

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

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Although bupropion and nicotine replacement therapy (NRT) are efficacious tobacco dependence treatments, there is substantial interindividual variability in therapeutic response and most smokers relapse. Pharmacogenetics research may improve treatment outcomes by identifying genetic variants predictive of therapeutic response. We investigated the roles of two functional genetic variants in the dopamine D2 receptor (*DRD2*) gene in response to pharmacotherapy for tobacco dependence among participants in two randomized clinical trials with a 6-month follow-up period: a double-blind placebo-controlled trial of bupropion ($n = 414$) and an open label trial of transdermal nicotine vs nicotine nasal spray ($n = 368$). At the end of the treatment phase, a statistically significant ($p = 0.01$) interaction between the *DRD2* -141C *Ins/Del* genotype and treatment indicated a more favorable response to bupropion among smokers homozygous for the *Ins* C allele compared to those carrying a *Del* C allele. By contrast, smokers carrying the *Del* C allele had statistically significantly ($p = 0.006$) higher quit rates on NRT compared to those homozygous for the *Ins* C allele, independent of NRT type. The C957T variant was also associated ($p = 0.03$) with abstinence following NRT. These results suggest that bupropion may be the preferred pharmacologic treatment for smokers homozygous for the *DRD2* -141 *Ins* C allele, while NRT may be more beneficial for those who carry the *Del* C allele. Study findings require confirmation in additional larger samples before they are applied in practice. *Neuropsychopharmacology* (2006) 31, 231–242. doi:10.1038/sj.npp.1300861; published online 10 August 2005

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INTRODUCTION

Currently, there are two FDA-approved pharmacotherapies for tobacco dependence, nicotine replacement therapy (NRT) and bupropion. Although both treatments have proven efficacy relative to placebo, there is substantial interindividual variability in therapeutic response and few smokers are able to maintain long-term abstinence (Dale

et al, 2001; Fiore *et al*, 1994; Hurt *et al*, 1997; Jorenby *et al*, 1999; Transdermal Nicotine Study Group, 1991). Emerging research in pharmacogenetics (Evans and Relling, 2004; Roses, 2000) may improve the outcomes of tobacco dependence treatment by allowing practitioners to individualize pharmacotherapy based on smokers' genotypes (Lerman and Niaura, 2002; Lerman *et al*, 2005).

Genetic variation in the dopamine pathway is a plausible target for pharmacogenetic studies of tobacco dependence and treatment response. There is abundant empirical support for the role of dopamine in the rewarding effects of nicotine (Balfour, 2004; Di Chiara and Imperato, 1988; Pontieri *et al*, 1996). Further, inhibition of dopamine reuptake is one putative mechanism for the beneficial effects of bupropion (Ascher *et al*, 1995; Sanchez and Hyttel, 1999). Consistent with evidence for the importance of dopamine in nicotine dependence, the dopamine D2

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receptor (*DRD2*) *Taq1* A1 variant is associated with an increased likelihood of nicotine abstinence symptoms (Gilbert *et al*, 2004), a reduced likelihood of sustained abstinence (Cinciripini *et al*, 2004), and greater efficacy of transdermal nicotine (TN) treatment relative to placebo (Johnstone *et al*, 2004; Yudkin *et al*, 2004). While initial findings relating the *DRD2* *Taq1* polymorphism to therapeutic response are promising, evidence for an association with smoking status in case-control studies has not been consistent (Bierut *et al*, 2000; Spitz *et al*, 1998). The functional significance of genetic variation at the *Taq1* locus has not been fully elucidated; however, the A1 allele correlates with altered receptor density and binding characteristics (Noble *et al*, 1991; Ritchie and Noble, 1996; Thompson *et al*, 1997). Furthermore, recent data indicate that the *Taq1* locus is actually 10 kb 3' to the end of the *DRD2* gene in a kinase gene of unknown function (*ANKK1*) (Neville *et al*, 2004).

In an effort to elucidate the functional consequences of genetic variation in *DRD2*, additional sequencing has identified single-nucleotide polymorphisms (SNPs) that may influence *DRD2* receptor expression by altering transcription or translation. An insertion/deletion variant in the *DRD2* promoter region (*DRD2* -141C *Ins/Del*) has been identified, with increased transcriptional efficiency observed with the more common -141C *Ins* allele as compared to the -141C *Del* allele (Arinami *et al*, 1997). In addition, Duan *et al* (2003) recently identified a functional synonymous SNP in *DRD2* (C957T) that decreases mRNA stability and protein synthesis. While previous studies have reported associations of the -141C *Ins/Del* variant with schizophrenia (Arinami *et al*, 1997; Breen *et al*, 1999), no studies have examined associations of the -141C *Ins/Del* or C957T SNPs with smoking behavior or response to pharmacotherapy.

We investigated the roles of the -141C *Ins/Del* and C957T *DRD2* variants in response to pharmacotherapy for tobacco dependence in two independent randomized trials. The first of these was a double-blind placebo-controlled trial of bupropion (Lerman *et al*, 2002, 2003), and the second was an open-label trial comparing TN to nicotine nasal spray (NS) (Lerman *et al*, 2004a, b).

MATERIALS AND METHODS

Participants and Procedures (Study 1, Bupropion)

Smokers responding to local media advertisements for free smoking cessation treatment and to physician referrals were screened for eligibility and recruited from April 1999 to October 2001 at Georgetown University (Washington, DC) and SUNY Buffalo (New York). The institutional review boards from both universities approved the research protocols. Figure 1 shows the flow of participants through the enrollment, treatment, and follow-up phases of the trial. Participants who were randomized, but did not enter treatment (ie those not included in the intent-to-treat analysis), did not differ significantly from those included in the outcome analysis in terms of demographics, smoking rate, nicotine dependence, or *DRD2* -141C genotype (all *p*-values >0.10) (genotyping for *DRD2* C957T was not performed for participants not entering the trial).

Participants in the final analysis included 414 smokers who were ≥18 years of age, reported smoking ≥10 cigarettes a day for the prior 12 months, and provided informed consent for both genotyping and treatment. Exclusion criteria included pregnancy, a history of DSM-IV axis I psychiatric disorder, seizure disorder, and current use of antidepressants or other psychotropic medications. Analyses were limited to persons of European ancestry in order to reduce bias due to racial admixture.

The study was a double-blind randomized clinical trial of bupropion vs placebo for smoking cessation. Randomization was determined by a computer-generated randomization scheme operated by a senior data manager; stratification was carried out by study site. Allocation was concealed from the counselors who delivered the interventions and from the study assistants who assessed the outcomes.

Participants at both sites received identical assessments and treatments. At an initial visit to the smoking clinic, participants provided a 40-ml blood sample and completed a set of standardized self-report questionnaires. All participants received 10 weeks of either placebo or bupropion. Bupropion treatment was delivered according to the standard therapeutic dose (150 mg/day for the first 3 days, followed by 300 mg/day). All participants also received seven sessions of standardized behavioral group counseling, focusing on self-monitoring and behavioral modification approaches. All participants were instructed to quit smoking on a target quit date (TQD) 2 weeks after initiating medication and counseling.

Smoking status was assessed by telephone interview at the end of treatment (EOT) (8 weeks post-TQD) and at 6 months after the TQD using a validated timeline follow-back method (Brown *et al*, 1998). Interviewers were blind to study group assignment. Participants who reported complete abstinence (not even a puff of a cigarette) for at least the 7 days prior to the assessment were asked to complete an in-person visit for biochemical verification of abstinence. Saliva cotinine testing was performed for participants who reported abstinence at a given time-point using a gas-liquid chromatography method (Feyerabend and Russell, 1990). Of 175 participants self-reporting abstinence at EOT, 146 (83%) provided a sample for cotinine verification; of 125 participants reporting abstinence at 6-months, 108 (86%) provided a sample.

Adverse events were self-reported on a weekly basis using a standardized measure of severity of potential side effects. Any severe or persistent side effects were reviewed with the study physician.

Participants and Procedures (Study 2, NRT)

Participants were recruited at Georgetown University (Washington, DC) and the University of Pennsylvania (Philadelphia, PA), from February 2000 to August 2003, using methods identical to those described above. The institutional review boards from both universities approved the research protocols. Figure 2 shows the flow of participants through the enrollment, treatment, and follow-up phases of the trial. Subjects who were randomized, but did not enter treatment (ie those not included in the intent-to-treat analysis), did not differ significantly from

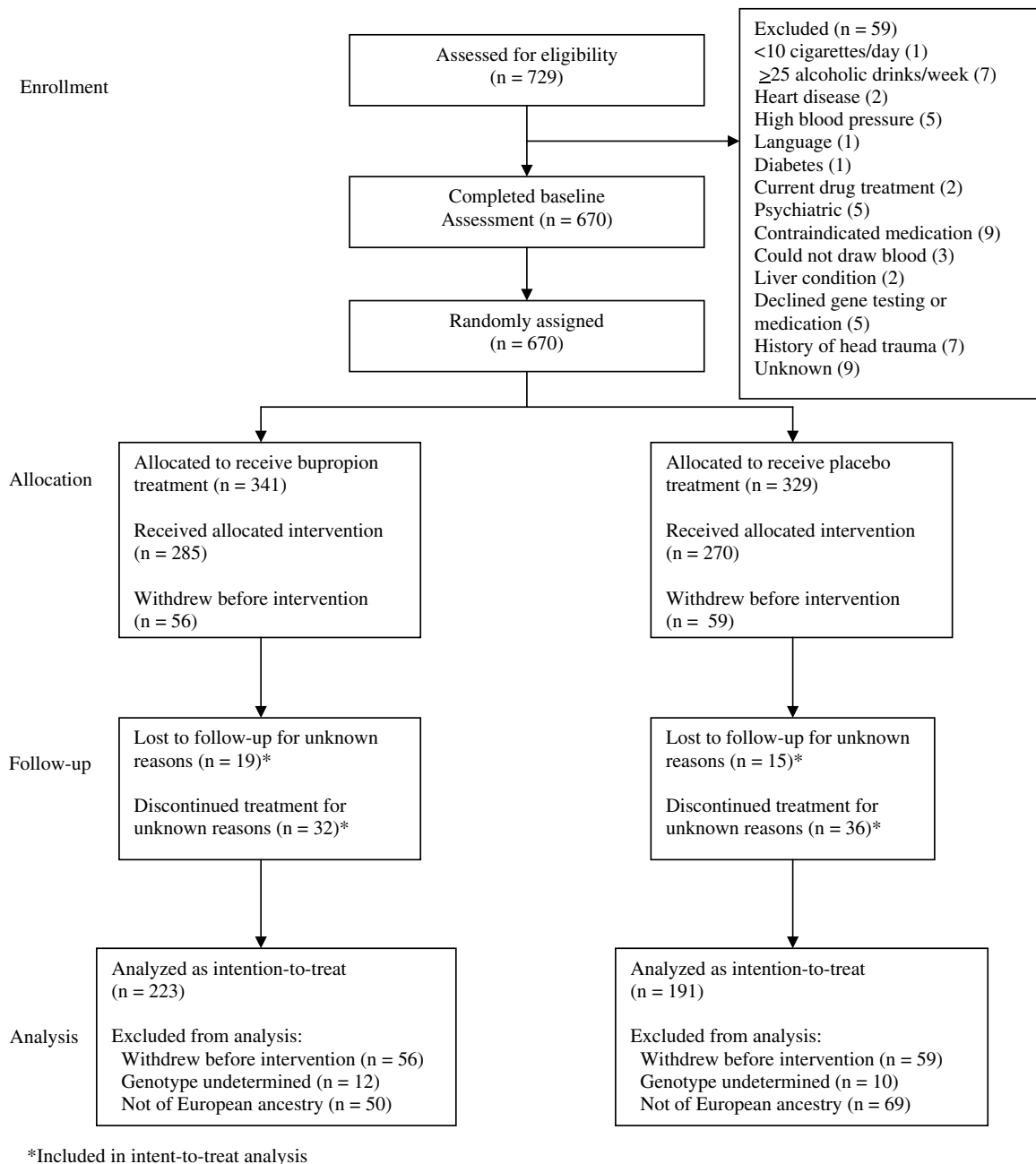


Figure 1 Flow diagram of participation for Bupropion Trial (Study 1).

those included in the outcome analysis in terms of demographics, smoking rate, or nicotine dependence (all p -values >0.20). There was a marginal association of $-141C$ *Ins/Del* genotype with entry into treatment ($p = 0.07$).

Participants were 368 current cigarette smokers of European ancestry who were ages ≥ 18 years, smoked at least 10 cigarettes per day for the prior 12 months, and provided informed consent for both genotyping and treatment. Exclusion criteria included: pregnancy or lactation, uncontrolled hypertension, unstable angina, heart attack or stroke within the past 6 months, current treatment or recent diagnosis of cancer, drug or alcohol dependence, current diagnosis or history of a DSM-IV axis I psychiatric

disorder, and current use of bupropion or nicotine-containing products other than cigarettes.

The trial was an open-label randomized clinical trial of two forms of NRT for smoking cessation: TN vs NS. Randomization was performed as described above for Study 1. Allocation to treatment could not be concealed from the counselors or study assistants who delivered the medication to participants. NS (Nicotrol[®]; Pharmacia, Helsingborg, Sweden) was initiated on the morning of the target quit date (TQD) (after 2 weeks of counseling) and provided to participants over an 8-week period according to the standard regimen. TN (Nicoderm[®] CQ; Glaxo Smith Kline, Research Triangle Park, NC) was used by participants over an 8-week treatment period, beginning with the morning of

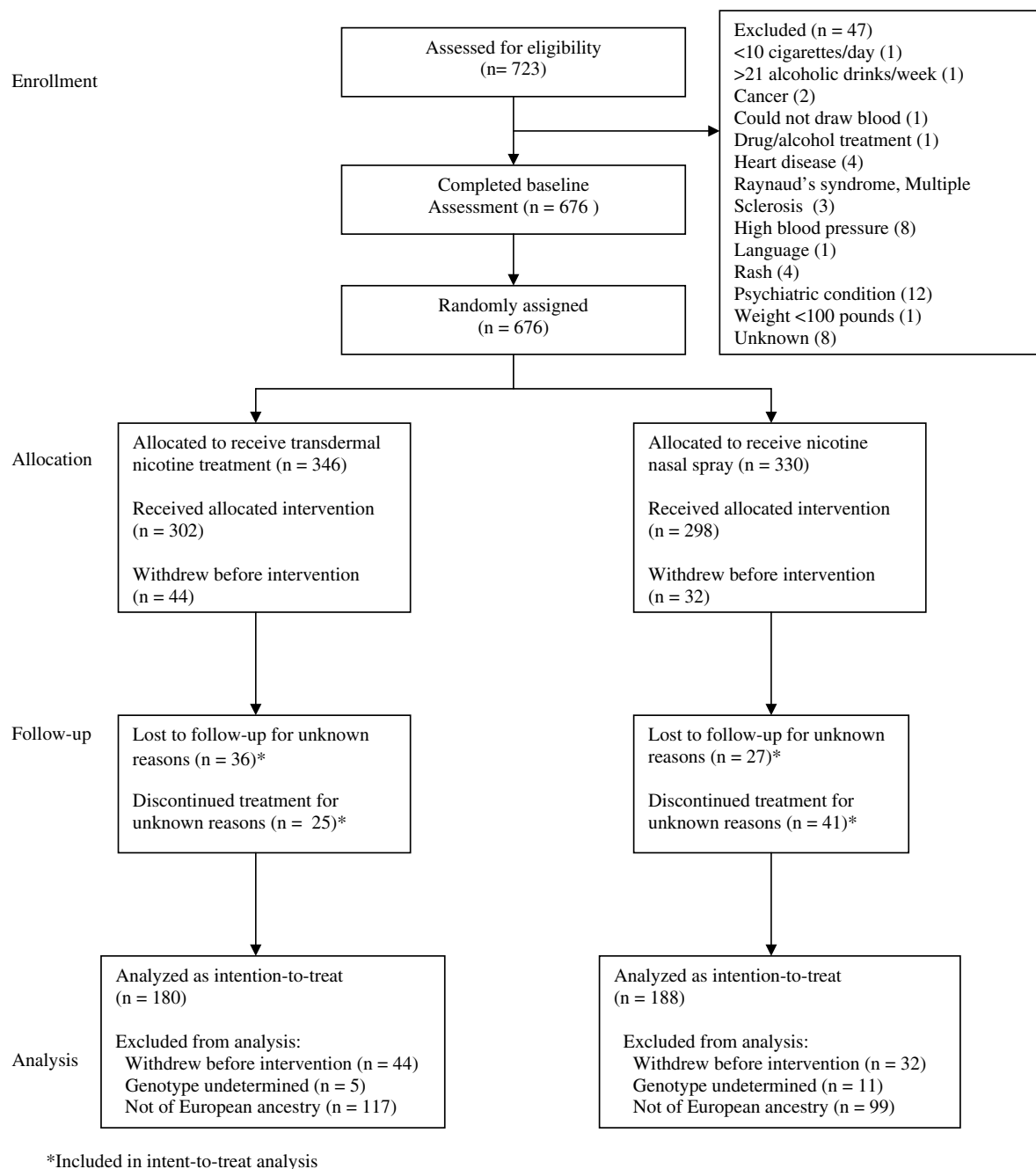


Figure 2 Flow diagram of participation for NRT Trial (Study 2).

the TQD, according to the standard regimen. All participants received seven sessions of standardized behavioral group counseling and adverse events were reported as described above for Study 1.

Self-report data on smoking status were obtained at EOT and 6-month follow-up, as described above for Study 1. Participants who reported complete abstinence (not even a puff of a cigarette) for at least the 7 days prior to the assessment were asked to complete an in-person visit for biochemical verification of abstinence using exhaled carbon monoxide (CO) reading (cotinine verification was not used because the treatments contained nicotine). Of 178 participants self-reporting abstinence at EOT, 136 (76%) provided

a CO sample for verification; of 115 participants reporting abstinence at 6-months, 83 (72%) provided a CO sample.

Measures (Studies 1 and 2)

Genotype. Genotyping for the *DRD2* -141C *Ins/Del* and C957T variants was completed using the ABI Prism[®] 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA). PCR was performed with 2.25 ng of DNA, 2.5 µl of ABI Taqman[®] Universal Mastermix (Foster City, CA), 0.125 µl of water, and 0.125 µl of 40X Assay by Design SNP Assay for the *DRD2* variants (ABI, Foster City, CA). The 5 µl reactions were performed in a 384-well plate (ABI,

Foster City, CA). The plates were thermal cycled using the following conditions: (1) 2 min hold at 50°C to activate the AmpErase[®] Uracil N-glycosylase (UNG), an enzyme in the Universal Mastermix used to prevent PCR contamination; (2) 10 min denaturation step at 95°C; (3) 50 cycles of 95°C for 15 s and 60°C for 1 min; (4) 4°C hold forever. The plates were scanned utilizing the Allelic Discrimination End-Point Analysis on the ABI Prism[®] 7900HT Sequence Detection System. The Allelic Discrimination data were analyzed by the AutoCall algorithm of the SDS v2.1 Software (ABI, Foster City, CA). The *DRD2* assays were validated by genotyping 40 samples from both the Coriell Caucasian Human Variation Panel of 50 (HD50CAU) and the Coriell African American Human Variation Panel of 50 (HD50AA) along with 15 samples from the Coriell CEPH/UTAH Pedigree 1333. The Human Variation Panel samples yielded high-quality PCR amplification and genotypes, which could be called with certainty by the ABI software for all 95 samples. The CEPH family genotypes were analyzed using PedCheck (http://watson.hgen.pitt.edu/register/soft_doc.html) and no Mendelian inheritance errors were found.

For the -141C *Ins/Del* variant, participants were classified based on the presence or absence of the N null (*Del*) allele (CC vs CN or NN); because of the very small number of NN cases in the analysis, we combined CN and NN into a single category. For the C957T variant, participants were classified as CC, CT, or TT.

Covariates. Sex, education, age, and smoking rate were assessed at baseline. Nicotine dependence was also assessed at baseline using the Fagerström Test for Nicotine Dependence (Heatherton *et al*, 1991). The Center for Epidemiological Studies Depression (CES-D) measure (Radloff, 1977) was used to assess baseline depression symptoms.

Primary outcome. In accordance with recommendations of the SRNT expert panel (Hughes *et al*, 2003; SRNT Subcommittee on Biochemical Verification, 2002), participants were classified as abstinent if they self-reported abstinence (not even a puff) for each of the 7 days immediately prior to the follow-up point (ie point-prevalence), and provided a cotinine reading of ≤ 15 ng/ml (Study 1) or a CO reading of ≤ 10 ppm (Study 2). Consistent with these recommendations, we presumed that those who failed to complete the follow-up, or failed to provide a biosample for biochemical verification, had relapsed.

Intermediate outcomes. A self-report measure of *withdrawal symptoms* assessed the severity (in the past 7 days) of common symptoms, including items such as irritability, difficulty concentrating, anxious/tense, insomnia, drowsiness, nausea, and general physical complaints (eg sweating, dizziness) (Hughes *et al*, 1984; Piasecki *et al*, 2000). Responses to items (from 0 = not at all to 3 = severe) were summed to create a withdrawal severity index. In the bupropion trial, this instrument was administered at baseline (approximately 3 weeks prior to the TQD), and again at 1 week pre-TQD, on the TQD, and at 1, 2, and 3 weeks post-TQD; in the NRT trial, withdrawal symptoms were assessed at baseline (approximately 3 weeks prior to

the TQD), and again at 1 week pre-TQD, on the TQD, and at 1 and 2 weeks post-TQD. A *side effects* checklist was used for each study to assess the severity of physical complaints potentially associated with treatment (17 items for the bupropion trial and 17 items for the NRT trial). Responses to items (from 0 = none to 3 = severe) were summed to create a side effects severity index. In the bupropion trial, this instrument was administered at 1-week pre-TQD, on the TQD, and at 1, 2, 3, and 5 weeks post-TQD; in the NRT trial, side effects were assessed on the TQD and again at 1, 2, 4, and 6 weeks post-TQD. *Depression symptoms* were reassessed at EOT and at 6-month follow-up using the CES-D as described above.

Compliance in the bupropion trial was assessed based on a standard pill count measure. In the NRT trial, participants assigned to TN recorded their daily application of patches, and those assigned to NS recorded the number of doses per day. Because use of NRT may be confounded with smoking status (ie participants may discontinue NRT if they relapse), we focused on average use of TN or NS during the first 2 weeks of treatment.

Statistical Analysis (Studies 1 and 2)

We calculated that a sample size of at least 165 participants per group was needed to detect an odds ratio of 4.7 for a genotype by treatment interaction with power ≥ 0.80 (Power and Statistical Analysis Software, NCSS, Kaysville, UT). All analyses were intent-to-treat. Bivariate associations of genotypes and treatment group with abstinence were assessed using χ^2 tests. Logistic regression models of abstinence at EOT and 6-month follow-up were then estimated. Predictors included the *DRD2* -141C *Ins/Del* and *DRD2* C957T genotypes, sex, nicotine dependence, treatment group, and interactions of each genotype with treatment group. Both genotypes were treated as dichotomous (0/1) variables, with codes of 1 representing CC (for -141C *Ins/Del*) and CC/CT (for C957T). In each model, all predictors were entered as a block, after which genotype by treatment interaction terms were allowed to drop out in stepwise fashion (p to enter = 0.05, p to remove = 0.10). To explore the longitudinal pattern of relapse by genotype, we examined time to failure (defined as 7 consecutive days of self-reported smoking) using Cox regression. Since a large number of people are left censored by not quitting on their target quit day (so they do not become part of the abstinent cohort), we also examined the left censoring dichotomy using logistic regression.

Genotype associations with changes in intermediate outcomes (withdrawal symptoms and side effects) were assessed using multivariate analysis of variance (MANOVA) to test genotype by time interactions. Genotype effects on post-treatment depression symptoms were assessed using linear regression models that controlled for baseline depression levels.

Haplotype Analysis

We estimated linkage disequilibrium (LD) between the *DRD2* -141C *Ins/Del* and C957T SNPs using maximum-likelihood methods through the PWLD STATA routine. The two SNPs were in moderate LD ($D' = 0.70$, $p < 0.001$),



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Table 1 Logistic Regression Models of Abstinence at EOT and 6-Month Follow-Up—Bupropion Trial (Study 1)

Predictor	EOT ^a			6 months		
	OR	95% CI	p	OR	95% CI	p
Sex (female = 1)	0.58	0.37, 0.92	0.02	0.79	0.49, 1.28	0.34
Treatment (bupropion = 1)	0.46	0.15, 1.47	0.19	0.60	0.18, 2.06	0.42
Nicotine dependence	0.88	0.80, 0.98	0.02	0.95	0.85, 1.06	0.36
DRD2 -141C (CC = 1)	0.42	0.19, 0.96	0.04	0.47	0.19, 1.13	0.09
DRD2 C957T (CC or CT = 1)	0.95	0.58, 1.57	0.86	0.59	0.35, 0.99	0.05
DRD2 -141C by treatment	4.99	1.42, 17.62	0.01	3.32	0.86, 12.80	0.08

^aEOT = end of treatment.

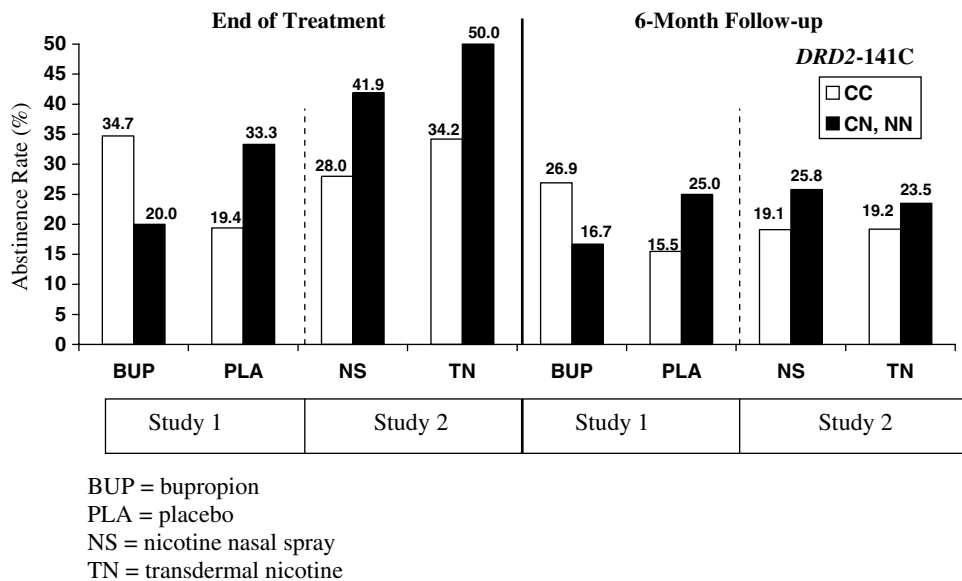


Figure 4 Abstinence rates by DRD2 -141C Ins/Del genotype and treatment.

12.80; $p = 0.08$). The form of the interaction was similar to that seen at EOT. In contrast to the univariate results above, the main effect of C957T genotype was significant in the regression model (OR = 0.59; CI = 0.35, 0.99; $p = 0.048$) (Table 1).

Application of survival methods revealed that the observed interaction between bupropion treatment and the -141C genotype results entirely from an improved ability of subjects with the CC genotype (compared to the CN/NN group) to quit on their target quit day in response to drug, an initial difference that carried through to 6 months. The results were confirmed by the logistic regression analysis of genotype by treatment effects on abstinence at the TQD (ie left censoring) (OR = 4.63; CI = 1.39–15.48; $p = 0.01$), while Cox regression to estimate differences in relapse over time revealed no significant interaction (HR = 0.59; CI = 0.26–1.39; $p = 0.23$).

Haplotype Analysis of Treatment Outcome

There were four possible haplotype combinations arising from the DRD2 -141C Ins/Del and C957T SNPs: C_C, C_T,

N_C, and N_T (position 1 represents the -141C Ins/Del allele, and position 2 represents the C957T allele). HAPIPF estimated their respective haplotype frequencies to be 40.2, 51.0, 7.4, and 1.4%. Based on the results from the logistic regression, we tested for haplotype by treatment interaction overall results using the log-linear analysis in HAPIPF; this provided evidence of a significant association between haplotype/treatment combinations and point prevalence abstinence at EOT ($-2LR(7) = 21.5$, $p = 0.003$), and at 6 months ($-2LR(7) = 15.8$, $p = 0.03$). To avoid using the rare haplotype (N_T) in the analysis, we used the frequency estimates from the haplotype \times treatment vs abstinence analysis to perform contingency table analysis without the rare haplotype. As in the original log-linear analysis, results were significant at EOT ($\chi^2(5) = 19.5$, $p = 0.002$), and at 6 months ($\chi^2(5) = 14.4$, $p = 0.02$). Consistent with the regression results described above, it appeared that haplotypes containing the -141 InsC allele were associated with better response to bupropion treatment while those containing the -141 DelC allele were associated with better response to placebo (Table 2).

Table 2 Estimated Haplotype Counts by Treatment in Bupropion Trial (Study 1)

Haplotype	Placebo			Bupropion		
	Estimated chromosome sample	Percent success at EOT ^a	Percent success at 6 months	Estimated chromosome sample	Percent success at EOT	Percent success at 6 months
C_C	153.2	19.3	14.0	179.7	34.0	23.4
C_T	188.8	21.4	18.3	233.3	33.9	28.7
N_C	32.8	37.8	25.9	28.3	21.2	17.7

^aEOT = end of treatment.

Haplotypes are written as -141 *Ins/Del* C allele first, followed by the C957T allele.

$p = 0.002$ for EOT, $p = 0.02$ for 6 months.

Genotype Associations with Intermediate Outcomes

The results of the MANOVAS revealed no evidence for genotype effects on changes in withdrawal symptoms or side effects (all p -values > 0.20). The two genotypes were unrelated to changes in depression symptoms from baseline to EOT or baseline to 6-month follow-up (all p -values > 0.20).

There was a nonsignificant difference in pill counts by -141C genotype (CC group = 63.8 ± 1.6 vs CN/NN group = 56.2 ± 3.9 ; $t = -1.89$, $p = 0.06$), but no difference by C957T genotype ($t = -0.54$, $p = 0.58$). Given the marginal association of genotype with pill counts, the logistic regression model was re-run including pill count as a covariate, and the results were unchanged. The odds ratio for the -141C genotype by treatment interaction effect on abstinence, controlling for pill counts, was 4.70 ($p = 0.018$), compared with OR = 4.99 ($p = 0.012$) in Table 1.

Study 2: NRT

Descriptive data. Of the 368 eligible participants, 46% were female, 50% were college graduates, and the mean age was 47 (SD = 11.1) years. On average, participants smoked 24 cigarettes per day (SD = 9.3). The mean score for nicotine dependence was 5.58 (SD = 2.22). For the -141C *Ins/Del* SNP, 303 (82.3%) participants were homozygous for the C allele (CC), 62 (16.8%) were heterozygous (CN), and 3 (0.8%) were homozygous for the N allele (NN). For the C957T SNP, 115 (31.3%) participants were homozygous for the T allele (TT), 182 (49.5%) were heterozygous (CT), and 71 (19.3%) were homozygous for the C allele (CC). Both loci were in Hardy-Weinberg equilibrium (p -values > 0.8).

Compared to participants in the bupropion trial, NRT trial participants were younger (44.6 vs 46.8 y, $p = 0.006$), had lower levels of nicotine dependence (FTND score = 5.2 vs 5.6 , $p = 0.008$), and were more likely to be female (54 vs 46% , $p = 0.04$).

In total, 188 (51.1%) participants were randomized to the NS treatment condition, and 180 (48.9%) to the TN condition. There were no significant differences in allele frequencies, genotypes, or covariates by treatment group. Of the 368 eligible participants, 350 (95.1%) provided data at EOT, and 353 (95.9%) provided data at 6-month follow-up. There were no significant differences in genotypes, covariates, or treatment assignment by EOT completion status

(p -values > 0.10). Participants lost to follow-up at 6 months were more likely to have been allocated to the TN condition ($\chi^2(1) = 3.73$, $p = 0.05$). There were no significant differences in genotypes or covariates by 6-month interview completion status.

Treatment Outcome

At the end of the NRT treatment phase (EOT), 124 (33.7%) participants were abstinent (37.2% of TN group vs 30.3% of NS group; $\chi^2(1) = 1.96$, $p = 0.16$). At the 6-month follow-up, 74 (20.1%) were abstinent (20.0% of TN group vs 20.2% of NS group; $\chi^2(1) = 0.01$, $p = 0.96$).

There was a significant association of -141C *Ins/Del* genotype with abstinence at EOT ($\chi^2(1) = 5.48$, $p = 0.02$). Overall, 46% of participants with CN/NN genotypes were abstinent compared to 31% of those with the CC genotype (Figure 4); when the CN and NN groups are not combined, the quit rates at EOT are 31% for the CC group, 44% for the CN group, and 100% for the NN group ($n = 3$). This effect was no longer significant at 6-month follow-up ($\chi^2(1) = 1.00$, $p = 0.32$). The C957T SNP was not significantly associated with abstinence at EOT ($\chi^2(2) = 2.33$, $p = 0.31$) or at 6-month follow-up ($\chi^2(2) = 0.12$, $p = 0.94$).

Results of the logistic regressions of abstinence at EOT and 6 months are shown in Table 3. Both *DRD2* SNPs were significantly associated with abstinence at EOT, controlling for treatment group, sex, and nicotine dependence. For the -141C *Ins/Del* locus, participants with CC genotypes were about one-half as likely to be abstinent at EOT as participants with CN/NN genotypes (OR = 0.44; CI = 0.25, 0.79; $p = 0.006$). For the C957T locus, participants with CT/CC genotypes were less than two-thirds as likely to be abstinent at EOT as participants with TT genotypes (OR = 0.59; CI = 0.36, 0.95; $p = 0.03$). Genotype by treatment interactions were not significant. In the model of abstinence at 6 months, neither genotype contributed significantly and there were no genotype by treatment interactions. Depression symptoms did not predict abstinence at either time point (p -values > 0.20).

The survival analysis suggested that the *DRD2* -141C genotype effects on abstinence occur as a result of incremental differences in relapse/failure over time. Females failed more quickly than males (HR = 1.57; CI = 1.19–2.08; $p = 0.002$), those with higher dependence failed more quickly (per unit FTND; HR = 1.09; CI = 1.02–1.16;

Table 3 Logistic Regression Models of Abstinence at EOT and 6-Month Follow-Up—NRT Trial (Study 2)

Predictor	EOT ^a			6 months		
	OR	95% CI	p	OR	95% CI	p
Sex (female = 1)	0.71	0.45, 1.11	0.14	0.75	0.44, 1.26	0.28
Treatment (NS = 1)	0.74	0.48, 1.16	0.19	1.06	0.63, 1.77	0.84
Nicotine dependence	0.93	0.84, 1.02	0.14	0.90	0.81, 1.02	0.09
DRD2 -141C (CC = 1)	0.44	0.25, 0.79	0.006	0.71	0.37, 1.37	0.31
DRD2 C957T (CC or CT = 1)	0.59	0.36, 0.95	0.03	1.02	0.58, 1.82	0.94

^aEOT = end of treatment.**Table 4** Estimated Haplotype Counts in NRT Trial (Study 2)

Follow-up	Haplotype	Failed	Abstained	% Success
EOT ^a ($\chi^2_{(2df)} = 14.5$, $p < 0.001$)	C_C	196.04	70.93	26.57
	C_T	256.96	144.07	35.93
	N_C	27.96	29.07	50.98
6 months ($\chi^2_{(2df)} = 1.3$, $p = 0.52$)	C_C	214.84	52.14	19.53
	C_T	321.16	79.86	19.92
	N_C	42.16	14.86	26.07

^aEOT = end of treatment.Haplotypes are written as -141 *Ins/Del* C allele first, followed by the C957T allele. $p = 0.0008$ for EOT, $p = 0.5$ at 6-months.

$p = 0.013$), and those with the -141 CC genotype failed more quickly than those with CN or NN genotypes (HR = 1.46; CI = 1.00–2.14; $p = 0.05$).

Haplotype Analysis of Treatment Outcome

There were four possible haplotype combinations arising from the -141 C *Ins/Del* and C957T SNPs: C_C, C_T, N_C, and N_T (position 1 represents the -141C *Ins/Del* allele, and position 2 represents the C957T allele). HAPIPF estimated their respective haplotype frequencies to be 37.3, 54.5, 7.7, and 1.5%. Overall results from the log-linear analysis in HAPIPF provided evidence of an association between haplotype and abstinence at EOT ($-2LR(3) = 15.97$, $p = 0.0011$), but no evidence of an association of haplotype with abstinence at 6 months ($-2LR(3) = 4.30$, $p = 0.23$). To avoid using the rare haplotype (N_T) in the analysis, we used the frequency estimates from the haplotype and abstinence analysis to perform contingency table analysis without the rare haplotype. The results are shown in Table 4, and as in the original analysis, the association is significant at EOT ($\chi^2(2) = 14.39$, $p = 0.0008$), but not at 6 months ($\chi^2(2) = 1.24$, $p = 0.5$). Consistent with the regression analysis described above, at EOT, haplotypes containing the -141C *Ins/Del* C allele were associated with a lower abstinence rate.

Genotype Associations with Intermediate Outcomes

The average change in withdrawal symptom scores from prequit to 1-week postquit was 1.71 ± 7.18 (range = -17–30), and this did not differ by -141C genotype ($t = 1.6$, $p = 0.11$) or C957T genotype ($t = 0.46$, $p = 0.64$). The average side effects score at 1-week postquit was 26.95 ± 5.15 (range = 18–53), and this measure also did not differ by -141C genotype ($t = -0.92$, $p = 0.36$) or by C957T genotype ($t = -0.11$, $p = 0.91$).

The results of MANOVAS revealed no evidence for genotype effects on changes in withdrawal or side effects (all p -values > 0.20). There were no significant effects of either genotype on changes in depression symptoms from baseline to EOT or baseline to 6-month follow-up (all p -values > 0.20).

There was a significant difference in days per week of patch use by -141C genotype (CC group = 6.4 ± 1.3 vs CN/NN group = 6.9 ± 0.17 ; $t = 4.41$, $p = 0.001$) and by C957T genotype (CC/CT group = 6.7 ± 0.74 vs TT group = 6.1 ± 1.79 , $t = -2.25$, $p = 0.028$). However, this is likely attributable to the fact that those participants who relapse to smoking are advised to discontinue patch use. The average number of nicotine spray doses was 9.59 ± 7.95 , and this did not differ by -141C genotype ($t = -0.52$, $p = 0.60$) or by C957T genotype ($t = 0.20$, $p = 0.84$).

Analysis of Population Stratification

The STRUCTURE program (Pritchard *et al*, 2000) was used to test for the presence of ethnic admixture in the two study populations using 41 random biallelic SNPs (list of SNPs available upon request). We tested the hypothesis of two or more strata (ie ethnic subpopulations) within each study sample, and the program attempted to classify individuals as belonging to population or the other. The results included class probabilities for each individual. For example, an individual might be assigned a probability of 0.6 for belonging to one population, and 0.4 for belonging to the other. A stratified population would separate into a bimodal distribution of assignment probabilities, with values close to 0 or 1. In both of our study samples, the values all were extremely close to 0.5, indicating that the program could not identify separate strata within either of our study samples. As shown in Figure 5, probabilistic assignment was evenly split for all individuals.

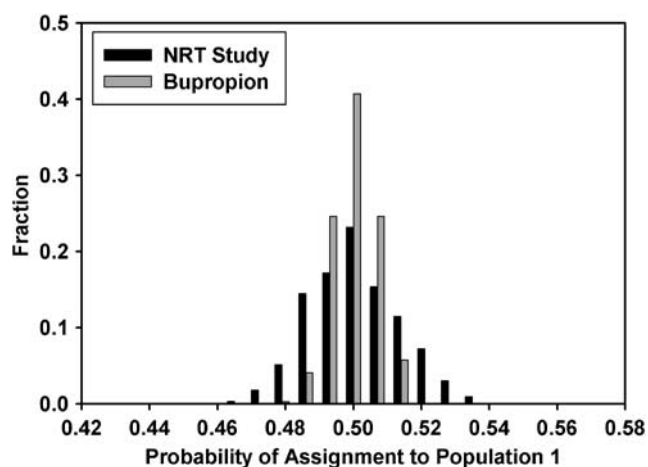


Figure 5 Assignment to population by the structure clustering program.

DISCUSSION

This study investigated the effects of two functional *DRD2* SNPs on response to pharmacotherapy in two smoking cessation randomized clinical trials, a double-blind placebo-controlled trial of bupropion and an open label trial of TN vs NS. The results of logistic regression and haplotype analyses revealed a significant bupropion treatment by genotype interaction at the EOT phase. Specifically, smokers homozygous for the *InsC* allele of the $-141C$ *Ins/Del* SNP (ie CC) responded better to bupropion plus counseling than did smokers carrying the *DelC* allele (CN or NN genotypes). The difference in abstinence rates between genotype groups at EOT was also clinically significant (bupropion condition: 35 vs 20% for CC vs CN/NN, respectively; placebo condition: 19 vs 33% for CC vs CN/NN, respectively). By contrast, smokers carrying one or more copies of the *DelC* allele at $-141C$ *Ins/Del* responded better to behavioral counseling alone (Study 1) and to NRT plus behavioral counseling (Study 2). A greater response to NRT was also observed among smokers who were homozygous for the T allele of the C957T SNP; however, this effect was significant only in the multivariable model when the effects of the $-141C$ *Ins/Del* SNP and other covariates were considered. Haplotypes for the two SNPs were strongly predictive of treatment response in the bupropion trial ($p=0.002$ and 0.02 for EOT and 6-months, respectively) and of abstinence at EOT in the NRT trial ($p=0.0008$). However, these effects were attributable primarily to the association of abstinence with the *DRD2* $-141C$ *Ins/Del* SNP. Finally, it should be noted that genotype effects on response to treatment are markedly attenuated at 6-month follow-up. This may be expected for a true pharmacogenetic effect, as participants have been without medication for an extended period of time.

From a neurobiological perspective, these results may be interpreted as follows. Nicotine introduced into the ventral tegmental area (VTA) releases dopamine from nerve terminals in the nucleus accumbens (NAC) (Nisell et al, 1994), contributing to nicotine's rewarding effects (Di Chiara and Imperato, 1988). Given that the $-141C$ *InsC* allele results in higher transcriptional efficiency compared to the *Del* (N) allele (Arinami et al, 1997), individuals with the $-141C$ *Ins/Del* CC genotype may have more D2 receptors

available to bind dopamine, and smoking may be more rewarding in this group. Therefore, smokers with the CC genotype may have a greater liability to relapse than those who carry the *DelC* allele, consistent with the observation that smokers carrying the *DelC* allele have higher quit rates than those with the CC genotype following behavioral counseling alone (study 1) or NRT (study 2). In contrast to NRT, bupropion treatment (via inhibition of dopamine reuptake) may result in sustained elevations in extracellular dopamine; higher dopamine levels, in turn, may replace the hedonic (pleasurable) effects of smoking more effectively than NRT (Balfour, 2004). Thus, bupropion treatment may overcome the liability to relapse in individuals with the $-141C$ CC genotype because they have more D2 receptors to bind dopamine.

The mechanisms of genotype effect on abstinence and treatment response in these trials merit further investigation. There were no significant effects of either genotype on changes in side effects or withdrawal symptoms, suggesting that these effects on therapeutic response are attributable to other mechanisms. Survival analysis in the bupropion trial indicated that the effect of $-141C$ *Ins/Del* genotype on response to treatment occurs mainly at the TQD, an effect maintained through 6-month follow-up. This suggests the hypothesis that bupropion treatment (which occurs for 2 weeks prior to the TQD) may increase motivation to quit, rather than altering abstinence-related symptoms. In contrast, in the NRT trial, the main effect of genotype appeared to occur as a result of incremental differences in relapse over time, perhaps due to medication effects not measured in the current trial.

These findings extend previous pharmacogenetic investigations of bupropion and NRT focusing on the *DRD2* *Taq1A* polymorphism. For example, greater beneficial effects of bupropion have been found among smokers with *DRD2* *Taq1* A2/A2 genotypes, as compared to those who carry the A1 allele (David et al, 2003). This finding is consistent with our results when one considers that a genotype associated with increased transcriptional efficiency in cell culture ($-141C$ *Ins/Del* CC) (Arinami et al, 1997) and a genotype correlated with normal (vs reduced) receptor density (*DRD2* *Taq1* A2A2) (Thompson et al, 1997) both predict a favorable response to bupropion relative to placebo. Since significant linkage disequilibrium between the $-141C$ *Ins/Del* and the *Taq1A* is not observed (Gelernter et al, 1998), the effects of these two variants on therapeutic response are likely to be at least partially independent. To confirm this, the logistic regression analyses reported above were rerun including *DRD2* *Taq1A* genotype as a covariate and the results were essentially unchanged (results not shown).

Data from the NRT trial are also compatible with previous reports that the *DRD2* *Taq1A* A1 genotype is associated with a more favorable response to NRT (Johnstone et al, 2004; Yudkin et al, 2004). This genotype has been associated with reduced D2 receptor binding (Thompson et al, 1997). As described above, we found that genotypes containing the *Del* allele (CN/NN), associated with decreased transcriptional efficiency for the D2 receptor (Arinami et al, 1997) predict better response to NRT. Thus, in the case of these two independent polymorphisms, smokers with genotypes associated with altered receptor availability or function achieved higher quit rates with NRT. We also found that

smokers homozygous for the C957T T allele, associated with decreased mRNA stability and translation (Duan et al, 2003), exhibited a better response to NRT. Taken together, the findings from the bupropion and NRT trials are compatible with the premise that genotypes associated with reduced D2 receptor availability or function predict better outcomes with NRT, while those associated with normal receptor expression or function predict a better response to bupropion.

The present study has both strengths and limitations. The strengths include the collection of DNA samples from all study participants, rather than retrospective DNA collection that may result in bias. An additional strength is the empirical evaluation of the presence of population stratification, the results of which provided no evidence for population substructure that could bias the study results. One limitation of the study is that we did not collect post-treatment samples to determine levels of bupropion to verify self-reported medication compliance. A second limitation is the restricted ethnic background of participants, all of whom were of European origin. Thirdly, although the sample sizes of 414 and 368 afforded sufficient power to detect a clinically meaningful genotype by treatment interaction, it was not possible to test for gene-gene interactions because of the small cells generated for participants with the less common alleles for both genotypes. Previous evidence for interacting effects of the *DRD2 Taq1A* and dopamine transporter (*SLC6A3*) variants on smoking cessation suggests that testing for epistatic effects is important (Lerman et al, 2003).

Larger scale pharmacogenetic trials confirming the findings of the present study in different ethnic groups may allow for genotype-based selection of pharmacotherapy for nicotine dependence. A recent survey of primary care physicians suggests a high level of interest in adoption of genetic testing to tailor smoking cessation treatment (Shields et al, in press). In parallel to the pharmacogenetics research effort in smoking cessation treatment, there will need to be an investigation of the psychosocial, ethical, and health policy issues raised by the translation of findings to clinical practice (Shields et al, 2004, 2005).

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