journal of Nuclear Medicine 2005;46:745–751. [PubMed: 15872345]

Vandenbergh DJ, Bennett CJ, Grant MD, Strasser AA, O'Connor R, Stauffer RL, Vogler GP, Kozlowski LT. Smoking status and the human dopamine transporter variable number of tandem repeats (VNTR) polymorphism: failure to replicate and finding that never-smokers may be different.[see comment]. Nicotine & Tobacco Research 2002;4(3):333–340. [PubMed: 12215242]

Wu X, Hudmon KS, Detry MA, Chamberlain RM, Spitz MR. D2 dopamine receptor gene polymorphisms among African-Americans and Mexican-Americans: a lung cancer case-control study. Cancer Epidemiology, Biomarkers & Prevention 2000;9(10):1021–1026.

Yoshida K, Hamajima N, Kozaki K, Saito H, Maeno K, Sugiura T, Ookuma K, Takahashi T. Association between the dopamine D2 receptor A2/A2 genotype and smoking behavior in the Japanese. Cancer Epidemiology, Biomarkers & Prevention 2001;10(4):403–405.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Table I

Allele Frequencies

Allele	Frequency	Percent
DRD2 TaqIA		
A2	1427	81.0
A1	335	19.0
DRD2 TaqIB		
B2	1521	86.3
B1	241	13.7
DRD2 C957T		
C	752	43.3
T	984	56.7
DRD2 -141C ins/del		
C	1548	88.0
-	212	12.0
SLC6A3		
10	1281	72.7
9	463	26.3

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Table II

Subjects distributed according to selected matching variables and genotypes

Characteristic	N	%	Smoking behavior on Follow-up (%) Not Abstinent (N=662)	Abstinent (N=219)	p-value
Overall	881	100	75.1	24.8	
Matching Variables					
Gender					0.858
men	423	48.0	48.2	47.5	
women	458	52.0	51.8	52.5	
Age (years)					0.981
50–59	530	60.2	60.3	59.8	
60–69	253	28.7	28.5	29.2	
70–79	98	11.1	11.2	11.0	
Time of Enrollment					0.751
01/01/2002 - 03/31/2002	22	2.5	2.0	4.1	
04/01/2002 - 06/30/2002	108	12.3	12.7	11.0	
07/01/2002 - 09/30/2002	108	12.3	13.0	10.0	
10/01/2002 - 12/31/2002	82	9.3	8.8	11.0	
01/01/2003 - 03/31/2003	88	10.0	9.7	11.0	
04/01/2003 - 06/30/2003	128	14.5	14.8	13.7	
07/01/2003 - 09/30/2003	106	12.0	12.1	11.9	
10/01/2003 - 12/31/2003	116	13.2	13.1	13.2	
01/01/2004 - 03/31/2004	80	9.1	9.1	9.1	
04/01/2004 - 06/30/2004	43	4.9	4.8	5.0	
Genotypes					
DRD2 Taq1A					0.001
A2/A2	595	67.5	65.6	73.5	
A2/A1	237	26.9	29.8	18.3	
A1/A1	49	5.6	4.7	8.2	
DRD2 Taq1B					0.060
B2/B2	650	73.8	72.8	76.7	

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

Characteristic	N	%	Smoking behavior on Follow-up (%) Not Abstinent (N=662)	Abstinent (N=219)	p-value
B2/B1	221	25.1	26.4	21.0	
B1/B1	10	1.1	0.8	2.3	
DRD2 C957T[*]					0.200
C/C	175	20.2	21.1	17.2	
C/T	402	46.3	46.9	44.7	
T/T	291	33.5	32.0	38.1	
DRD2 -141C Ins/Del[**]					0.870
C/C	692	78.6	78.2	79.8	
C/ -	164	18.6	19.0	17.4	
− / −	24	2.7	2.7	2.8	
SLC6A3 [***]					0.773
10/10	481	55.7	54.8	53.9	
10/9	307	35.5	34.3	36.5	
9/9	76	8.8	8.9	7.8	

[*] excludes 13 participants for whom DRD2 C957T data are unavailable

[**] excludes 14 participants for whom DRD2 -141C Ins/Del data are unavailable

[***] excludes 17 participants with alleles other the 10-repeat or 9-repeat

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Table III

Crude and adjusted odds ratios for the effect of genotype on one-year smoking status.

Genotype	Abstinent	Not Abstinent	Crude OR 95% CI	Crude p-value^	Model set 1* OR 95% CI	Model set 1* p-value^^	Model 2** OR 95% CI	Model 2** p-value^^
TaqIA				0.030		0.027		0.033
A2/A2	1 61	434	1.00 (reference)		1.00 (reference)		1.00 (reference)	
Any A1	58	228	0.69 (0.49–0.96)		0.68 (0.48–0.96)		0.47 (0.24–0.94)	
TaqIB				0.256		0.239		0.184
B2/B2	168	482	1.00 (reference)		1.00 (reference)		1.00 (reference)	
Any B1	51	180	0.81 (0.57–1.16)		0.80 (0.56–1.16)		1.66 (0.79–3.51)	
C957T				0.214		0.153		0.261
TT	82	209	1.00 (reference)		1.00 (reference)		1.00 (reference)	
Any C	133	444	1.29 (0.86–1.93)		1.35 (0.90–2.03)		1.29 (0.83–2.02)	
-141C Ins/Del				0.624		0.561		0.900
C/C	174	518	1.00 (reference)		1.00 (reference)		1.00 (reference)	
Any Del	44	144	0.91 (0.62–1.33)		0.892 (0.61–1.31)		0.98 (0.65–1.45)	
Any 9-repeat allele				0.900		0.822		
No	97	290	1.00 (reference)		1.00 (reference)			
Yes	122	372	1.02 (0.75–1.39)		1.04 (0.76–1.41)			

*Model Set 1 uses logistic regression to adjust the genotype-smoking outcome associations (expressed as odds ratios) for factors (gender, decade of age, and quarter of enrollment) used to match the case and controls groups. N=881 for the TaqIA, TaqIB and SLC6A3 models. N=868 fo the C957T model. N=880 for the -141C Ins/Del model.

**Model 2 uses logistic regression to adjust the genotype-smoking outcome associations (expressed as odds ratios) for matching factors and for each of the other DRD2 genetic risk factors. N=867

^Chi-square test

^^Wald Test

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

Table IV

Most frequent DRD2 haplotypes for all 881 participants

Haplotype Number	Alleles Included in Each Haplotype				Frequency
	Taq1A	*Taq1B*	*C957T*	*-141C Ins/Del*	
1	A2	B2	C	C	0.20
2	A2	B2	C	Del	0.08
4	A2	B2	T	C	0.50
8	A1	B1	C	C	0.12
10	A1	B2	T	C	0.08

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

Table V

Odds of quitting, adjusted for decade of age, gender, and time of enrollment (3 month intervals), based on the number of copies of DRD2 haplotype 4, limited to participants with haplotype pairs that were determined with a certainty of more than 75% (N=830)

Number of copies of haplotype 4	OR	95% CI
1 versus 0	1.03	(0.68, 1.54)
2 versus 0	1.57	(1.02, 2.41)
2 versus 1	1.55	(1.07, 2.23)