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Pharmacogenetic clinical trial of sustained-release bupropion for smoking cessation

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Abstract

This randomized, double-blinded, placebo-controlled trial examined genetic influences on treatment response to sustained-release bupropion for smoking cessation. Smokers of European ancestry ($N=291$), who were randomized to receive bupropion or placebo (12 weeks) plus counseling, were genotyped for the dopamine D2 receptor (*DRD2*-Taq1A), dopamine transporter (*SLC6A3* 3' VNTR), and cytochrome P450 2B6 (*CYP2B6* 1459 C→T) polymorphisms. Main outcome measures were cotinine-verified point prevalence of abstinence at end of treatment and at 2-, 6-, and 12-month follow-ups post quit date. Using generalized estimating equations, we found that bupropion, compared with placebo, was associated with significantly greater odds of abstinence at all time points (all p values $<.01$). We found a significant *DRD2* × bupropion interaction ($B=1.49$, $SE=0.59$, $p=.012$) and a three-way *DRD2* × bupropion × craving interaction on 6-month smoking cessation outcomes ($B=-0.45$, $SE=0.22$, $p=.038$), such that smokers with the A2/A2 genotype demonstrated the greatest craving reduction and the highest abstinence rates with bupropion. Furthermore, there was a significant *DRD2* × *CYP2B6* interaction ($B=1.43$, $SE=0.56$, $p=.01$), such that individuals with the *DRD2*-Taq1 A2/A2 genotype demonstrated a higher odds of abstinence only if they possessed the *CYP2B6* 1459 T/T or C/T genotype. Because the sample size of this study was modest for pharmacogenetic investigations, the results should be interpreted with caution. Although these results require replication, the data suggest preliminarily that the *DRD2*-Taq1A polymorphism may influence treatment response to bupropion for smoking cessation and, further, that exploration of gene × gene and gene × craving interactions in future, larger studies may provide mechanistic insights into the complex pharmacodynamics of bupropion.

Introduction

Despite major public health gains in the past 50 years, smoking remains the leading cause of preventable death in western countries (Peto et al., 2000; Peto et al., 1996). To improve the

efficacy of current smoking cessation methods, investigators have begun to focus on the potential for individually tailored pharmacotherapies (Baker et al., 2003). With abundant evidence from animal and human studies suggesting that dopamine is an important substrate in the rewarding properties of nicotine (Benwell & Balfour, 1992;Corrigall, Coen, & Adamson, 1994) and in the behavioral conditioning process common to addiction to all drugs of abuse (Balfour, 2002;Robinson & Berridge, 2001), attention has increasingly been directed to how individual variation in dopamine neurotransmission affects the efficacy of pharmacological therapies for smoking cessation such as bupropion and nicotine replacement therapy (Baker et al., 2003;Lerman & Niaura, 2002). Twin studies indicate that smoking behavior has a major heritable component (Li, Cheng, Ma, & Swan, 2003), and association studies suggest that polymorphisms for genes in the dopamine and serotonin pathways, as well as in the CYP2B6 enzyme, are associated with nicotine dependence (Munafò, Clark, Johnstone, Murphy, & Walton, 2004). Thus novel research into the efficacy of tailored therapies seeks to identify how individual differences in genotype and gender influence drug targets and drug metabolism, thereby affecting clinical treatment outcome (Lerman & Niaura, 2002;Munafò, Bradburn, Bowes, & David, 2004a,2004b;Scharf & Shiffman, 2004).

Prior to the recent U.S. Food and Drug Administration (FDA) approval of varenicline (Chantix, Pfizer Inc.), sustained-release (SR) bupropion had demonstrated the highest efficacy of any medication approved by the FDA for smoking cessation. Six-month abstinence rates ranging from 25% to 35%, as compared with 15%–20% for placebo (Hurt et al., 1997;Jorenby et al., 1999). A recent meta-analysis of 16 clinical trials indicated that smokers using bupropion hydrochloride were twice as likely to be abstinent at 6 months, compared with those receiving placebo ($OR=1.97$, 95% $CI=1.67-2.34$; Hughes, Stead, & Lancaster, 2003). Bupropion is an antidepressant medication with somewhat complex pharmacodynamic and pharmacokinetic properties. It is a weak inhibitor of dopamine and norepinephrine reuptake (Ascher et al., 1995) and may serve as a noncompetitive inhibitor of nicotinic acetylcholine receptors (Fryer & Lucas, 1999). These pharmacodynamic properties are consistent with known effects of bupropion on nicotine withdrawal (Lerman, Roth et al., 2002), which appear to be moderated by genetic variation in the dopamine D2 receptor gene (David et al., 2003). With regard to its pharmacokinetic properties, bupropion is metabolized to a primary metabolite, hydroxybupropion, by the cytochrome P450 enzyme 2B6 (*CYP2B6*;Faucette et al., 2000), an enzyme that also contributes to nicotine metabolism during states of elevated serum nicotine, such as the period immediately following the smoking of one or more cigarettes (Yamanaka et al., 2005).

Given the pharmacodynamics of bupropion, genes involved in dopamine neurotransmission and bupropion metabolism are logical candidates for studying pharmacogenetic response for smoking cessation. Indeed, heritable predisposition to smoking behavior may be mediated partly by genetic variation in the dopamine pathway (Munafò et al., 2004). This hypothesis is supported by studies linking dopaminergic candidate genes with smoking behavior.

The *DRD2*-Taq1A2 restriction fragment length polymorphism (RFLP) is located approximately 10 kilobases (kb) downstream from the *DRD2* gene in the neighboring “ankyrin repeat and kinase domain containing 1” (*ANKK1*) gene. This RFLP results in an amino acid substitution (Glu713Lys) in a serine/threonine kinase, which may affect substrate binding to the DRD2 receptor (Neville, Johnstone, & Walton, 2004), is associated with decreased striatal D2 receptors (Noble, Blum, Ritchie, Montgomery, & Sheridan, 1991;Noble, Gottschalk, Fallon, Ritchie, & Wu, 1997;Pohjalainen et al., 1998), and has been associated with being a smoker in some studies (Comings et al., 1996;Noble et al., 1994;Spitz et al., 1998) but not all (Bierut et al., 2000). In addition to the *DRD2*-Taq1A polymorphism, two single nucleotide polymorphisms (SNPs) in the *DRD2* gene—the -141C insertion/deletion (-141C ins/del; Arinami, Gao, Hamaguchi, & Toru, 1997) and 957 C→T (Duan et al., 2003) SNPs—have been

shown to affect transcriptional activity. Of these two SNPs, only the *DRD2* -141C ins/del polymorphism has been evaluated for association with smoking. However, Yoshida and colleagues (2001) found no association between smoking status and the *DRD2* -141C ins/del polymorphism, whereas they did find a significant association with the *DRD2*-Taq1A RFLP.

The absence of the 9-repeat allele in the 3'-untranslated region of the dopamine transporter gene (*SLC6A3* "null" or ".*") has been associated with smoking in some studies (Lerman et al., 1999; Sabol et al., 1999); however, this association has not been replicated in more recent studies (Bierut et al., 2000; Jorm et al., 2000; Vandenbergh et al., 2002).

The 1459 C→T SNP in the *CYP2B6* gene results in an amino acid change (arginine/cysteine), is common in Whites, and has been reported to reduce CYP2B6 protein expression in human liver (Lang et al., 2001) and brain (Miksys, Lerman, Shields, Mash, & Tyndale, 2003). Further, this SNP is associated with reduced bupropion hydroxylation in humans (Hesse et al., 2004). At least four additional amino acid-altering SNPs are found within the *CYP2B6* gene (64 C→T, Arg22Cys; 516 G→T, Gln172His; 777 C→A, Ser259Arg; and 785 A→G, Lys262Arg; Lang et al., 2001). However, only two of these SNPs are common in Whites (785 A→G/33%, 516 G→T/29%; Lang et al., 2001), and none appear to affect gene expression (Lang et al., 2001) or bupropion metabolism (Hesse et al., 2004).

Lerman and colleagues (Lerman et al., 2003; Lerman, Shields et al., 2002) found a significant interaction between the *DRD2*-Taq1A polymorphism and the *SLC6A3* variable number of tandem repeats (VNTR) polymorphism on smoking cessation during the treatment phase of a bupropion smoking cessation trial (Lerman et al., 2003) and that the *CYP2B6* 1459 C→T polymorphism was predictive of smoking cessation on bupropion at the end of treatment (12 weeks) in women but not in men (Lerman, Shields et al., 2002). However, Lerman and colleagues also demonstrated that the *DRD2* -141C ins genotype was associated with increased quit rates at the end of treatment on bupropion but, conversely, that it was associated with decreased efficacy with nicotine replacement therapy for smoking cessation when compared with the *DRD2* -141C del genotype (Lerman et al., 2006). Neither study demonstrated genotype main effects or genotype × treatment interactions beyond the treatment phase of cessation (i.e., extending to 6 months or more of follow up).

Swan and colleagues (2005) used a different study design to examine the influence of the *DRD2*-Taq1A polymorphism on effectiveness of bupropion SR for smoking cessation in an open-label trial. Smokers ($N=496$) were randomized to receive one of four combinations of bupropion SR and counseling: 150 mg bupropion SR with either less intensive or more intensive counseling, or 300 mg bupropion with either less intensive or more intensive counseling. These investigators observed that smokers with *DRD2*-Taq1A2/A2 were more likely to be abstinent at 12 months than were A1 allele carriers (OR for smoking at 12 months = 0.76, 95% $CI=0.56-1.03$; $p<.076$). In addition, A1 carriers were more likely to drop out of the study due to side effects of bupropion ($OR=1.91$, 95% $CI=1.01-3.60$; $p=0.04$). Both of these effects were observed only in women. Interestingly, the *DRD2* × bupropion interaction in this study persisted long after end of treatment to 12 months of follow-up. This observation raises the question of why a pharmacogenetic interaction might persist for months beyond termination of medication administration. Persistent pharmacogenetic effects are not biologically implausible. Indeed, *in vitro* evidence indicates that compounds that block dopamine transport stimulate presynaptic neuroadaptations vis-à-vis c-fos expression (Yatin, Miller, & Madras, 2005; Yatin, Miller, Norton, & Madras, 2002); furthermore, in at least one clinical trial, bupropion demonstrated persistent effects on relapse prevention beyond the end of treatment (Hays et al., 2001).

Both the Lerman and Swan studies demonstrated that smokers with A2/A2 genotypes had the highest quit rates on bupropion (Lerman et al., 2003; Swan et al., 2005). In addition, a smaller, laboratory-based cue-reactivity trial of bupropion demonstrated significant reductions in craving only among smokers using bupropion who had the *DRD2*-Taq1A2/A2 genotype (David et al., 2003). In addition, Cinciripini and colleagues (2004) found that treatment response to venlafaxine, another antidepressant for smoking cessation that has somewhat different pharmacodynamic properties, appeared to be influenced by *DRD2*-Taq1A genotype.

These pharmacogenetic examinations suggest preliminarily that at least two genotypes portending increased dopaminergic tone (*DRD2*-Taq1A2 and *DRD2* -141C ins) may be associated with enhanced treatment response to bupropion for smoking cessation.

Given this background, we chose to test the hypothesis that bupropion would demonstrate greater efficacy for smoking cessation among smokers with the *DRD2*-Taq1A2/A2 genotype than among those with the *DRD2*-Taq1A1/A1 or A1/A2 genotypes in a randomized, placebo-controlled trial of bupropion. Furthermore, we sought to determine whether the *SLC6A3* 3' VNTR or *CYP2B6* 1459 C→T polymorphisms would interact with the *DRD2*-Taq1A/A2 polymorphism to affect smoking cessation outcomes.

Method

The present study was a pharmacogenetic analysis of treatment outcome in a randomized, double-blinded, placebo-controlled trial of bupropion hydrochloride for smoking cessation.

Participants

Participants included 138 male and 145 female smokers of White European ancestry who reported smoking 10 cigarettes/day or more. They were recruited using flyers and newspaper advertisements for a free smoking cessation research program from November 1997 to January 2001. Potential participants were screened for eligibility first by telephone and then during an in-person medical and psychiatric session. Exclusion criteria included pregnancy; a history of mood or thought disorder, based on the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (*DSM-IV*; American Psychiatric Association, 1994); seizure disorder; current use of psychotropic medications; history of seizures or eating disorders; previous use of l-dopa or monoamine oxidase inhibitors; age less than 18 years; or score less than 5 on a 10-point Likert scale of willingness to quit. Statistical analyses for the genetic component of this study were limited to Whites of European ancestry to reduce bias due to racial admixture.

Procedure

Participants were enrolled at the Miriam Hospital and at Butler Hospital, both teaching affiliates of Brown Medical School, in Providence, Rhode Island. All participants provided written consent for genotyping and smoking cessation treatment. After initial telephone screening, participants were invited to informational meetings where individuals were randomized to one of two treatment sites at which assessments and group sessions were conducted. At the initial assessment session, participants provided informed consent, provided a 40-ml blood sample, and were screened for exclusion criteria and current *DSM-IV* diagnoses, as assessed by the Structured Clinical Interview for *DSM-IV* Non-Patient Edition (SCID-NP; Spitzer, Williams, Gibbon, & First, 1989), administered by trained Ph.D.-level raters. Participants then received a brief medical examination by the study physician, who assessed medical exclusion criteria.

Participants were then randomized in double-blind fashion to bupropion ($n=133$) or placebo ($n=150$), according to gender, current depressive symptoms, and levels of nicotine dependence, using the urn randomization technique (Wei & Lachin, 1988). Bupropion treatment was

delivered according to the standard therapeutic dose (150 mg/day for the first 3 days, followed by 300 mg/day) for a total of 12 weeks. All bupropion and placebo pills were prepared by the manufacturer of Zyban, GlaxoSmithKline, and were identical in shape, size, and color.

Participants also were randomized to receive one of two intensive group-counseling interventions: either standard, cognitive-behavioral smoking cessation treatment (ST) or standard cessation treatment combined with cognitive-behavioral group therapy for depression (CBT). The CBT versus ST comparison was included because CBT has been shown to have an incremental benefit over ST for smoking cessation among smokers with major depression. Thus a secondary hypothesis of the study was to examine whether CBT had an incremental benefit for smoking cessation in nondepressed smokers (Brown et al., 2001). Both treatment conditions provided 12 two-hour sessions and were equated for participant and contact time with Ph.D.-level behavioral therapists. Both group treatments incorporated self-monitoring, self-management, and other behavioral modification techniques, and all participants were instructed to quit smoking on a target quit date 2 weeks after initiating medication, which coincided with session 7 of behavioral counseling. The CBT condition included the preceding components, as well as cognitive-behavioral coping skills for depression, described in detail elsewhere (Brown et al., 2007). Self-reported data on smoking status were recorded weekly, at the end of treatment, and at 2-, 6-, and 12-month follow-ups (Brown, Burgess, Sales, & Whiteley, 1998). Monetary incentives were provided for study participation and attendance at scheduled follow-up sessions. Biochemical verification of self-report data was performed with salivary cotinine testing for participants who reported abstinence at the end of treatment and at the 2-, 6-, and 12-month follow-ups using a gas-liquid chromatography method (Feyerabend & Russell, 1990).

Measures

Smoking outcomes—We considered 7-day point prevalence at the end of treatment and at 2-, 6-, and 12-months post end of treatment as the primary outcomes. Seven-day point prevalence reflects smoking status for the prior 7 days and was verified biochemically on the basis of cotinine levels. Participants with cotinine levels of 15 ng/ml or less were classified as abstinent for the point-prevalence outcome. Participants lost to follow-up were classified as smokers for all outcome analyses, consistent with established statistical practice adopted by the Cochrane Tobacco Addiction Group (Hughes, Stead, & Lancaster, 2004).

Genotype—Using methods published previously by our group (Lerman et al., 1999; Lerman, Shields et al., 2002), we genotyped participants for the *DRD2*-Taq1A (11q23, GeneID:1813), *SLC6A3* 3' VNTR (5p15.3, GeneID: 6531), and *CYP2B6* 1459 C→T (19q13.2, GeneID:1555) polymorphisms. Consistent with previous reports (Comings et al., 1996; Lerman et al., 1999; Lerman, Shields et al., 2002), participants were classified in the following way:

- The *SLC6A3* genotype was classified as the presence or absence of the 3' VNTR 9-repeat allele. We refer to non-9-repeat alleles (mostly 10-repeat) as null (*) and therefore dichotomized as 9/9 or 9/* vs. 9/9 or 9/*.
- The *DRD2*-Taq1A genotype was classified as the presence or absence of the A1 allele (A1/A1 or A2/A1 vs. A1/A1 or A1/A2).
- The *CYP2B6* genotype was classified as the presence or absence of the T allele (1459 T/T or C/T vs. T/T or C/T).

The justification for dichotomization of these polymorphisms in this fashion is supported by functional evidence for each polymorphism. Preclinical studies of the *DRD2/ANKK1*, *CYP2B6*, and *SLC6A3* 3' VNTR demonstrated significant differences in function comparing carriers of one or more copies of each functional polymorphism to homozygous wild-type

individuals (striatal D2 availability using positron emission tomography; Jonsson et al., 1999), *in vitro* expression of the CYP2B6 enzyme (Hesse et al., 2000; Lang et al., 2001; Miksys et al., 2003), and dopamine transporter binding using cerebral perfusion magnetic resonance imaging (Szobot et al., 2005). In addition, association studies for each of these polymorphisms suggest dominant, rather than additive, models of inheritance (Lerman et al., 1999; Lerman, Shields et al., 2002). The genetic assays were validated by confirming a polymorphic inheritance pattern in seven human family lines that encompassed three generations (National Institute of Genetic Medicine, 2004). Quality control procedures included positive and negative controls with each assay and independent repeat genotyping for 20% of the results.

Demographic factors—Gender, education, marital status, age, and ethnic ancestry were assessed by self-report during the pretreatment assessment visit.

Nicotine dependence—The Fagerström Test for Nicotine Dependence (FTND) was administered during the pretreatment assessment visit (Heatherton et al., 1991). The FTND is a six-item self-report measure of nicotine dependence derived from the Fagerström Tolerance Questionnaire.

Mood and craving—Mood was assessed via several measures. Initially, participants were screened with the SCID-NP (Spitzer et al., 1989) and excluded for any *DSM-IV* Axis I mood or thought disorders, including depression. Depressive symptoms were assessed with the Center for Epidemiological Studies Depression Scale (CES-D; Radloff, 1977). Tobacco craving was evaluated with the Shiffman-Jarvik Craving Scale (SJCS; Shiffman & Jarvik, 1976), a five-item questionnaire with well-established validity (Shiffman et al., 1998), administered at each follow-up visit until the end of treatment. For the purposes of evaluating bupropion $\times$ craving interactions on smoking cessation outcomes, the SJCS score at the end of treatment (12 weeks) was included as a covariate in the general estimating equation (GEE) analysis described below (Liang & Zeger, 1986; Zeger & Liang, 1986).

Data analyses

Data analyses were performed using SAS/STAT version 8.2. The success of the randomization procedure in balancing the covariate distribution by treatment arm was assessed at baseline using chi-square tests for discrete covariates and two-tailed *t* tests for continuous covariates. In addition, the number of follow-ups completed was tested for association with condition and genotype. Intention-to-treat methods were used to evaluate trial outcome, with treatment condition (placebo or bupropion) classified according to initial treatment assignment based on randomization, and participants lost to follow-up coded as smokers. Participants lost to follow-up were coded as smokers because of the high correlation between loss to follow-up and smoking relapse and because this approach is considered to be more rigorous than other approaches and is the standard practice adopted by the Cochrane Collaboration (Stead & Lancaster, 2000) and the U.S. Department of Health and Human Services (2000). Although an intention-to-treat approach is usually thought to be conservative, it may have an anticonservative bias, if dropout rates are differential by treatment arm. Therefore, a chi-square test of association was used to compare dropout rates in the placebo and bupropion arms.

Analyses of smoking cessation outcomes were conducted using GEEs (Liang & Zeger, 1986; Zeger & Liang, 1986) for repeated-measures analyses of categorical outcomes with point-prevalence abstinence at the four time periods as the dependent variable. GEEs allow for inclusion of both categorical and continuous independent variables and for appropriate modeling of covariance structures when observations are correlated across time. Analyses were conducted in SAS using PROC GENMOD with the Logit link function and an unstructured correlation matrix specified.

Prior to the primary analyses, univariate association models with smoking abstinence were first fit for each potential confounder using GEEs. Together with treatment group and genotype, all variables with suggestive ($p < .10$) associations with outcome were added as main effects in subsequent marker-specific multivariate GEE models. The first model included a linear effect of time, FTND score, and medication condition. We also tested the effects of CBT vs. ST treatment, but this effect was nonsignificant in all analyses (p values $> .10$) and was not retained. The next set of models tested separately the main effects of genotype using dummy coding. For the *SLC6A3*, *DRD2*, and *CYP2B6* markers, the reference groups were */*, A2/A2, and C/C, respectively. The third set of models included the genotype $\times$ condition interaction to test the hypothesis that genotype would moderate the effects of bupropion. The fourth set of models included a time $\times$ genotype $\times$ medication interaction to determine whether any differential effect of medication $\times$ genotype changed over the course of follow-up. Finally, we tested potential interactions between both *SLC6A3* 3' VNTR and the *CYP2B6* 1459 C $\rightarrow$ T with the *DRD2*-Taq1A2/A2 polymorphism. To examine the effects of gene $\times$ bupropion interactions on withdrawal-related negative mood and craving on quit date, 3 days after quit date, and 7 days after quit date, we conducted repeated-measures analyses for continuous outcomes using GEEs. These models covaried for FTND score and the corresponding variable at the beginning of treatment, as well as whether the participant reported complete abstinence during the week after quit date. Analyses mirrored those used to examine point-prevalence abstinence; the first set of models tested main effects, the second tested gene $\times$ bupropion interactions, and the third tested gene $\times$ bupropion $\times$ time interactions. These analyses included 263 participants who provided data at any of the three assessment times.

Results

Participants for the present study were part of a larger, randomized clinical trial of 524 participants (Brown et al., 2007). Of these participants, 319 (59.4%) consented to and underwent blood collection for DNA analysis. Of the subjects who were genotyped, 291 were of White European decent, thus constituting the final study sample. The mean age of the sample was 45.4 years ($SD=10.7$), and the mean number of years of education was 13.6 years ($SD=2.2$). Participants reported smoking on average 24.6 cigarettes/day ($SD=9.9$) and had smoked for an average of 27.1 years ($SD=11.6$). The sample mean score on the FTND was 6.4 ($SD=1.9$). The majority of participants (92.3%) had made at least one quit attempt that lasted more than 24 h. The sample baseline mean score on the CES-D was 6.3 ($SD=6.7$). A history of MDD was reported by 22.1% of participants.

The randomization procedure was successful in balancing the covariate distribution (age, education, cigarettes smoked per day, years of smoking, mean FTND score, having made a 24-h quit attempt, baseline CES-D score, and history of MDD) between the placebo and bupropion groups, with no p values smaller than 0.26 (Table 1). Subjects contributing DNA were significantly more likely to be female (51% vs. 40%) and older (45.4 vs. 43.2 years), and had been smoking longer (27.1 vs. 24.8 years). In addition, genotypic frequencies for all three markers (*SLC6A3*, *DRD2*, and *CYP2B6*) were balanced between treatment arms and did not diverge significantly from Hardy-Weinberg proportions. Of the 291 White participants with DNA data, 5 were missing valid data on at least one genotype and 3 were missing data from the FTND, which was used as a covariate in the main analyses. To keep the sample consistent across analyses, these participants were dropped from further analysis as their data could be considered missing at random, leaving a final sample size of 283.

Of the 283 participants who began treatment, 233 participants or their significant others (82.3%) provided complete outcome data at all follow-ups: end of treatment, 2 months, 6 months, and 12 months. A total of 30 participants (10.6%) lacked complete outcome data at one of the four follow-ups, 9 (3.2%) lacked data at two follow-ups, 5 (1.8%) lacked data at

three follow-ups, and 6 (2.1%) lacked data at all follow-ups. The odds of completing follow-ups were not significantly related to treatment condition or genotype or any of the potential confounder variables (p values $>.10$). For our primary analyses, only individuals with smoking abstinence confirmed at a given follow-up were considered abstinent; those with missing data were considered nonabstinent. In this way, our reported abstinence rates correspond to the reporting of abstinence in most smoking cessation trials and allow for cross-study comparisons. However, in the GEE analyses, we also ran analyses in which no assumptions were made about missing data, using only available data for each participant. Those analyses excluded the six participants who provided no follow-up data. Results using no assumptions regarding missing data were highly concordant with those using a worst-case assumption and are therefore not detailed here.

Smoking outcomes

Figure 1 demonstrates the point-prevalence rates by medication condition for each of the three genotype dichotomies. In all three panels, the main effect of bupropion across genotype is clear, although the effect weakens by 12 months. The differences in outcomes between genotypes are small in both conditions. The most notable exception to this pattern is for outcome at 6 months, in which those with one A1 allele who received bupropion showed little treatment effect compared with those who received placebo.

In the first GEE model, the linear effect of time ($B=-0.37$, $SE=0.05$, $OR=0.69$, $p<.0001$) and higher FTND scores ($B=-0.16$, $SE=0.062$, $OR=0.85$, $p=.001$) were associated with lower odds of abstinence, whereas bupropion, compared with placebo, was associated with significantly greater odds of abstinence ($B=0.76$, $SE=0.22$, $OR=2.14$, $p=.0007$). None of the genotype dichotomies were associated with the overall odds of abstinence across the end of treatment and follow-up (p values $>.20$). Likewise, in the second set of models, none of the medication $\times$ genotype interactions were significant when added to the models (p values $>.40$). In the third set of models, none of the medication $\times$ genotype $\times$ time interactions approached significance (p values $>.40$). However, outcomes diverged at 6 months in the bupropion $\times$ *DRD2* genotype analysis, suggesting that among those with the *DRD2*-Taq1A2/A2 genotype, those on bupropion continued to have markedly higher quit rates than those on placebo, whereas virtually no difference was observed between treatment groups in the A1 allele carriers. Therefore, to examine, in preliminary fashion, whether gene $\times$ gene and gene $\times$ treatment interactions were present at the 6-month follow-up, we ran a separate logistic regression predicting abstinence at that follow-up. Point prevalence of abstinence at 6 months following the end of treatment is plotted for all follow-up time points based on treatment assignment and genotype in Figure 1.

The logistic regression predicting abstinence at 6 months included FTND score, medication condition, *DRD2*-Taq1A genotype, and the genotype $\times$ bupropion interaction. The overall model was significant, $-2LL=289.65$, $\chi^2(4)=16.1$, $p=.003$. In this model, the medication $\times$ genotype interaction was significant ($B=1.49$, $SE=0.59$, $p=.012$), and its inclusion in the model improved the percent predicted correctly from 61.7% to 63.3%, compared with a model with only main effects. However the 0.05 alpha level used in this study, Bonferroni adjustment for multiple comparisons resulted in a critical p value of .0166 (for tests of three genes). Thus, the *DRD2*-Taq1A genotype $\times$ bupropion interaction was significant. Model coefficients indicated that the effect of bupropion in those with at least one A1 allele was nonsignificant ($B=-0.18$, $SE=0.43$, $OR=0.83$, $p=.67$). By contrast, for those with two A2 alleles, bupropion was associated with a significantly higher odds of abstinence, compared with placebo ($B=1.31$, $SE=0.41$, $OR=3.73$, $p=.001$).

To test interactions between *DRD2* and the other genotypes, we included in a GEE model all three genotype dichotomies, FTND, bupropion condition, and interaction terms between

DRD2 and *SCL6A3* and between *DRD2* and *CYP2B6*. In this model, the interaction between *DRD2* and *CYP2B6* was significant ($B=1.43$, $SE=0.56$, $p=.01$), whereas the interaction between *DRD2* and *SCL6A3* did not approach significance ($B=-0.04$, $SE=0.45$, $p=.93$). The *DRD2* $\times$ *CYP2B6* interaction indicated that, although there was no main effect on abstinence odds for either genotype, the nature of the effect of *DRD2* changed depending on whether participants were homozygous for the *CYP2B6* C allele. When tested in a GEE model including only those homozygous for the C allele and controlling for time, bupropion, and FTND, having two A2 alleles was associated with a lower odds of abstinence ($B=-0.47$, $SE=0.25$, $OR=0.62$, $p=.06$). By contrast, among those with at least one *CYP2B6* 1459 T allele, having two A2 alleles was associated with a higher odds of abstinence ($B=0.93$, $SE=0.51$, $OR=2.51$, $p=.07$).

Given this finding, we dropped the *DRD2* $\times$ *SCL6A3* interaction term from the GEE model and added interactions between bupropion and both *DRD2* and *CYP2B6*, as well as a three-way interaction between bupropion and these genotypes. The three-way interaction was nonsignificant ($p=.75$). In Figure 2, we present the abstinence rates across follow-up regardless of bupropion condition for the four *DRD2* and *CYP2B6* combinations.

Mood and craving

In the first set of GEE models, the main effects of genotype on both negative mood and craving in the first week of quitting were nonsignificant. Of the genotype $\times$ bupropion interactions, only the *DRD2* $\times$ bupropion interaction effect on craving was significant ($B=-0.45$, $SE=0.22$, $p=.038$). This result suggests that bupropion, compared with placebo, was associated with a relatively greater reduction in craving for those homozygous for the A2 allele compared with those with at least one A1 allele. All three genotype $\times$ bupropion $\times$ time interactions were nonsignificant.

Discussion

As demonstrated consistently in other clinical trials (Hughes, Stead, & Lancaster, 2004), including the larger trial population from which these participants were drawn (Brown et al., 2007), bupropion was effective for smoking cessation across all follow-up time points post quit date, with attenuation by 12 months. However, our primary line of enquiry was the examination of potential gene $\times$ bupropion interactions on sustained abstinence following a quit attempt. As noted, the differences in outcomes across all time points between genotype dichotomies were small in both the bupropion and placebo conditions for all three candidate gene variants (*DRD2*-Taq1A, *SLC6A3* 3' VNTR, *CYP2B6* 1459 C $\rightarrow$ T). However, outcomes diverged at 6 months according to *DRD2* genotype. Specifically, participants who received bupropion and possessed the *DRD2*-Taq1A2/A2 genotype were nearly three times as likely to be abstinent from smoking at the 6-month follow-up, compared with their A2/A2 counterparts who received placebo. In contrast, bupropion did not appear to be effective among participants with at least one A1 allele.

It is quite conventional to analyze outcomes at the end of treatment and more distant posttreatment follow-ups separately, as seen in other pharmacogenetic trials (Lerman et al., 2003; Lerman, Shields et al., 2002; Swan et al., 2005; Yudkin et al., 2004). This is the case because after cessation of treatment, genes would not, by definition be interacting with treatment per se, but rather would be interacting with persistent neuropsychological adaptations resulting from treatment. Therefore, it would be biologically plausible for divergence of gene $\times$ "treatment" effects, between the end of treatment and subsequent follow-up. Even so, the precise mechanisms underlying the *DRD2* $\times$ bupropion interaction on 6-month point prevalence from smoking cannot be determined from this study.

In the present study, participants with two A2 alleles treated with bupropion demonstrated lower self-reported cigarette craving than those with one or more A1 alleles who were treated with bupropion. These results are consistent with a nested, laboratory-based substudy from this clinical trial indicating that the *DRD2*-Taq1A2/A2 genotype predicted a reduction in cessation-related craving (David et al., 2003) and cue-elicited craving among smokers attempting to quit with bupropion (David et al., 2001). However, the gene $\times$ treatment $\times$ craving interaction demonstrated early in the treatment phase, during the period of heightened nicotine withdrawal, does not explain why this interaction would affect 6-month smoking cessation outcomes. It is conceivable that, combined with the intensive CBT, persistent biobehavioral neuroadaptations translated into less persistent cigarette craving beyond the treatment phase combined with better coping skills for resisting and preventing relapse triggers. However, this possibility is purely speculative given that we do not have self-reported craving or coping data at the 6-month follow up. That said, Hays and colleagues (2001) demonstrated that bupropion was effective in preventing relapse as long as 6 months beyond the end of treatment. Examination of the neurobehavioral mechanisms underlying the observed *DRD2* $\times$ bupropion $\times$ craving interaction, if robust, will require collection of additional cognitive and affective data well beyond the end of treatment and might be augmented by application of functional neuroimaging.

The mechanisms underlying the observed *DRD2* $\times$ *CYP2B6* interaction may not be intuitively apparent. As noted in the introduction, the hepatic P450 isoenzyme *CYP2B6* contributes to nicotine metabolism, particularly during states of high serum nicotine, such as the period immediately following cigarette smoking (Yamanaka et al., 2005). Individuals with one or more *CYP2B6* 1459 T alleles have been shown to express *CYP2B6* at substantially lower levels than C/C counterparts in liver (Lang et al., 2001) and brain (Miksys et al., 2003). Furthermore, individuals with slow nicotine metabolism—resulting from specific variants in the *CYP2A6* gene—tend to smoke fewer cigarettes per day and are more likely to quit smoking than normal or rapid metabolizers (Munafò et al., 2004; Schoedel, Hoffmann, Rao, Sellers, & Tyndale, 2004). Given the lower expression of the *CYP2B6* enzyme in *CYP2B6* 1459 T allele carriers, it is conceivable that these individuals would be slower metabolizers of nicotine and subsequently smoke fewer cigarettes than their C/C counterparts. Therefore, it is not surprising that the individuals demonstrating the highest long-term abstinence rates would be those with *DRD2*-Taq1A A2/A2 genotypes (conferring relatively “normal” striatal dopamine set points) and *CYP2B6* 1459 C $\rightarrow$ T alleles, the latter perhaps resulting in lower nicotine tolerance and cigarette consumption. Although we do not report the analysis here, significant differences were found in total FTND score or number of cigarettes per day according to *CYP2B6* 1459 C $\rightarrow$ T genotype (p values $>.10$).

Another plausible explanation would be that altered nicotine metabolism resulting from the *CYP2B6* 1459 C $\rightarrow$ T polymorphism would affect accumbal dopamine release, potentially influencing the neuroadaptations resulting in incentive sensitization to nicotine. If this were the case, the *DRD2*-Taq1A genotype would potentially interact with the *CYP2B6* 1459 C $\rightarrow$ T genotype at the level of the nucleus accumbens. Again, such a mechanism cannot be concluded based on the data in the present study but would require the combination of pharmacogenetic and functional neuroimaging for resolution in future work.

The present results differ somewhat from those of existing pharmacogenetic studies of bupropion. In particular, Lerman and colleagues (2003) examined the role of the *SLC6A3* 3' VNTR and *DRD2*-Taq1A polymorphisms in a clinical trial of bupropion with a design and sample similar to those in the present study. No significant genotype $\times$ treatment effects on prolonged abstinence or 7-day point prevalence were observed at the end of treatment (10–12 weeks) or 6-month follow-up. Instead, a *SLC6A3* $\times$ *DRD2* interaction predicted prolonged abstinence at the end of treatment (independent of treatment condition), an effect not seen in

the present study. In addition, as described above, bupropion is metabolized by cytochrome P450 2B6 (CYP2B6) enzyme. In another examination from the same trial (Lerman, Shields et al., 2002), the *CYP2B6* 1459 C allele increased the likelihood of smoking relapse; however, the effect was attenuated by bupropion among women. In the present study, the *CYP2B6* C→T substitution did not predict prolonged abstinence as a main effect or interaction with bupropion treatment. Thus, although consistent with one prior study suggesting a gene × bupropion effect on craving, the results of the present study differ from the Lerman, Shields et al. (2002) study with regard to the effects of the *CYP2B6* 1459 C→T polymorphism on smoking cessation.

Certain limitations to the present study should be highlighted. The present sample size was modest, which will have reduced the power of our study and may have limited the generalizability of our results, particularly given the somewhat specific demographics of our participant sample. Approximately 60% of the subjects in the original clinical trial agreed to blood collection for genotyping. Although the subject characteristics appeared to be quite similar between those who agreed to genotyping and those who did not, other differences between these groups may not have been ascertainable. However, compared with other pharmacogenetic studies using retrospective genotyping, our study had a blood collection rate within the norm (David et al., 2002; Johnstone et al., 2004; Yudkin et al., 2004).

Finally, the intervention used was more intensive than might be typical, including guided group therapy. A usual consequence of such intensive therapy is an increase in quit rates, and if a more typical treatment model were used, the attenuation in global quit rates might also attenuate the genotype × treatment interaction effect reported here. This issue should be addressed in future studies that attempt to replicate our findings.

The precise mechanism of action of bupropion remains unclear (Ascher et al., 1995), but it is widely accepted that it acts (although not exclusively) on the dopaminergic pathway, serving to inhibit reuptake. For this reason, dopaminergic genes represent good candidates for the pharmacogenetic study of potential genetic moderators of bupropion treatment efficacy.

Our data support the view that likely genetic moderators will consist, at least in part, of genes that influence dopaminergic biosynthesis and receptor systems. It is somewhat surprising, however, given this theoretical perspective, that we did not demonstrate an effect of the dopamine transporter on treatment response. However, multiple candidate polymorphisms would ideally be included in a comprehensive pharmacogenetic evaluation of bupropion and dopamine system genes.

This point brings up one of the weaknesses in the present study's design, namely that the functional significance of the *DRD2*-Taq1A RFLP and the *SLC6A3* 3' VNTR remains speculative. As noted previously, the Taq1A RFLP is present in the *ANKK1* gene (approximately 10 kb 3' from the end of the *DRD2* gene) rather than in the *DRD2* gene itself (Neville et al., 2004). Jonsson and colleagues (1999) demonstrated that the *DRD2*-Taq1A polymorphism influences striatal *DRD2* receptor density, but the mechanisms underlying how a polymorphism in an adjacent tyrosine kinase gene affects striatal D2 receptor expression are not known. Indeed, two functional SNPs shown to influence transcriptional efficiency—*DRD2* -141 ins/del (Arinami et al., 1997) and *DRD2* C957T (Duan et al., 2003)—may also affect bupropion efficacy and, ideally, should be considered in future exploration of *DRD2* × bupropion interactions on smoking cessation.

Furthermore, even though the *SLC6A3* 3' VNTR does not appear to affect expression of the transporter *in vitro* (Greenwood & Kelsoe, 2003; Mill, Asherson, Craig, & D'Souza, 2005), other SNPs and mutations do appear to influence dopamine transporter expression (Horschitz, Hummerich, Lau, Rietschel, & Schloss, 2005; Kelada et al., 2005; Lin & Uhl, 2003). This observation may account in part for the lack of replication in studies of associations between

smoking phenotypes and the *SLC6A3* 3' VNTR polymorphism (Bierut et al., 2000;Jorm et al., 2000;Lerman et al., 1999;Sabol et al., 1999;Vandenbergh et al., 2002). Also, other reasonable SNP candidates in the *CYP2B6* gene should be considered for study. Thus, until future studies are designed with sufficient statistical power to include all known functional SNPs in genes throughout the dopamine system, conclusions on the presence or absence of these gene $\times$ gene interactions are premature.

Interest is growing in other potential moderators of treatment efficacy, most notably possible sex differences in treatment response to bupropion (Scharf & Shiffman, 2004) and nicotine replacement therapy (Munafò et al., 2004a;Shiffman, Sweeney, & Dresler, 2005). However, the investigation of multiple potential treatment moderators brings with it the usual dangers associated with multiple statistical testing and should not proceed without firm theoretical grounds for investigating specific variables (Munafò, Johnstone, Murphy, & Walton, 2001). In this respect, dopaminergic genes represent strong candidates, but if multiple candidates are to be investigated simultaneously this will require explicitly designed pharmacogenetic studies adequately powered to detect effect sizes likely to be modest.

The increased understanding of the mechanisms of nicotine addiction and smoking behavior that has developed from the study of genetic influences on these phenotypes has led to a growing interest in the possibility of offering personalized smoking cessation therapy to patients based on their genotype (Audrain et al., 1997;Lerman & Niaura, 2002). Our data reinforce this possibility, in particular because the patients in the present study who possessed at least one copy of the A1 allele did not appear to gain any benefit from bupropion relative to placebo. Any strong claims regarding translational implications of our research would be premature, however, and these data should be regarded as preliminary until replicated. Even assuming that such replication does occur, the potential implication of these data—that there might be grounds for not offering bupropion treatment to those statistically unlikely to benefit on the basis of their genotype—brings with it profound ethical, social, and policy implications. Not least, the acceptability of such an approach to those seeking treatment for smoking cessation remains to be determined.

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Figure 1.

Smoking cessation outcomes by treatment assignment and genotype. Biochemically verified 7-day point prevalence of abstinence at end of treatment (EOT) and at 2, 6, and 12 months are presented for participants randomized to bupropion or placebo according to genotype for (a) *SLC6A3* 3' VNTR, (b) *DRD2* Taq1A1/A2, and (c) *CYP2B6* 1459 C→T genotypes.

Figure 2.

DRD2 × *CYP2B6* interaction on 6-month abstinence. Biochemically verified 7-day point prevalence of abstinence at 6 months for subjects with *CYP2B6* 1459 C/C, T/T, or C/T alleles according to *DRD2*-Taq1A A1/A1 or A1/A2 and A2/A2 alleles.

Table 1

Baseline covariate distribution by treatment arm.

	Placebo		Bupropion		Overall		p value
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	
Female gender, per cent	49.67	NA	53.03	NA	51.24	NA	.66
Combined group therapy, per cent	40.40	NA	35.61	NA	38.16	NA	.48
Age, years	45.92	10.94	45.03	10.53	45.51	10.74	.49
Age at onset of smoking, years	14.61	3.03	14.48	4.38	14.55	3.71	.77
Years of smoking	31.31	11.07	30.66	10.57	31.01	10.83	.61
FTND score	6.42	1.89	6.23	1.83	6.33	1.86	.38
Number of quit attempts	3.34	2.27	3.27	2.31	3.30	2.28	.80
Longest quit period	3.43	3.41	4.13	4.52	3.79	4.00	.50
Cigarettes/day	23.87	9.08	22.55	7.86	23.26	8.54	.19
CES-D score	5.93	6.61	6.68	6.82	6.28	6.70	.35

Note. CES-D=Center for Epidemiological Studies on Depression scale; FTND=Fagerström Test for Nicotine Dependence; NA=not applicable.