Title

Does sex alter the relationship between *CYP2B6* variation, hydroxybupropion concentration, and bupropion-aided smoking cessation in African Americans? A moderated mediation analysis.

Authors

Meghan J. Chenoweth, PhD[1,2], Annie R. Peng, PhD[1,2], Andy Z. X. Zhu, PhD[1,2], Lisa Sanderson Cox, PhD[3], Nikki L. Nollen, PhD[3], Jasjit S. Ahluwalia, MD, MPH[4], Neal L. Benowitz, MD[5], Jo Knight, PhD[6.7], Walter Swardfager, PhD[2,8], Rachel F. Tyndale, PhD[1,2,7]

Affiliations

[1]Campbell Family Mental Health Research Institute, Centre for Addiction and Mental Health, Toronto, Ontario, Canada

[2]Department of Pharmacology and Toxicology, University of Toronto, Toronto, Ontario, Canada

[3]Department of Population Health, University of Kansas School of Medicine, Kansas City, Kansas, USA

[4]Departments of Behavioral and Social Sciences and Medicine, Brown University, Providence, Rhode Island, USA

[5]Department of Medicine, University of California, San Francisco, San Francisco, California, USA

[6]Data Science Institute, Lancaster University Medical School, Lancaster, England, UK

[7]Department of Psychiatry, University of Toronto, Toronto, Ontario, Canada

[8]Sunnybrook Research Institute, Sunnybrook Health Sciences Centre, Toronto, Ontario, Canada

Corresponding Author

Dr. Rachel F. Tyndale

Medical Sciences Building Room 4326

Department of Pharmacology and Toxicology, University of Toronto

1 King's College Circle

Toronto, ON, Canada, M5S 1A8

Telephone: 416-978-6374

Fax: 416-978-6395

E-mail: r.tyndale@utoronto.ca

Running head: Hydroxybupropion mediates smoking cessation

Declaration of competing interests

R. F. Tyndale has consulted for Quinn Emanuel and Ethismos. Dr. Benowitz has consulted with Achieve Life Sciences and Pfizer, companies that market or are developing smoking cessation medications, and has been a paid expert witness in litigation against tobacco companies. The other authors declare no conflicts of interest. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Funding

This work was funded by CIHR Catalyst T2023973 (Tyndale, Chenoweth, Swardfager, Knight) and Foundation FDN-154294 (Tyndale) grants, NIH grants CA091912 (Sanderson Cox, Nollen, Ahluwalia), DA031815 (Sanderson Cox, Nollen, Ahluwalia), and COBRE P20GM130414 (Ahluwalia), a Canada Research Chair in Pharmacogenomics (Tyndale), and the Campbell Family Mental Health Research Institute (Tyndale, Chenoweth), CAMH.

Keywords: Pharmacogenetics, CYP2B6, Smoking Cessation, Bupropion, Hydroxybupropion, Sex Differences, Mediation Analyses

Abstract

Background and Aims: CYP2B6, a genetically variable enzyme, converts bupropion to its active metabolite hydroxybupropion. CYP2B6 activity and bupropion-aided cessation differ between women and men. The aim of this study was to determine whether genetically normal (vs. reduced) *CYP2B6* activity increases bupropion-aided cessation in African American smokers via higher hydroxybupropion concentration, and whether this differs by sex. **Design and setting:** Secondary analysis of a smoking cessation clinical trial (NCT00666978). **Participants/Cases:** African American light-smokers (≤10 cigarettes/day). **Interventions:** Participants were treated with bupropion for 7 weeks. **Measurements:** Participants with detectable bupropion and/or hydroxybupropion concentrations were divided into normal (n=64) and reduced (n=109) *CYP2B6* activity groups based on the presence of reduced-function *CYP2B6*6* and *CYP2B6*18* alleles. Biochemically-verified smoking cessation was assessed at week 3, end-of-treatment (7 weeks), and follow-up (26 weeks). **Findings:** Normal (vs. reduced) *CYP2B6* activity was associated with increased cessation at week 7, which was mediated by higher hydroxybupropion concentration (odds ratio (OR)=1.25, 95% confidence interval (CI)=1.03, 1.78); this mediation effect persisted at week 26 (OR=1.23, 95% CI=1.02, 1.70). The mediation effect was similar in women (n=116; OR=1.33, 95% CI=1.01, 2.30) and men (n=57; OR=1.33, 95% CI=0.92, 3.87). Moreover, sex did not appear to moderate the mediation effect, although this should be tested in a larger sample. **Conclusions:** In African American light-smokers with verified early bupropion use, genetically normal *CYP2B6* activity appears to be indirectly associated with greater smoking cessation success in a relationship mediated by higher hydroxybupropion concentration. The mediating effect of higher hydroxybupropion concentration on smoking cessation persists beyond the active treatment phase and does not appear to differ by sex.

INTRODUCTION

Cigarette smoking remains a leading cause of preventable illness and death (1). There are three FDA-approved pharmacotherapies (nicotine replacement therapy, bupropion, and varenicline) for treating nicotine dependence but <25% of smokers achieve long-term cessation (2, 3). Tailored approaches considering individual variation in treatment response may improve therapeutic outcomes (4).

Cigarette smoking behaviours, including cessation outcomes, differ between women and men (5, 6). Women have higher quit rates on varenicline compared to nicotine patch or bupropion, while men have similar quit rates on all three treatments (5). In evaluating treatment effects against placebo, women have relatively lower quit success on bupropion and the nicotine patch compared to men, but equivalent quit success on varenicline (5). In a separate study, women had relatively lower odds of quitting than men in both the bupropion and placebo arms (6).

Smoking behaviours are also influenced by genetics. Smoking cessation is ~50-60% heritable (7) and genetic studies have highlighted numerous loci associated with smoking-related traits (8). For example, variation in pharmacogenes (e.g. *CYP2B6*) may influence cessation (9). *CYP2B6* is genetically polymorphic (see pharmvar.org/gene/CYP2B6), with many function-altering variants including the prevalent (~15-60% frequency) reduced-function *CYP2B6*6* allele (10). The CYP2B6 enzyme metabolically activates bupropion to hydroxybupropion (11). Hydroxybupropion can be further metabolized via glucuronidation by UGT enzymes (12). CYP2B6 metabolizes other drugs such as efavirenz, methadone, ketamine, and propofol, and the Clinical Pharmacogenetics Implementation Consortium (CPIC) recently published a guideline for efavirenz dosing based on *CYP2B6* genotype (10).

CYP2B6 can be induced by estradiol (13), and higher CYP2B6 mRNA and protein levels have been observed in the livers of females compared to males (14, 15), suggesting women, especially in higher estradiol states (e.g. pregnancy), may have faster CYP2B6 activity compared to men. In pharmacokinetic studies, higher hydroxybupropion concentrations were observed in women compared to men following 7 days of bupropion (16), and after single bupropion dosing in adolescents (17) but not adults (18). The

influence of *CYP2B6* variation on CYP2B6 expression and activity may also differ by sex (14). A sex difference in the effect of a genetic variant on expression and activity of *CYP3A4*, another pharmacogene, has also been observed (19). CPIC treatment guidelines do not consider potential sex differences in pharmacogenetic effects on treatment response (10).

In this study, we leveraged data from the Kick-it-at-Swope (KIS)-3 clinical trial, which evaluated bupropion efficacy in African American light-smokers (≤10 cigarettes/day) (20). African Americans are more likely to attempt to quit smoking but have lower success (21) and disproportionately higher rates of tobacco-related morbidity and mortality (22, 23), compared to European Americans. The vast majority of genomics research has analyzed participants of European ancestry (24) and has not investigated sex differences (25).

In KIS3, quit rates did not differ between the bupropion (13.3%) and placebo (10.0%) arms at 6 months (20). The high frequency of the *CYP2B6*6* allele in African Americans (26, 27), which reduces the amount of active hydroxybupropion formed (11), may have contributed to the lack of overall efficacy. We previously showed 1) *CYP2B6* variation influences hydroxybupropion concentration, and 2) hydroxybupropion concentration predicts abstinence (11); however, we did not investigate sex differences. Therefore, we used mediation analysis as a novel approach to examine whether genetically normal (vs. reduced) *CYP2B6* activity increases cessation via higher hydroxybupropion concentration. We posited that potential sex differences in hydroxybupropion formation (16) and/or gender differences in quitting (5, 6) could affect relationships between *CYP2B6* variation and cessation. Therefore, we also examined whether the mediating effect of hydroxybupropion on cessation differed between women and men.

METHODS

Study participants

Kick-it-at-Swope (KIS3; NCT00666978) Smoking Cessation Clinical Trial

This was a secondary analysis of data from KIS3 (20). KIS3 was a single-centre trial (University of Kansas Medical Center) in n=540 smokers randomized to placebo (n=270) or bupropion SR (150mg twice/day) (n=270). Participants were light-smoking (≤10 cigarettes/day (20)) adults of self-identified African American ancestry, and >95% of participants had genetic ancestries concordant with self-identified ancestry (28). Light-smokers represent a growing segment of the smoking population, particularly among African Americans (29). Participants who provided written informed consent for DNA collection and genotyping were included in analyses. Procedures were approved by IRBs at the University of Toronto, University of California, San Francisco, and University of Kansas (20). The current analyses included participants with biochemically verified early (i.e. at week 3, two weeks following the target quit date) bupropion use (n=173), as described below.

Biomarker Measurements and Smoking Abstinence

The concentrations of bupropion and hydroxybupropion (half-lives of ~10-20h and ~20h, respectively (30-32) were measured from plasma samples collected at week 3 (11). The limit of detection for each assay was 1 ng/ml. Self-reported smoking abstinence at week 7 (i.e. end-of-treatment) was biochemically verified using salivary cotinine (≤15 ng/ml) using established LC-MS/MS assays (20). In the bupropion arm (n=270), 175 (64.8%) had verified bupropion use at week 3, defined as quantifiable bupropion and/or hydroxybupropion plasma concentrations (≥1 ng/ml of either analyte); two participants were excluded due to missing *CYP2B6* data (n=1) or baseline cotinine data (n=1), yielding a final analytic sample of n=116 women and n=57 men. Participants were analyzed as intention-to-treat, whereby those with cotinine values >15 ng/ml and those lost to follow-up were considered non-abstinent (20). In addition to week 7 (i.e. end-of-treatment), as secondary end-points, we examined cotinine-verified abstinence at

week 3, when bupropion and hydroxybupropion concentrations were assessed, and at week 26, to test whether any mediating effects on abstinence extended beyond the active treatment phase.

CYP2B6 Genotyping and Activity Group Assignments

Participants were genotyped for *CYP2B6*6* and *CYP2B6*18*, which are common in African ancestry populations (~33-68% and ~4-12% frequency, respectively (26, 27)). *CYP2B6*6* contains both the c.785A>G and c.516G>T single nucleotide variants (SNVs) (see pharmvar.org/gene/CYP2B6). The *CYP2B6*6* haplotype has been assigned reduced function by CPIC (10). The *CYP2B6*18* allele contains the core SNV c.983T>C and is associated with null function (10). Genotyping was performed at the University of Toronto using established two-step PCR assays. *CYP2B6*6* and *CYP2B6*18* genotypes were determined using a previously validated haplotyping assay (genotypes for c.785A>G and c.516G>T) and SNV genotyping assay (genotypes for c.983T>C), respectively. The first step amplifies the *CYP2B6* gene (to reduce confounding by the *CYP2B7* pseudogene), while the second step amplifies the specific allele(s). Full details are described elsewhere (9, 11).

Individuals with no copies of the reduced-function **6* or **18* alleles were grouped as *CYP2B6* normal metabolizers, those with one copy of **6* or **18* were grouped as *CYP2B6* intermediate metabolizers, while those with two copies of **6* and/or **18* were grouped as *CYP2B6* slow metabolizers (11). For the main analyses, intermediate and slow metabolizers were combined into a single group (i.e. reduced metabolizers) to increase power, however we also examined the three metabolism groups separately.

Descriptive Statistics

Categorial variables were compared using Pearson Chi-square tests, while continuous variables were compared using Mann-Whitney U tests.

Mediation Path Analysis

Inferential bias-corrected bootstrapping procedures (10,000 replications) were used to assess an indirect effect of *CYP2B6* activity group (coded as 2=normal, 1=reduced) on abstinence at week 7 (coded as 1=abstinent, 0=still smoking) through hydroxybupropion concentration (in µg/ml). The indirect effect is the product of two unstandardized linear regression coefficients: the first is the effect of X (*CYP2B6* genotype) on the mediator (hydroxybupropion concentration) (i.e. path a), and the second is the effect of the mediator on Y (abstinence at week 7) (i.e. path b) in a model controlling for X. Secondary analyses examined week 3 and week 26 abstinence. The direct effect measured the effect of X on Y (i.e. path c') after controlling for the mediation effect and covariates. Analyses were conducted using Model #4 in PROCESS (version 2.16), a macro implementation of moderation and mediation analyses for SPSS (version 27; IBM, Armonk, New York, USA) (33, 34). PROCESS was written by Dr. Andrew F. Hayes. A mediation effect was considered significant if the bias-corrected 95% confidence interval did not contain 0. These analyses were restricted to individuals who had detectable bupropion and/or hydroxybupropion concentrations (measured at week 3). Covariates in the main model included age, mentholated cigarette smoking, and baseline cotinine concentration (14), regressed against both the mediator and outcome. Secondary models controlled for 1) smoking duration and the number of study visits attended (35), and 2) the total number of bupropion pills taken during the past three days (assessed at week 3 via timeline follow-back). The total effect (path c) was calculated separately using logistic regression models (controlling for covariates) as the total effect model was not available in PROCESS for dichotomous Y variables. Mediation analyses were conducted in the total group (controlling for sex) and after stratifying by sex. We also conducted a moderated mediation analysis in the total group using Model #7 in PROCESS (version 2.16) that tested for sex moderation of the effect of *CYP2B6* metabolism group on hydroxybupropion concentration.

Sensitivity Analyses

We conducted four sensitivity analyses in the full sample. Sensitivity analyses included age, mentholated cigarette smoking, baseline cotinine concentration, and sex as covariates. In sensitivity analysis #1, we tested whether *CYP2B6* activity group (normal versus reduced) interacted with hydroxybupropion concentration to influence the likelihood of abstinence at weeks 3, 7, and 26 using logistic regression: the model included main effects of *CYP2B6* activity group (normal versus reduced) and hydroxybupropion concentration, and a *CYP2B6* activity group x hydroxybupropion interaction term. In sensitivity analysis #2, we split the *CYP2B6* reduced metabolism group into intermediate and slow metabolizers. We then assessed an indirect effect of *CYP2B6* activity (coded as 3=normal, 2=intermediate, and 1=slow) on abstinence at weeks 3, 7, and 26 through hydroxybupropion concentration. To determine whether our results were robust to relatively low levels of adherence, in sensitivity analysis #3, we excluded participants with hydroxybupropion concentrations <100 ng/ml and evaluated the indirect effect of *CYP2B6* activity (i.e. normal versus reduced) on abstinence at weeks 3, 7, and 26 through hydroxybupropion. In sensitivity analysis #4, we tested bupropion concentration (in μg/ml) as the mediator, and examined an indirect effect of *CYP2B6* activity group (normal versus reduced) on abstinence at weeks 3, 7, and 26 through bupropion concentration.

The analysis plan was not pre-registered on a publicly available platform, therefore the results should be considered exploratory.

RESULTS

Participant Characteristics

Characteristics of the final analytic sample (n=173) are shown in **Table 1**. A similar proportion of women (67.8%) and men (59.4%) demonstrated early bupropion use (P=0.16). There were no differences between the women and men in age, baseline cotinine concentration, menthol cigarette smoking, *CYP2B6* group distribution, hydroxybupropion concentration, or abstinence rates (**Table 1**). There were 64 normal metabolizers, 76 intermediate metabolizers, and 33 slow metabolizers (i.e. 109 reduced metabolizers). Abstinence rates at weeks 3, 7, and 26 are shown in **Table S1** and participant characteristics according to *CYP2B6* group are shown in **Table S2.** The distribution of hydroxybupropion concentration is shown in **Figure S1**. Hydroxybupropion concentration did not differ between women (median 543 ng/ml) and men (570 ng/ml) (Mann-Whitney U test P=0.88). The influence of *CYP2B6* activity group on hydroxybupropion concentration is shown for the total group (**Figure S2**) and after stratifying by sex and age (**Figure S3**). The ratio of hydroxybupropion to bupropion (both measured in ng/ml), a marker of CYP2B6 activity, was higher in women versus men (18.8 vs. 12.5, respectively; Mann-Whitney U test P=0.05). In a logistic regression model evaluating end-of-treatment (week 7) abstinence, there was a main effect of *CYP2B6* activity group (normal vs. reduced; OR=3.40, 95% CI=1.01, 11.38), no main effect of sex (OR=1.67, 95% CI=0.64, 4.36), and no interaction effect (*CYP2B6* activity x sex; OR=0.23, 95% CI=0.05, 1.01).

Mediation Path Analysis

In the analysis of women and men together, there was a significant indirect effect of *CYP2B6* activity on week 7 abstinence, mediated by hydroxybupropion concentration; genetically normal *CYP2B6* activity was associated with a greater likelihood of abstinence in a relationship mediated by higher hydroxybupropion concentration (OR=1.25, 95% CI=1.03, 1.78) **(Figure 1 and Table 2)**. When including smoking duration and number of study visits attended as additional covariates, the indirect effect did not

change (OR=1.23, 95% CI=1.02, 1.78) **(Table 2)**. Moreover, additionally controlling for the total number of bupropion pills taken during the past 3 days did not alter the indirect (i.e. mediation) effect (OR=1.21, 95% CI=1.01, 1.69), nor the relationship between *CYP2B6* activity and hydroxybupropion concentration (P=0.002) **(Table 2)**. The indirect effect of *CYP2B6* activity group on abstinence was also significant at week 3 (OR=1.27, 95% CI=1.05, 1.71) and week 26 (OR=1.23, 95% CI=1.02, 1.70) **(Table 2)**. As expected, *CYP2B6* activity group was not directly associated with abstinence after controlling for the mediation effect and covariates (OR at week 7=0.99, 95% CI=0.47, 2.05; P=0.97) **(Table 2)**; we anticipated that any influence of *CYP2B6* genetic variation on cessation would occur indirectly via altered hydroxybupropion concentrations. The total effect (path c; i.e. the influence of *CYP2B6* activity on abstinence after controlling for covariates) was also not significant; importantly, this lack of association does not preclude the conduction of mediation analysis, nor the interpretation of the indirect effect (36).

Sensitivity Analyses

In sensitivity analysis #1, which tested for an interaction between *CYP2B6* activity group and hydroxybupropion concentration on abstinence, no interaction was observed at weeks 3, 7, or 26: the P-values for the interaction term (*CYP2B6* activity group x hydroxybupropion concentration) at weeks 3, 7, and 26 were 0.89, 0.79, and 0.81, respectively. In sensitivity analysis #2, where *CYP2B6* activity was evaluated as three groups (normal vs. intermediate vs. slow), the indirect effect of *CYP2B6* activity on abstinence remained significant **(Table 2)**. The indirect effects were OR=1.18 (95% CI=1.04, 1.45), 1.18 (95% CI=1.03, 1.48), and 1.16 (95% CI=1.02, 1.43) at weeks 3, 7, and 26, respectively. Sensitivity analysis #3 excluded participants with hydroxybupropion concentrations <100 ng/ml (n=35) (**Figure S1**). In the subset of participants with hydroxybupropion concentrations ≥100 ng/ml (n=138), a significant indirect effect of *CYP2B6* activity on abstinence was observed at weeks 3 and 7 **(Table 2)**. The indirect effects were OR=1.25 (95% CI=1.02, 1.74), 1.24 (95% CI=1.01, 1.85) and 1.13 (95% CI=0.89, 1.53) at weeks 3, 7, and 26, respectively. In sensitivity analysis #4, which tested for a mediating effect of bupropion

concentration on abstinence, we found no evidence of mediation **(Table 2)**: the indirect effect of *CYP2B6* activity on abstinence via bupropion concentration was not significant at weeks 3 (OR=0.95, 95% CI=0.77, 1.02), 7 (OR=0.95, 95% CI=0.83, 1.06), or 26 (OR=0.91, 95% CI=0.76, 1.07).

Examination of Potential Sex and Gender Differences

When we conducted the mediation path analysis in women and men separately, the indirect effect of *CYP2B6* activity group on week 7 abstinence was similar in women (OR=1.33, 95% CI=1.01, 2.30) and men (OR=1.33, 95% CI=0.92, 3.87) **(Table 2)**. There was no direct effect of *CYP2B6* activity on abstinence in women (P=0.19) or men (P=0.09) after controlling for the indirect effect and covariates **(Table 2)**. Because of the effect of estradiol on inducing CYP2B6 (13), we ran an exploratory sub-group analysis comparing women aged ≤50 years (n=66) to those aged >50 years (n=50), age groups enriched for pre- and post-menopausal women, respectively (37). The overall quit rate at week 7 was 26% in the younger women compared to 34% in the older women (P=0.33). The median hydroxybupropion concentration was 480 ng/ml in younger women and 604 ng/ml in older women (Mann-Whitney U test P=0.11). To test formally for age (≤50 years versus >50 years) moderation of the effect of *CYP2B6* activity on hydroxybupropion concentration in women, we performed a moderated mediation analysis, where there was no evidence of age moderation (index of moderated mediation 0.16, 95% CI=-0.30, 0.94) **(Table 2).**

We also ran a moderated mediation analysis to test formally for sex moderation of the effect of *CYP2B6* activity on hydroxybupropion concentration. The indirect effect was significant in women (OR=1.30, 95% CI=1.03, 1.98) but not men (OR=1.15, 95% CI=0.89, 1.71), however there was no evidence of sex moderation (index of moderation mediation 0.13, 95% CI=-0.22, 0.58) **(Table 2)**. Furthermore, there was no interaction between *CYP2B6* activity group and sex on hydroxybupropion concentration **(Figure 2)**.

DISCUSSION

In African American light-smokers with verified early bupropion use, genetically normal *CYP2B6* activity was indirectly associated with greater smoking cessation success in a relationship mediated by higher hydroxybupropion concentration. The indirect effect of *CYP2B6* activity on abstinence through hydroxybupropion concentration was significant at week 3, end-of-treatment (i.e. week 7), and at follow up (week 26), suggesting that higher hydroxybupropion concentration during the treatment phase promotes long-term smoking cessation. The magnitude of the indirect effect of *CYP2B6* activity on abstinence at end-of-treatment was similar in women and men. Moreover, there was no evidence of sex moderation of the mediation effect.

We anticipated observing a sex difference in hydroxybupropion concentration (16, 17), however hydroxybupropion concentration did not differ between women and men, and our moderated mediation model found no evidence of a sex-by-*CYP2B6* interaction effect on hydroxybupropion, although the lower number of men in the trial reduced power. We did find evidence, however, of higher CYP2B6 activity (ratio of hydroxybupropion/bupropion) among women. The lack of sex effect on hydroxybupropion concentration in our study could be due to age (57% of women were >50 years old) and/or differences in adherence. While one might expect to observe lower hydroxybupropion concentrations among older women, older smokers may be more adherent to pharmacotherapy (38), consistent with the positive association between age and hydroxybupropion concentration observed in women in this trial (**Figure S4**). However, age did not moderate the effect of *CYP2B6* on hydroxybupropion concentration in women. Moreover, the influence of *CYP2B6* activity group on hydroxybupropion concentration remained significant after controlling for the number of bupropion pills taken (measured at week 3), suggesting an influence of *CYP2B6* on hydroxybupropion concentration independent of self-reported adherence. Future studies that are powered to examine effects of age in addition to sex and *CYP2B6* genotype on CYP2B6 activity and adherence will help clarify their relative contributions to smoking cessation.

In contrast to the mediating effect of hydroxybupropion, bupropion itself did not mediate associations between *CYP2B6* activity group and abstinence. The role of hydroxybupropion in smoking cessation success is supported by preclinical studies in rats, where hydroxybupropion reduced the positive reinforcing effects of nicotine (39). For patients with reduced *CYP2B6* activity, who form less hydroxybupropion from a given bupropion dose, a higher dose of bupropion could be administered to increase hydroxybupropion concentration (11). Clinically, bupropion is also used in the treatment of major depression (30). As was found for smoking cessation, higher hydroxybupropion concentrations were associated with greater therapeutic success in depression (40). Proof-of-concept clinical trials in smoking cessation and depression could evaluate clinical outcomes following *CYP2B6* genotyped-guided bupropion dosing. For example, in smokers with elevated rates of nicotine inactivation due to fast CYP2A6 activity, a proof-of-concept trial involving nicotine replacement therapy suggested that doubling the standard dose of the nicotine patch from 21 mg to 42 mg may improve quit rates (41).

Compared to self-reported measures, measurement of drug and/or metabolite concentrations in biospecimens provides a more accurate indication of drug taking (38). Our current analyses included participants with week 3 detectable bupropion and/or hydroxybupropion concentrations; participants with undetectable bupropion but detectable hydroxybupropion concentrations were included in analyses due to the long half-life of hydroxybupropion, the ~10-fold higher concentration of hydroxybupropion relative to bupropion at steady state, and the role of hydroxybupropion in smoking cessation (11, 30). Of the 270 KIS3 participants in the bupropion arm, 65% demonstrated bupropion use at week 3. In a varenicline trial, the overall proportion of participants with detectable drug concentrations was similar (66%) (38). Low medication adherence is a challenge in smoking cessation trials, is even more prevalent in real-world settings, and predicts poorer cessation outcomes (42). Biological assessments of drug and metabolite concentrations should be completed wherever possible to accurately identify predictors of non-adherence and potential mitigation strategies (42).

Strengths of our study include the use of mediation modelling, sex-based analyses, assessment of drug and metabolite concentrations, evaluation of abstinence at multiple time-points, and sensitivity analyses that tested the robustness of our findings. Importantly, the lack of significant direct (path c') and total (path c) effects in our analysis does not affect the validity of our mediation models (36). To help support a claim of mediation (43), we included several covariates associated with hydroxybupropion concentration and/or cessation success in order to reduce confounding in our models. In addition, our mediation model satisfies the temporality requirement, as *CYP2B6* genotype is determined before birth, hydroxybupropion concentration was measured at week three, and abstinence was measured at week seven and week 26. We also ensured that the mediation effect was not better explained by a moderation effect by demonstrating no interaction between *CYP2B6* activity group and hydroxybupropion concentration. Finally, the exposure (*CYP2B6* group) was significantly associated with the mediator (hydroxybupropion concentration), the mediator was significantly associated with the outcome (abstinence), and the mediating effect was also significant, further supporting a claim of mediation (43).

A limitation of our study is the relatively small sample size, including the proportion of participants with detectable drug concentrations (65% of the bupropion arm), although this is consistent with medication use in other cessation trials (38). There were also fewer men (~34% of total KIS3 trial were men (20)). Although we did not find evidence of sex differences at week 7, we were unable to examine sex differences in the indirect effect at week 26 due to the relatively low quit rates and low number of men who were abstinent **(Table S1)**. Thus, these findings should be considered exploratory. In addition, the trial did not collect information on menstrual cycle phase or menopausal status, thus we were unable to assess their impacts. Moreover, we genotyped common reduced-function *CYP2B6* variants. Future studies in larger samples will have greater power to examine rarer function-altering *CYP2B6* variants, to detect potential sex differences, and to extend this to smokers from other ancestral backgrounds.

In conclusion, we showed that genetically normal *CYP2B6* activity is indirectly associated with greater smoking cessation success in a relationship mediated by higher hydroxybupropion concentration in African American light-smokers. The indirect effect of *CYP2B6* activity on abstinence was apparent in women and men. These findings may set the stage for *CYP2B6* genotype-guided dosing of bupropion for smoking cessation, as has been done for efavirenz-containing antiretroviral therapy (10). Our work demonstrates the utility of measuring drug and metabolite concentrations to clarify relationships between variation in pharmacogenes and clinical outcomes, and deepens our understanding of the biological underpinnings of individual variation in smoking cessation success.

Acknowledgements

The authors acknowledge Qian Zhou for genotyping.

Author Contributions

MJC, ARP, AZXZ, JK, WS, and RFT were responsible for the study concept and analysis plan. LSC, NLN, JSA, NLB, CL, and RFT oversaw the design of the original studies. MJC performed data analysis. All authors contributed to the interpretation of the findings. MJC and RFT drafted the manuscript and revised it based on co-author comments. All authors critically reviewed the manuscript and approved the final version for publication.

References

1. Jha P, Peto R. Global effects of smoking, of quitting, and of taxing tobacco. N Engl J Med. 2014 Jan 2;370(1):60-8.
2. West R. Tobacco smoking: Health impact, prevalence, correlates and interventions. Psychol Health. 2017 Aug;32(8):1018-36.
3. Cahill K, Lindson-Hawley N, Thomas KH, Fanshawe TR, Lancaster T. Nicotine receptor partial agonists for smoking cessation. Cochrane Database Syst Rev. 2016 May 9(5):CD006103.
4. Lerman C, Schnoll RA, Hawk LW, Jr., Cinciripini P, George TP, Wileyto EP, et al. Use of the nicotine metabolite ratio as a genetically informed biomarker of response to nicotine patch or varenicline for smoking cessation: a randomised, double-blind placebo-controlled trial. Lancet Respir Med. 2015 Feb;3(2):131-8.
5. Smith PH, Weinberger AH, Zhang J, Emme E, Mazure CM, McKee SA. Sex Differences in Smoking Cessation Pharmacotherapy Comparative Efficacy: A Network Meta-analysis. Nicotine Tob Res. 2017 Mar 1;19(3):273-81.
6. Scharf D, Shiffman S. Are there gender differences in smoking cessation, with and without bupropion? Pooled- and meta-analyses of clinical trials of Bupropion SR. Addiction. 2004 Nov;99(11):1462-9.
7. Broms U, Silventoinen K, Madden PA, Heath AC, Kaprio J. Genetic architecture of smoking behavior: a study of Finnish adult twins. Twin Res Hum Genet. 2006 Feb;9(1):64-72.
8. Liu M, Jiang Y, Wedow R, Li Y, Brazel DM, Chen F, et al. Association studies of up to 1.2 million individuals yield new insights into the genetic etiology of tobacco and alcohol use. Nat Genet. 2019 Feb;51(2):237-44.
9. Lee AM, Jepson C, Hoffmann E, Epstein L, Hawk LW, Lerman C, et al. CYP2B6 genotype alters abstinence rates in a bupropion smoking cessation trial. Biol Psychiatry. 2007 Sep 15;62(6):635-41.
10. Desta Z, Gammal RS, Gong L, Whirl-Carrillo M, Gaur AH, Sukasem C, et al. Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for CYP2B6 and Efavirenz-Containing Antiretroviral Therapy. Clin Pharmacol Ther. 2019 Oct;106(4):726-33.
11. Zhu AZ, Cox LS, Nollen N, Faseru B, Okuyemi KS, Ahluwalia JS, et al. CYP2B6 and bupropion's smoking-cessation pharmacology: the role of hydroxybupropion. Clin Pharmacol Ther. 2012 Dec;92(6):771-7.
12. Gufford BT, Lu JB, Metzger IF, Jones DR, Desta Z. Stereoselective Glucuronidation of Bupropion Metabolites In Vitro and In Vivo. Drug Metab Dispos. 2016 Apr;44(4):544-53.
13. Koh KH, Jurkovic S, Yang K, Choi SY, Jung JW, Kim KP, et al. Estradiol induces cytochrome P450 2B6 expression at high concentrations: implication in estrogen-mediated gene regulation in pregnancy. Biochem Pharmacol. 2012 Jul 1;84(1):93-103.
14. Lamba V, Lamba J, Yasuda K, Strom S, Davila J, Hancock ML, et al. Hepatic CYP2B6 expression: gender and ethnic differences and relationship to CYP2B6 genotype and CAR (constitutive androstane receptor) expression. J Pharmacol Exp Ther. 2003 Dec;307(3):906-22.
15. Al Koudsi N, Tyndale RF. Hepatic CYP2B6 is altered by genetic, physiologic, and environmental factors but plays little role in nicotine metabolism. Xenobiotica. 2010 Jun;40(6):381-92.
16. Benowitz NL, Zhu AZ, Tyndale RF, Dempsey D, Jacob P, 3rd. Influence of CYP2B6 genetic variants on plasma and urine concentrations of bupropion and metabolites at steady state. Pharmacogenet Genomics. 2013 Mar;23(3):135-41.
17. Stewart JJ, Berkel HJ, Parish RC, Simar MR, Syed A, Bocchini JA, Jr., et al. Single-dose pharmacokinetics of bupropion in adolescents: effects of smoking status and gender. J Clin Pharmacol. 2001 Jul;41(7):770-8.
18. Hsyu PH, Singh A, Giargiari TD, Dunn JA, Ascher JA, Johnston JA. Pharmacokinetics of bupropion and its metabolites in cigarette smokers versus nonsmokers. J Clin Pharmacol. 1997 Aug;37(8):737-43.
19. Schirmer M, Rosenberger A, Klein K, Kulle B, Toliat MR, Nurnberg P, et al. Sex-dependent genetic markers of CYP3A4 expression and activity in human liver microsomes. Pharmacogenomics. 2007 May;8(5):443-53.
20. Cox LS, Nollen NL, Mayo MS, Choi WS, Faseru B, Benowitz NL, et al. Bupropion for smoking cessation in African American light smokers: a randomized controlled trial. J Natl Cancer Inst. 2012 Feb 22;104(4):290-8.
21. Nollen NL, Mayo MS, Cox LS, Benowitz NL, Tyndale RF, Ellerbeck EF, et al. Factors That Explain Differences in Abstinence Between Black and White Smokers: A Prospective Intervention Study. Jnci-J Natl Cancer I. 2019 Oct;111(10):1078-87.

22. Cunningham TJ, Croft JB, Liu Y, Lu H, Eke PI, Giles WH. Vital Signs: Racial Disparities in Age-Specific Mortality Among Blacks or African Americans - United States, 1999-2015. MMWR Morb Mortal Wkly Rep. 2017 May 5;66(17):444-56.
23. Haiman CA, Stram DO, Wilkens LR, Pike MC, Kolonel LN, Henderson BE, et al. Ethnic and racial differences in the smoking-related risk of lung cancer. N Engl J Med. 2006 Jan 26;354(4):333-42.
24. Popejoy AB, Fullerton SM. Genomics is failing on diversity. Nature. 2016 Oct 13;538(7624):161-4.
25. Powers MS, Smith PH, McKee SA, Ehringer MA. From sexless to sexy: Why it is time for human genetics to consider and report analyses of sex. Biol Sex Differ. 2017;8:15.
26. Zanger UM, Klein K. Pharmacogenetics of cytochrome P450 2B6 (CYP2B6): advances on polymorphisms, mechanisms, and clinical relevance. Front Genet. 2013;4:24.
27. Rajman I, Knapp L, Morgan T, Masimirembwa C. African Genetic Diversity: Implications for Cytochrome P450-mediated Drug Metabolism and Drug Development. EBioMedicine. 2017 Mar;17:67-74.
28. Chenoweth MJ, Ware JJ, Zhu AZX, Cole CB, Sanderson Cox L, Nollen N, et al. Genome-wide association study of a nicotine metabolism biomarker in African American smokers: impact of chromosome 19 genetic influences. Addiction. 2018 March;113(3):509-23.
29. Reyes-Guzman CM, Pfeiffer RM, Lubin J, Freedman ND, Cleary SD, Levine PH, et al. Determinants of Light and Intermittent Smoking in the United States: Results from Three Pooled National Health Surveys. Cancer Epidemiol Biomarkers Prev. 2017 Feb;26(2):228-39.
30. Jefferson JW, Pradko JF, Muir KT. Bupropion for major depressive disorder: Pharmacokinetic and formulation considerations. Clin Ther. 2005 Nov;27(11):1685-95.
31. Johnston AJ, Ascher J, Leadbetter R, Schmith VD, Patel DK, Durcan M, et al. Pharmacokinetic optimisation of sustained-release bupropion for smoking cessation. Drugs. 2002;62 Suppl 2:11-24.
32. Findlay JW, Van Wyck Fleet J, Smith PG, Butz RF, Hinton ML, Blum MR, et al. Pharmacokinetics of bupropion, a novel antidepressant agent, following oral administration to healthy subjects. Eur J Clin Pharmacol. 1981;21(2):127-35.
33. Hayes AF. Introduction to mediation, moderation, and conditional process analysis: a regression-based approach. Second ed. New York: The Guilford Press; 2018.
34. Cogo-Moreira H, Swardfager W. On Mediation Models in Clinical Neurology Studies. JAMA Neurol. 2019 Jan 1;76(1):116-7.
35. Faseru B, Nollen NL, Mayo MS, Krebill R, Choi WS, Benowitz NL, et al. Predictors of cessation in African American light smokers enrolled in a bupropion clinical trial. Addict Behav. 2013 Mar;38(3):1796-803.
36. Hayes AF. Beyond Baron and Kenny: Statistical Mediation Analysis in the New Millennium. Communication Monographs. 2009;76(4):408-20.
37. Gold EB. The Timing of the Age at Which Natural Menopause Occurs. Obstet Gyn Clin N Am. 2011 Sep;38(3):425-+.
38. Peng AR, Morales M, Wileyto EP, Hawk LW, Jr., Cinciripini P, George TP, et al. Measures and predictors of varenicline adherence in the treatment of nicotine dependence. Addict Behav. 2017 Dec;75:122-9.
39. Malcolm E, Carroll FI, Blough B, Damaj MI, Shoaib M. Examination of the metabolite hydroxybupropion in the reinforcing and aversive stimulus effects of nicotine in rats. Psychopharmacology (Berl). 2015 Aug;232(15):2763-71.
40. Laib AK, Brunen S, Pfeifer P, Vincent P, Hiemke C. Serum concentrations of hydroxybupropion for dose optimization of depressed patients treated with bupropion. Ther Drug Monit. 2014 Aug;36(4):473-9.
41. Schnoll RA, Wileyto EP, Leone FT, Tyndale RF, Benowitz NL. High dose transdermal nicotine for fast metabolizers of nicotine: a proof of concept placebo-controlled trial. Nicotine Tob Res. 2013 Feb;15(2):348-54.
42. Pacek LR, McClernon FJ, Bosworth HB. Adherence to Pharmacological Smoking Cessation Interventions: A Literature Review and Synthesis of Correlates and Barriers. Nicotine Tob Res. 2018 Sep 4;20(10):1163-72.
43. Mascha EJ, Dalton JE, Kurz A, Saager L. Statistical grand rounds: understanding the mechanism: mediation analysis in randomized and nonrandomized studies. Anesth Analg. 2013 Oct;117(4):980-94.

Table 1. Characteristics of the final analytic sample of participants with detectable bupropion and/or hydroxybupropion concentrations at week 3 from the KIS3 clinical trial

Characteristic	Women (n=116)	Men (n=57)	P-value
Age (years), mean (SD), median; range	47.9 (11.6), 49.0; 22 - 73	48.2 (10.2), 48.0; 27-71	0.97[b]
Cotinine (ng/ml), mean (SD), median; range	258.1 (136.2), 247.1; 5.0 – 689.1	271.6 (166.9), 265.2; 5.0 – 737.8	0.94[b]
Menthol cigarette smoking (n; %)			
Yes	93 (80.2)	42 (73.7)	0.33[a]
No	23 (19.8)	15 (26.3)	
CYP2B6 metabolism group (n; %):			
Normal (no copies of **6* or **18*)	43 (37.1)	21 (36.8)	0.44[a]
Intermediate (one copy of either **6* or **18*)	48 (41.4)	28 (49.1)	0.98[c]
Slow (two copies of **6* and/or **18*)	25 (21.6)	8 (14.0)	
Hydroxybupropion concentration at week 3 (μg/ml), mean (SD), median; range	0.62 (0.53), 0.54; 0.003 – 2.74	0.58 (0.44), 0.57; 0.003 – 2.15	0.89[b]
Abstinence status at week 3 (n; %):			
Still smoking	85 (73.3)	39 (68.4)	0.51[a]
Abstinent	31 (26.7)	18 (31.6)	
Abstinence status at week 7 (n; %):			
Sill smoking	82 (70.7)	39 (68.4)	0.76[a]
Abstinent	34 (29.3)	18 (31.6)	
Abstinent status at week 26 (n; %):			
Still smoking	96 (82.8)	50 (87.7)	0.40[a]
Abstinent	20 (17.2)	7 (12.3)	

Abbreviations: KIS, Kick-It-At-Swope.
[a]Categorical variables were compared using Pearson Chi-square tests
[b]Continuous variables were compared using Mann-Whitney U tests
[c]P-value derived from Pearson Chi-square test comparing frequency of normal versus reduced (intermediate + slow) metabolizers between women and men

Table 2. Results from the Mediation Path Analyses in participants with detectable bupropion and/or hydroxybupropion concentrations at week 3 from the KIS3 clinical trial

Analysis	Effect of X on Mediator (Path a) (Beta, SE; P-value)	Effect of Mediator on Y (Path b) (OR, 95% CI; P-value)	Total Effect (Path c) (OR, 95% CI; P-value)	Direct Effect (Path c') (OR, 95% CI; P-value)	Indirect Effect (OR, 95% CI)
Main Model[a]; Week 7 Abstinence	**0.218, 0.076; P=0.005**	**2.80, 1.35-5.78; P=0.006**	1.28, 0.64-2.55; P=0.49	0.99, 0.47-2.05; P=0.97	**1.25, 1.03-1.78**
Main Model[a]; Week 3 Abstinence	**0.218, 0.076; P=0.005**	**3.01, 1.47-6.19; P=0.003**	1.12, 0.56-2.22; P=0.75	0.86, 0.42-1.79; P=0.69	**1.27, 1.05-1.71**
Main Model[a]; Week 26 Abstinence	**0.218, 0.076; P=0.005**	**2.58, 1.12-5.95; P=0.03**	1.52, 0.64-3.57; P=0.34	1.18, 0.48-2.91; P=0.72	**1.23, 1.02-1.70**
Main Model, additional covariates[b]; Week 7 Abstinence	**0.239, 0.07; P=0.002**	**2.35, 1.11-4.98; P=0.03**	1.27, 0.62-2.59; P=0.52	0.98, 0.46-2.11; P=0.96	**1.23, 1.02-1.78**
Main Model, additional covariates[c]; Week 7 Abstinence	**0.218, 0.07; P=0.002**	**2.37, 1.08-5.22; P=0.03**	1.28, 0.64-2.58; P=0.49	1.03, 0.49-2.15; P=0.95	**1.21, 1.01-1.69**
Sensitivity Analysis 2[d]; Week 7 Abstinence	**0.152, 0.05; P=0.003**	**2.95, 1.42-6.15; P=0.004**	1.05, 0.66-1.68; P=0.83	0.86, 0.53-1.42; P=0.56	**1.18, 1.03-1.48**
Sensitivity Analysis 3[e]; Week 7 Abstinence	**0.211, 0.08; P=0.008**	**2.75, 1.16-6.48; P=0.02**	1.15, 0.54-2.41; P=0.72	0.91, 0.41-1.99; P=0.81	**1.24, 1.01-1.85**
Sensitivity Analysis 4[f]; Week 7 Abstinence	-0.009, 0.006; P=0.17	374.1, .07-1.9x10^6; P=0.17	1.28, 0.64-2.55; P=0.49	1.33, 0.66-2.68; P=0.42	0.95, 0.83-1.06
Main Model, Women[g]; Week 7 Abstinence	**0.264, 0.10; P=0.007**	**2.92, 1.21-7.09; P=0.02**	0.77, 0.33-1.83; P=0.56	0.53, 0.20-1.38; P=0.19	**1.33, 1.01-2.30**
Main Model, Men[h]; Week 7 Abstinence	0.211, 0.12; P=0.10	3.82, 0.87-16.8; P=0.08	**3.88, 1.07-14.1; P=0.04**	3.17, 0.83-12.1; P=0.09	1.33, 0.92-3.87
Sex Moderated Mediation Model[i]; Week 7 Abstinence	0.134, 0.13; P=0.32	**2.78, 1.35-5.76; P=0.006**	1.28, 0.64-2.55; P=0.49	0.99, 0.47-2.05; P=0.97	**Women: 1.30, 1.03-1.98** Men: 1.15, 0.89-1.71
Age Moderated Mediation Model[j], Women; Week 7 Abstinence	0.202, 0.13; P=0.12	**2.92, 1.21-7.09; P=0.02**	0.77, 0.33-1.83; P=0.56	0.53, 0.20-1.38; P=0.19	Older women: 1.46, 0.98-3.50 Younger women: 1.24, 0.96-2.03

Abbreviations: KIS, Kick-It-At-Swope.

[a]Main mediation path analysis model: Women and men analyzed together; Covariates included sex, age, baseline cotinine concentration, mentholated cigarette smoking. Model #4 in PROCESS was used for this analysis.

[b]Main mediation path analysis model, additionally controlling for smoking duration and the number of study visits attended. Model #4 in PROCESS was used for this analysis.

[c]Main mediation path analysis model, additionally controlling for the number of bupropion pills taken during the previous 3 days. Model #4 in PROCESS was used for this analysis.

[d]Sensitivity analysis #2 evaluated three *CYP2B6* activity groups (i.e. normal vs. intermediate vs. slow metabolizers). Model #4 in PROCESS was used for this analysis.

[e]Sensitivity analysis #3 excluded participants with hydroxybupropion concentrations <100 ng/ml. Model #4 in PROCESS was used for this analysis.

[f]Sensitivity analysis #4 tested bupropion concentration (in µg/ml) as the mediator instead of hydroxybupropion concentration (in µg/ml). Model #4 in PROCESS was used for this analysis.

[g]Main mediation path analysis model, restricted to women. Model #4 in PROCESS was used for this analysis.

[h]Main mediation path analysis model, restricted to women. Model #4 in PROCESS was used for this analysis.

[i]There was no sex x *CYP2B6* group interaction effect on hydroxybupropion concentration (beta=0.125, se=0.164; P=0.45). The index of moderated mediation was 0.13 (95% CI: -0.22 to 0.58). Model #7 in PROCESS was used for this analysis.

[j]There was no age x *CYP2B6* group interaction effect on hydroxybupropion concentration (beta=0.149, se=0.197; P=0.45) in women. The index of moderated mediation was 0.16 (95% CI: -0.30 to 0.94). Model #7 in PROCESS was used for this analysis.

Figure Legends

Figure 1. Mediation analysis in smokers with detectable bupropion and/or hydroxybupropion concentrations at week 3 from the KIS3 clinical trial: women and men analyzed together (n=173). This model shows the relationship between *CYP2B6* activity group (coded as 2 = normal activity, 1 = reduced activity) and smoking abstinence at week 7 (coded as 1 = abstinent, 0 = still smoking) via hydroxybupropion concentration at week 3 (in µg/ml). Thick solid black arrows denote significant pathways at P<0.05. The (a) pathway shows the association between *CYP2B6* activity group and week 3 hydroxybupropion concentration. The (b) pathways shows the association between week 3 hydroxybupropion concentration and week 7 abstinence. The mediating effect of week 3 hydroxybupropion concentration on week 7 abstinence is shown in the (a b) pathway (i.e. indirect effect pathway) and is shaded in grey. The (c) pathway shows the direct effect of *CYP2B6* activity group on week 7 abstinence adjusted for covariates, while the (c') pathway shows the direct effect of *CYP2B6* activity group on week 7 abstinence adjusted for covariates and the indirect effect. Sex, baseline cotinine concentration, age, and menthol cigarette smoking were included as covariates in the model. Dotted grey arrows denote significant covariate effects at P<0.05. Non-significant covariate effects are not shown. Abbreviations: B = unstandardized coefficient, OR = odds ratio, CI = confidence interval.

Figure 2. Moderated mediation analysis in smokers with detectable bupropion and/or hydroxybupropion concentrations at week 3 from the KIS3 clinical trial: women and men analyzed together (n=173). This model shows the relationship between *CYP2B6* activity group (coded as 2 = normal activity, 1 = reduced activity) and smoking abstinence at week 7 (coded as 1 = abstinent, 0 = still smoking) via hydroxybupropion concentration at week 3 (in µg/ml). This model tested for sex moderation of the relationship between *CYP2B6* activity group and hydroxybupropion concentration: no significant moderation by sex was observed (P=0.91). The main effects of sex (B=0.029, SE=0.26, P=0.91) and *CYP2B6* activity group (B=0.14, SE=0.10, P=0.14) on hydroxybupropion concentration were not significant. The thick solid black arrow denotes a significant pathway at P<0.05. The mediating effect of week 3 hydroxybupropion concentration on week 7 abstinence is shown in the (a b) pathway (i.e. indirect effect pathway) and is shaded in dark grey in women, and in light grey in men. The index of moderated mediation was 0.13 (95% CI = -0.22, 0.58). The (a) pathway shows the association between *CYP2B6* activity group and week 3 hydroxybupropion concentration. The (b) pathways shows the association between week 3 hydroxybupropion concentration and week 7 abstinence. The (c) pathway shows the direct effect of *CYP2B6* activity group on week 7 abstinence adjusted for covariates, while the (c') pathway shows the direct effect of *CYP2B6* activity group on week 7 abstinence adjusted for covariates and the indirect effect. Baseline cotinine concentration, age, and menthol cigarette smoking were included as covariates in the model. Dotted grey arrows denote significant covariate effects at P<0.05. Non-significant covariate effects are not shown. Abbreviations: B = unstandardized coefficient, OR = odds ratio, CI = confidence interval.

Supplementary Information Titles

Table S1. Smoking abstinence rates, by sex and by *CYP2B6* activity group, at week 3, week 7 (end-of-treatment), and week 26 among participants with detectable bupropion and/or hydroxybupropion concentrations at week 3 (n=173) from the KIS3 clinical trial

Table S2. Characteristics of the final analytic sample of participants with detectable bupropion and/or hydroxybupropion concentrations at week 3 from the KIS3 clinical trial, according to *CYP2B6* genotype group

Figure S1. Distribution of hydroxybupropion concentration, measured at week 3, in participants with detectable bupropion and/or hydroxybupropion concentrations at week 3 (n=173) from the KIS3 clinical trial.

Figure S2. Association between *CYP2B6* activity group and hydroxybupropion concentration in participants with detectable bupropion and/or hydroxybupropion concentrations at week 3 (n=173) from the KIS3 clinical trial.

Figure S3. Association between *CYP2B6* activity group and hydroxybupropion concentration, stratified by sex and age, in participants with detectable bupropion and/or hydroxybupropion concentrations at week 3 (n=173) from the KIS3 clinical trial.

Figure S4. Association between age and hydroxybupropion concentration, stratified by sex, in participants with detectable bupropion and/or hydroxybupropion concentrations at week 3 (n=173) from the KIS3 clinical trial. Age was significantly associated with hydroxybupropion concentration in women (Spearman's rho=0.21; P=0.03) but not in men (Spearman's rho=0.09; P=0.49).