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# Title Page

## Gene variants in *CYP2C19* are associated with altered *in vivo* bupropion pharmacokinetics but not bupropion assisted smoking cessation outcomes

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# Running Title Page

## Gene variants in *CYP2C19* alter bupropion pharmacokinetics

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List of Abbreviations
HLM: Human liver microsomes
CYP2B6: Cytochrome P450 2B6
OH-BUP: Hydroxybupropion
OH-BUP/BUP: Hydroxybupropion/Bupropion
EB: Erythrohydrobupropion
TB: Threohydrobupropion

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## ABSTRACT

Bupropion is used clinically to treat depression and to promote smoking cessation. It is metabolized by CYP2B6 to its active metabolite hydroxybupropion, yet alterations in CYP2B6 activity have little impact on bupropion plasma levels. Furthermore, less than 10% of a bupropion dose is excreted as urinary bupropion and its characterized metabolites hydroxybupropion, threohydrobupropion and erythrohydrobupropion, suggesting that alternative metabolic pathways may exist. *In vitro* data suggested CYP2C19 could metabolize bupropion. The current study investigated the impact of functional *CYP2C19* genetic variants on bupropion pharmacokinetics and treatment outcomes. In 42 healthy volunteers, *CYP2C19*2* (a reduced activity allele) was associated with higher bupropion AUC, but similar hydroxybupropion AUC. The mean bupropion AUC was 771 versus 670h.ng/mL in individuals with, and without, *CYP2C19*2* respectively (P=0.017). *CYP2C19*2* was also associated with higher threohydrobupropion and erythrohydrobupropion AUC (P<0.005). Adjusting for *CYP2B6* genotype did not alter these associations, and *CYP2C19* variants did not alter the utility of the hydroxybupropion/bupropion ratio as a measure of CYP2B6 activity. Finally, in a clinical trial of 540 smokers, *CYP2C19* genotype was not associated with smoking cessation outcomes supporting the hypothesis that bupropion response is mediated by hydroxybupropion, which is not altered by CYP2C19. In conclusion, our study reports the first *in vivo* evidence that reduced CYP2C19 activity significantly increases the steady state exposure to bupropion and its reductive metabolites threohydrobupropion and erythrohydrobupropion. These pharmacokinetic changes were not associated with differences in bupropion's ability to promote smoking cessation in smokers, but may influence the side effects and toxicity associated with bupropion.

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## INTRODUCTION

Bupropion (Wellbutrin and Zyban) is used clinically to treat depression, to promote smoking cessation and to treat obesity (Hurt et al., 1997; Greenway et al., 2010; Moreira, 2011). Bupropion is extensively metabolized in humans with less than 10% of the dose recovered in urine as the parent compound (Lai and Schroeder, 1983; Laizure et al., 1985; Benowitz et al., 2013). *In vivo* pharmacokinetic studies have indicated the existence of multiple metabolites with three identified in plasma as hydroxybupropion (OH-BUP), erythrohydrobupropion (EB) and threohydrobupropion (TB) (Laizure and DeVane, 1985). Among these metabolites, OH-BUP had the highest steady state plasma levels and has been shown to be pharmacologically active (Fig. 1) (Damaj et al., 2004; Damaj et al., 2010; Zhu et al., 2012; Benowitz et al., 2013). Higher levels of OH-BUP, but not bupropion, were associated with greater rates of smoking cessation in smokers receiving bupropion treatment (Zhu et al., 2012). Previous *in vitro* studies, using cDNA-expressed recombinant CYP enzymes and human liver microsomes, demonstrated that Cytochrome P450 2B6 (CYP2B6) has been shown to mediate the metabolism of bupropion to OH-BUP (Fig. 1) (Faucette et al., 2001). Consistent with this, an *in vivo* association between *CYP2B6* genotype and OH-BUP levels and the OH-BUP to bupropion ratio (OH-BUP/BUP ratio, an indicator of bupropion hydroxylation activity) has been shown. However, no associations between *CYP2B6* genotype and the levels of the parent drug bupropion were observed (Kirchheiner et al., 2003; Zhu et al., 2012; Benowitz et al., 2013). Furthermore, when the potent CYP2B6 inhibitors clopidogrel and ticlopidine were given to healthy volunteers, their OH-BUP formation decreased dramatically (>80%), but there was little alteration in bupropion levels (Turpeinen et al., 2004; Turpeinen et al., 2005). Together, these data have suggested that

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alternative bupropion clearance pathways exist, which may be able to compensate for the reduction in CYP2B6 mediated bupropion metabolism.

The best characterized alternative bupropion metabolic routes are the reduction of bupropion to EB and TB. Despite their substantial plasma levels (Fig.1) (Laizure and DeVane, 1985; Zhu et al., 2012), a very small percentage of bupropion was recovered in urine as EB, TB and their glucuronides (Fig. 1) (Benowitz et al., 2013), and differences in the CYP2B6-mediated bupropion hydroxylation pathway did not result in any compensatory changes in EB and TB levels (Zhu et al., 2012; Benowitz et al., 2013). Together, these data suggest that other poorly characterized metabolic routes may play an important role in bupropion clearance (Fig. 1).

Recently, an *in vitro* study using human liver microsome and cDNA expressed recombinant CYPs suggested the existence of additional hydroxyl bupropion metabolites which were likely formed by CYP2C19 (Fig. 1) (Chen et al., 2010). Their existence could explain the relatively small amount of bupropion dose recovered as OH-BUP, EB, TB and their metabolites (Fig. 1). However, the role of CYP2C19 in bupropion pharmacokinetics has not been explicitly investigated in humans.

The human *CYP2C19* gene is polymorphic with more than 25 known variants alleles (http://www.cypalleles.ki.se/cyp2c19.htm). The most common loss-of-function allele is *CYP2C19*2* (c.681G>A; rs4244285), which has a 15% allele frequency in Caucasians & African Americans and a 30% allele frequency in Asians (Scott et al., 2011; Scott et al., 2013). Other reduced or loss-of-function *CYP2C19* alleles have also been identified, but their allele frequencies are below 1% in Caucasian and African Americans (Scott et al., 2011; Scott et al., 2013). *CYP2C19*3* (c.636G>A; rs4986893) is generally found in Asians with an allele frequency of 2-9%. A gain of function allele, *CYP2C19*17* (c.-806C>T; rs12248560), has recently been

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identified resulting in higher activity as a consequence of enhanced CYP2C19 transcription (Sim et al., 2006). It has an allele frequency of 21% in Caucasians, 16% in African Americans, and 3% in Asians (Sim et al., 2006; Scott et al., 2011; Scott et al., 2013).

In this study, we investigated the influence of these *CYP2C19* genetic variants on steady state *in vivo* bupropion pharmacokinetics. We hypothesized that the loss-of-function *CYP2C19* genetic variants, *CYP2C19*2* and *CYP2C19*3*, would result in lower bupropion oral clearance and higher steady state bupropion exposure (i.e. AUC), whereas the gain-of-function *CYP2C19* genetic variant, *CYP2C19*17*, would result in faster bupropion oral clearance and lower steady state bupropion exposure. Furthermore, we explored the influence of *CYP2C19* genetic variation on the plasma levels of bupropion metabolites OH-BUP, EB and TB. Lastly, we sought to evaluate the pharmacodynamic impact of *CYP2C19* genotype on bupropion's ability to promote smoking cessation in smokers.

## MATERIALS AND METHODS

### Participants and procedures

*Study 1: Steady State Bupropion Pharmacokinetics in Healthy Volunteers*

Healthy volunteers (n=42, 62% were men) were recruited for the pharmacokinetic study (Benowitz et al., 2013), which included 21 Caucasians, 14 African Americans and 7 Asians. The exclusion criteria included being younger than 18 years of age taking regular medications (including oral contraceptives), using alcohol or illicit drugs, or having a history of seizures, head trauma, or eating disorders. Eligible and consented participants were given 150 mg bupropion XL (once per day sustained release) daily for 7 days, allowing bupropion and its metabolites to reach plasma steady state. The participants were asked to call the clinic daily to confirm their bupropion adherence. The participants were admitted into a clinical research inpatient unit on day

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7, and their plasma samples were taken every 4 hours for a 24 hour period. A complete 24 hour urine sample was also collected. Supplementary table S1 summarizes the baseline demographics and *CYP2C19* genotype frequencies in Study 1. All participants provided written informed consent in accordance with the principles expressed in the Declaration of Helsinki, and the study protocol was approved by the Institutional Review Boards at the University of Toronto and the University of California, San Francisco.

*Study 2: Bupropion Treatment Efficacy in Smokers*

The association between C*YP2C19* variants and bupropion's treatment efficacy were investigated in a randomized, double-blind, and placebo-controlled clinical trial of 540 African-American smokers (DNA was available for 535 individuals) (Cox et al., 2012). This study consisted of a placebo arm (n=270) and a bupropion arm (n=270, 150mg/day for 3 days, then 150 mg b.i.d for 7 weeks). The inclusion criteria included being ≥ 18 years of age, having smoked an average of 10 or fewer cigarettes per day for at least 6 months before enrollment, and having smoked at least 25 of the past 30 days. The exclusion criteria were consistent with the contraindications of bupropion use (Cox et al., 2012). Cotinine verified (≤15ng/mL) smoking cessation was assessed at week 3 during treatment, at the end of the 7 weeks treatment and at week 26 follow-up consistent with accepted practice (SRNT Subcommittee on Biochemical Verification, 2002). The baseline demographics and *CYP2C19* genotype frequencies in Study 2 can be found in Supplementary table S3.

**Genotyping**

*CYP2C19*2*, *CYP2C19*3* and *CYP2C19*17* were genotyped using ABI Viia 7 real time PCR machine (Applied Biosystems, Foster City, CA). The genotyping reaction was performed with 5 μL TaqMan GTXpress master mix and 5 μL of water containing 10 ng of DNA and 0.5 μL

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of 20x Taqman drug metabolism analysis probes (*CYP2C19*2*: rs4244285, C__25986767_70; *CYP2C19*3*: rs4986893, C__27861809_10; and *CYP2C19*17*: rs12248560, C____469857_10, Applied Biosystems, Foster City, CA). The allele discrimination data were analyzed using Viia 7 software version 1.2. The *CYP2B6* genotyping was described and performed previously (Zhu et al., 2012; Benowitz et al., 2013).

**Analytical chemistry**

Plasma and urine concentration of bupropion and its metabolites were measured using liquid chromatography-tandem mass spectroscopy as described previously (Haas et al., 2004; Benowitz et al., 2013).

Briefly, all plasma samples (500 μL), standards, and controls were first spiked with a deuterium-labeled internal standard (100 μL), then deproteinized with 3% aqueous perchloric acid and extracted with 0.5 mL of 50% w/v aqueous tripotassium phosphate and 4 mL pentane:ethyl acetate:isopropanol (80:15:5). The organic layer was evaporated to dryness and reconstituted with 200 μL of 0.1% formic acid, 10 mM ammonium formate in water:methanol (75:25). The samples were analyzed by Agilent 1200 liquid chromatograph interfaced to a Thermo-Finnigan TSQ Quantum Ultra mass spectrometer using a BDS Hypersil Phenyl column (150x4.6 mm, Phenomenex) with a gradient mobile phase of mobile phase A (10 mM ammonium formate, 0.1% formic acid in HPLC grade water) and mobile phase B (10 mM ammonium formate, 0.1% formic acid in methanol) under 0.8 mL/min flow rate (75% mobile phase A at 0 min, 55% mobile phase A at 1.5 min, 50% mobile phase A at 7 min, 0% mobile phase A at 8 min, 0% mobile phase A at 11 min, and 75% mobile phase A at 11.1 min). The limit of quantitation for bupropion, OH-BUP, EB and TB was 1 ng/mL. Data on within-run and between-run accuracy and precision have been published previously (see supplementary table 1 of

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(Benowitz et al., 2013). Urine samples were measured as above, with and without glucuronide deconjugation. The deconjugation procedure was conducted by incubating 125 μL urine with 125 μL of β-glucuronidase (contain 750 units) in 0.9% saline and 250 μL of 0.1M pH 5.0 sodium acetate solution at 37C overnight (Petsalo et al., 2007). Concentrations of conjugated bupropion or metabolites were determined as the difference between concentrations measured with and without deconjugation.

**Pharmacokinetic and statistical analyses**

The steady state exposure to bupropion and its metabolites was measured using the area under the plasma concentration–time curve for 24 hours on day 7 ($AUC_{24}$). Urinary excretion data were presented both as absolute concentrations and the molar percentage of each metabolite in relation to the total steady state bupropion dose. Average steady state concentration ($C_{SS}$) was computed as $AUC_{24}$/24hrs. A linear regression approach was used to examine the effects of having zero, one or two copies of variant *CYP2C19* alleles on bupropion and metabolites exposure with and without adjusting for *CYP2B6* genotype. The statistical impact (i.e. P-values) of the *CYP2C19* variant alleles was derived from linear regression analyses. This approach gives an estimation of the effect size of each *CYP2C19* allele while controlling for the effect of other *CYP2C19* alleles and the *CYP2B6* genotype. The *CYP2C19*2* genotype was coded in the regression model as **1/*1*=0, **1/*2*=1, and **2/*2*=2. The *CYP2C19*17* genotype was coded in the regression model as **1/*1*=0, **1/*17*=1, and **17/*17*=2. The *CYP2B6* genotype was coded in the regression model as normal metabolizers (**1/*1*)=0, intermediate metabolizers (**1/*6* or **1/*18*) =1 and slow metabolizers (**6/*18*, **6/*6* & **18/*18*)=2 (Zhu et al., 2012). The regression residuals were normally distributed, thus the pharmacokinetic parameters were not log transformed. Chi2 tests were used to evaluate the association between *CYP2C19* genotype and

smoking abstinence in Study 2. Likelihood ratio test (the 'lrtest' procedure in Stata) was used to evaluate the impact of adjusting *CYP2C19* genotype on the association between *CYP2B6* genotype and the OH-BUP/BUP ratio. Statistical analyses were performed using R v2.5.3 (The R Project for Statistical Computing) and Stata version 12 (College Station, TX).

## RESULTS

### Study 1: *CYP2C19*2* was associated with significantly higher steady state bupropion, EB and TB exposure

In the pharmacokinetic study, there were 17 individuals with *CYP2C19*1/*1* genotype (i.e. without *CYP2C19*2, CYP2C19*3* or *CYP2C19*17*), 10 individuals with *CYP2C19*1/*2* genotype, 9 individuals with the **1/*17* genotype, 5 individuals with **2/*17* genotype and 1 individual with **17/*17* genotype (Supplementary Table S1). Individuals with a *CYP2C19*2* allele had a significantly higher steady state plasma bupropion AUC compared to those without any variants (771 h•ng/mL & 670 h•ng/mL, respectively $P$=0.017, Fig. 2A&Table 1A). Steady state plasma OH-BUP exposure did not differ between those with *CYP2C19*2* allele and those without any variants (Fig. 2B&Table 1A). Steady state EB and TB plasma AUCs were significantly higher in individuals with *CYP2C19*2* allele compared to those without any variants (EB: 947 h•ng/mL and 732 h•ng/mL, respectively $P$=0.001, Fig.2C&Table 1A; TB: 5427 h•ng/mL and 3867 h•ng/mL, respectively, $P$=0.002, Fig. 2D& Table 1A). Adjusting for *CYP2B6* genotypes did not statistically alter the association between *CYP2C19**2 and plasma BUP, EB and TB AUC (Table 1B, statistical inference was derived by the likelihood ratio tests in Table 1C).

The direction of the *CYP2C19*17* effect was consistent with the expected gain of function associated with this allele, but the effect size of *CYP2C19*17* was small and did not

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reach statistical significance in the regression analyses (Fig. 3 and Table 1A). Individuals with the gain of function *CYP2C19*17* allele had similar plasma BUP (with *CYP2C19*17*: 663 h•ng/mL and without any *CYP2C19* variants: 670 h•ng/mL), EB (with *CYP2C19*17*: 714 h•ng/mL and without any *CYP2C19* variants: 732 h•ng/mL), and TB (with *CYP2C19*17*: 3669 h•ng/mL and without any *CYP2C19* variants: 3867 h•ng/mL) exposure. The absolute and relative amounts of bupropion excreted as bupropion, OH-BUP, EB and TB in urine did not significantly differ between *CYP2C19* genotypes (Supplementary table S2). *CYP2C19*3* was genotyped in this study, but none of the participants had a *CYP2C19*3* allele.

The ratio of plasma OH-BUP levels to plasma bupropion levels (the OH-BUP/BUP ratio) following an acute bupropion administration or at steady state is often used in humans as an indicator of *in vivo* CYP2B6 activity (Kirchheiner et al., 2003; Fradette et al., 2004; Hesse et al., 2004). We investigated whether variation in *CYP2C19* would have a meaningful impact on the utility of the OH-BUP/BUP ratio as an indicator of CYP2B6 activity. Controlling for *CYP2C19* genotype did not meaningfully alter the association between *CYP2B6* genotype and the steady state OH-BUP/BUP plasma AUC ratio (*CYP2B6* genotype's effect without controlling for *CYP2C19* genotype: β=-0.38&P=0.01, Table 2A; *CYP2B6* genotype's effect after controlling for *CYP2C19* genotype: β=-0.40&P=0.01, Table 2B. Likelihood ratio test P=0.49, Table 2C). Thus, *CYP2C19* genotype is unlikely to alter the utility of the OH-BUP/BUP ratio as a measure of CYP2B6 activity.

**Study 2: *CYP2C19* genotype was not associated with bupropion's ability to promote smoking cessation**

Next, we examined the clinical impact of *CYP2C19* variants on bupropion's ability to promote smoking cessation in smokers. There were 197 individuals with *CYP2C19*1/*1*

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genotype (i.e. without *CYP2C19*2* or *CYP2C19*17*), 120 individuals with *CYP2C19*1/*2* genotype, 14 individuals with *CYP2C19*2/*2* genotype, 137 individuals with the **1/*17* genotype, 47 individuals with **2/*17* genotype and 20 individual with **17/*17* genotype (Supplementary Table S2). In this double-blind, placebo-controlled, randomized clinical trial, *CYP2C19* genotype was not significantly associated with smoking cessation outcomes (Figure 4A and Figure 4B).

## DISCUSSION

We present the first evidence that *CYP2C19* variants, particularly *CYP2C19*2*, can significantly alter the steady state exposure (AUC) to bupropion and it metabolites EB and TB while having no impact on OH-BUP. Consistent with the limited pharmacological activity associated with BUP, EB and TB, *CYP2C19* genotype did not have a significant impact on bupropion's efficacy in promoting smoking cessation in smokers.

### CYP2C19, bupropion metabolism and pharmacokinetics

The CYP2B6-mediated terminal hydroxylation of bupropion to OH-BUP was hypothesized to be a major bupropion metabolic clearance pathway (Hesse et al., 2000; Kirchheiner et al., 2003). However, a number of studies have reported that variation in CYP2B6 activity significantly altered OH-BUP pharmacokinetics, but not bupropion pharmacokinetics (Kirchheiner et al., 2003; Turpeinen et al., 2004; Turpeinen et al., 2005; Zhu et al., 2012; Benowitz et al., 2013). Our study suggested that CYP2C19-mediated hydroxylation of bupropion is a quantitatively important bupropion metabolic clearance pathway since reduced CYP2C19 activity resulted in a significant increase in bupropion exposure (Chen et al., 2010). Our findings extend previous *in vitro* reports that CYP2C19 can metabolize bupropion (Chen et al., 2010), and suggest that the OH-BUP (made by CYP2B6) pathway is not a quantitatively important

bupropion clearance pathway hence explaining the lack of association between CYP2B6 activity and bupropion levels (Kirchheiner et al., 2003; Turpeinen et al., 2004; Turpeinen et al., 2005). Since more than 20 different bupropion metabolites have been identified in urine after a bupropion administration, the quantitative and enzymatic aspects of additional bupropion pathways remain to be further clarified (Petsalo et al., 2007).

In the pharmacokinetic study, the direction of the *CYP2C19* genotype effects on bupropion, EB and TB pharmacokinetics were consistent with the known functional impact of these variants. For example, *CYP2C19*2*, a loss-of-function *CYP2C19* allele (Scott et al., 2013), was associated with higher bupropion, EB and TB exposure suggesting a reduction in the metabolic clearance of each of these compounds. Interestingly, no metabolic rerouting toward OH-BUP was observed in *CYP2C19* slow metabolizers, which might be because the *CYP2B6* genotypes were not evenly balanced between *CYP2C19* genotypes. Only 1 out of 17 *CYP2C19* normal metabolizers was *CYP2B6* slow metabolizer, whereas 4 out of 15 *CYP2C19* slow metabolizers were *CYP2B6* slow metabolizers reducing the ability to detect metabolic switching if it occurred.

While a significant association between the gain of function *CYP2C19*17* allele and bupropion pharmacokinetics was not observed. The lack of association between *CYP2C19*17* and bupropion pharmacokinetics could be due to the low number of homozygous *CYP2C19*17* individuals in our study (there was one) as *CYP2C19*17* can act recessively toward some substrates (Gawronska-Szklarz et al., 2012). For example, individuals with *CYP2C19*1/*17* genotypes had similar pantoprazole exposure compared to the *CYP2C19*1/*1* individuals whereas those with *CYP2C19*17/*17* genotype had significantly lower pantoprazole exposure compared to the *CYP2C19*1/*1* individuals (Gawronska-Szklarz et al., 2012).

### *CYP2C19* and bupropion treatment efficacy

Bupropion is used clinically to promote smoking cessation. Animal and human data suggest that bupropion's ability to promote smoking cessation is at least partially mediated by its major plasma metabolite OH-BUP (Damaj et al., 2010; Zhu et al., 2012). At steady state, plasma OH-BUP levels are 10-20 times higher than plasma bupropion levels and OH-BUP has higher affinity for the α4β2 nAChRs than bupropion (Damaj et al., 2004). In Study 2, no significant associations between *CYP2C19* genotype and smoking cessation outcomes were found. This finding supports the hypothesis that bupropion's smoking cessation pharmacology is primarily mediated by its CYP2B6-mediated active metabolite OH-BUP which did not differ by *CYP2C19* genotype (Zhu et al., 2012). Previously we have found that there was no relationship between the levels of the parent drug bupropion and smoking cessation outcomes, while levels of OH-BUP were predictive (Zhu et al., 2012). Thus, the present observations of a difference in bupropion, but not OH-BUP levels, due to *CYP2C19*2* are consistent with a lack of significant impact of parent bupropion levels on smoking cessation outcomes; our current sample size was statistically powered to reject a 17% difference in smoking cessation outcomes.

### Implications

Although not directly associated with bupropion treatment outcomes, genetic variation in *CYP2C19* may still influence side effects, or toxicity, associated with bupropion treatment. For example, the cardiotoxicity associated with bupropion is likely mediated by bupropion itself rather than OH-BUP, and the levels of bupropion could be altered by CYP2C19 (Al-Abri et al., 2013). Furthermore, the reductive metabolites of bupropion, TB and EB, are potent CYP2D6 inhibitors (Parkinson et al., 2010). Therefore, it is possible that variation in CYP2C19 activity could modulate the drug-drug interaction between bupropion and CYP2D6 substrates.

Another potential implication of the involvement of CYP2C19 in bupropion metabolism is that *CYP2C19* may alter the utility of the OH-BUP/BUP ratio as a marker of CYP2B6 activity, as *CYP2C19* alters bupropion levels. However, data from the pharmacokinetic study suggests that *CYP2C19* genotype did not affect the association between the steady state OH-BUP/BUP ratio and *CYP2B6* genotype. It is not clear whether this would also be the case in single-dosing paradigms, or among other ethnic groups with a higher frequency of reduced function *CYP2C19* alleles.

In conclusion, our study reports the first *in vivo* evidence that CYP2C19 is involved in the metabolism of bupropion, EB and TB, and that loss-of-function *CYP2C19* variants could significantly increase the steady state exposure to bupropion and its reductive metabolites. These pharmacokinetic changes were not associated with differences in bupropion's ability to promote smoking cessation in smokers, but may influence the side effects, and toxicity, associated with bupropion.

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## AUTHORSHIP CONTRIBUTION

Participated in research design: Cox, Benowitz, and Tyndale

Conducted experiments: Zhu, Zhou, Cox, Ahluwalia, and Benowitz

Contributed new reagents or analytic tools: Benowitz

Performed data analysis: Zhu

Wrote to contributed to the writing of the manuscript: Zhu, Zhou, Cox, Ahluwalia, Benowitz, and Tyndale

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**Footnotes**

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N.L.B. serves as a consultant to several pharmaceutical companies that market smoking cessation medications and has been a paid expert witness in litigation against tobacco companies. R.F.T. has participated in one-day advisory meetings for Novartis and McNeil.

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# Figure Legends

**Figure 1.** A quantitative scheme of bupropion metabolism profile in humans. The percentage below each metabolite represents the percentages of the total oral bupropion dose (150 mg in this case) excreted in urine as a % of daily dose of bupropion as the compound at steady state. These numbers were estimated using a 24 hours urine collection. The quantitative aspects were estimated using the study published by Benowitz et al., 2013 (Benowitz et al., 2013). The metabolic pathway data were compiled from (Butz et al., 1981; Laizure et al., 1985; DeVane et al., 1990; Sweet et al., 1995; Hsyu et al., 1997; Faucette et al., 2000; Johnston et al., 2002; Chen et al., 2010; Meyer et al., 2013). More than 20 bupropion metabolites have been identified, some poorly characterized secondary and tertiary metabolites are not shown on this figure. After 7 days of 150 mg/day bupropion treatment, the steady state plasma level of bupropion is 29 ng/mL, OH-BUP is 400 ng/mL, EB is 32 ng/mL and TB is 175 ng/mL (Benowitz et al., 2013). However, these metabolites account for a very small percentage of the total bupropion dose in urine, this suggests that other uncharacterized bupropion metabolites may still be quantitatively important in bupropion clearance. OH-BUP=hydroxybupropion; EB=erythrohydrobupropion; TB=threohydrobupropion.

**Figure 2.** Steady state plasma concentrations (mean ± SEM) of bupropion **(A)** and its metabolites hydroxybupropion (OH-BUP) **(B),** erythrohydrobupropion (EB) **(C)** and threohydrobupropion (TB) **(D)** over 24 hours by *CYP2C19*2* genotype. The **1/*1* group (n=17) represents individuals without any *CYP2C19*2, *3* and **17* allele. The **1/*2* group represents individuals with one copy of *CYP2C19*2* (n=15). The p-values were derived from the linear regression analyses presented in Table 1, which including five people with *CYP2C19*2/*17* genotype.

**Figure 3.** Steady state plasma concentrations (mean ± SEM) of bupropion (**A**) and its metabolites hydroxybupropion (OH-BUP) (**B**), erythrohydrobupropion (EB) (**C**) and threohydrobupropion (TB) (**D**) over 24 hours by *CYP2C19*17* genotype. The **1/*1* group (n=17) represents individuals without any known *CYP2C19*2, *3* and **17* alleles. The **1/*17* & **17/*17* group represent individuals with one or two copies of *CYP2C19*17* (n=15, there was one individual with the **17/*17* genotype). There were no significant effects as described by the linear regression analyses presented in Table 1, which including five people with *CYP2C19*2/*17* genotype.

**Figure 4.** *CYP2C19*2* **(A)** and **17* **(B)** were not significantly associated smoking cessation outcomes in smokers treated with bupropion (i.e. within the bupropion arm of Study 2). The value below the bar represents the total number of smokers in each group. The P-values were derived from Chi2 tests.

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**Table 1.** A regression analysis of the association between *CYP2C19* genotype and plasma bupropion or its metabolites AUC (Study 1).

| **A** | **BUP $AUC_{24}$** ($r^2$=0.146) | | **HB $AUC_{24}$** ($r^2$=0.007) | | **EB $AUC_{24}$** ($r^2$=0.277) | | **TB $AUC_{24}$** ($r^2$=0.274) | |
|---|---|---|---|---|---|---|---|---|
| | B (95%CI) | *P*-value | B (95%CI) | *P*-value | B (95%CI) | *P*-value | B (95%CI) | *P*-value |
| ***CYP2C19 *2*** | 132 (25,239) | **0.017** | 613 (-1966,3191) | 0.634 | 266 (113.3,418.3) | **0.001** | 1838 (744,2933) | **0.002** |
| ***CYP2C19 *17*** | -23 (-118.6,73.5) | 0.638 | -236 (-2557,2085) | 0.838 | -91 (-228,47) | 0.189 | -756 (-1741,229) | 0.129 |
| **B** | **BUP $AUC_{24}$** ($r^2$=0.151) | | **HB $AUC_{24}$** ($r^2$=0.313) | | **EB $AUC_{24}$** ($r^2$=0.333) | | **TB $AUC_{24}$** ($r^2$=0.317) | |
| | B (95%CI) | *P*-value | B (95%CI) | *P*-value | B (95%CI) | *P*-value | B (95%CI) | *P*-value |
| ***CYP2C19 *2*** | 134 (26, 243) | **0.016** | 1080 (-11023, 263) | 0.323 | 284 (135, 433) | **0.001** | 1955 (875, 3035) | **0.001** |
| ***CYP2C19 *17*** | -24 (-137, 88) | 0.664 | 1468 (-8093, 744) | 0.200 | -36 (-191, 120) | 0.646 | -493 (-1620, 633) | 0.381 |
| ***CYP2B6*** **NM>IM>SM** | -13 (-90, 64) | 0.733 | -3144 (-4688, -1600) | **0.001** | -103 (-208, 3) | 0.06 | -630 (-1394, 134) | 0.103 |
| **C** | **Likelihood ratio test comparing the model in (A) vs. (B)** | | | | | | | |
| | **BUP $AUC_{24}$** | | **HB $AUC_{24}$** | | **EB $AUC_{24}$** | | **TB $AUC_{24}$** | |
| | Likelihood Ratio $Chi^2$ | *P*-value | Likelihood Ratio $Chi^2$ | *P*-value | Likelihood Ratio $Chi^2$ | *P*-value | Likelihood Ratio $Chi^2$ | *P*-value |
| | 0.16 | 0.6902 | 14.53 | **0.0001** | 3.84 | 0.0501 | 2.91 | 0.0881 |

*CYP2B6* NM=Normal metabolizers, IM=Intermediate metabolizers, and SM=Slow metabolizers (Zhu et al., 2012; Benowitz et al., 2013).

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**Table 2.** The impact of *CYP2C19* genetic variation on the association between *CYP2B6* genotype and plasma steady state OH-BUP to bupropion ratio (Study 1).

| **A.** The association between *CYP2B6* genotype and plasma steady state OH-BUP/BUP ratio | | | | |
|---|---|---|---|---|
| OH-BUP/BUP model without *CYP2C19* | B | β | 95% CI | P-value |
| ***CYP2B6*** **NM>IM>SM** | **-6.48** | **-0.38** | **-11.5 to -1.43** | **0.01** |
| **B.** The association between *CYP2B6* genotype and plasma steady state OH-BUP/BUP ratio after controlling for *CYP2C19* genotype | | | | |
| OH-BUP/BUP model with *CYP2C19* | B | β | 95% CI | P-value |
| ***CYP2B6*** **NM>IM>SM** | **-6.88** | **-0.40** | **-12.3 to -1.51** | **0.01** |
| *CYP2C19*2* | -2.13 | -0.09 | -9.78 to 5.51 | 0.58 |
| *CYP2C19*17* | 2.50 | 0.11 | -4.64 to 9.64 | 0.48 |
| **C.** Comparing the likelihood of the model in (A) vs. (B), adjusting for *CYP2C19* did not significantly alter the association between *CYP2B6* and plasma steady state OH-BUP/BUP ratio | | | | |
| Likelihood Ratio $Chi^2$ | 1.44 | | | |
| *P*-value | 0.49 | | | |

*CYP2B6* NM=Normal metabolizers, IM=Intermediate metabolizers, and SM=Slow metabolizers (Zhu et al., 2012; Benowitz et al., 2013).

**Figure 1**

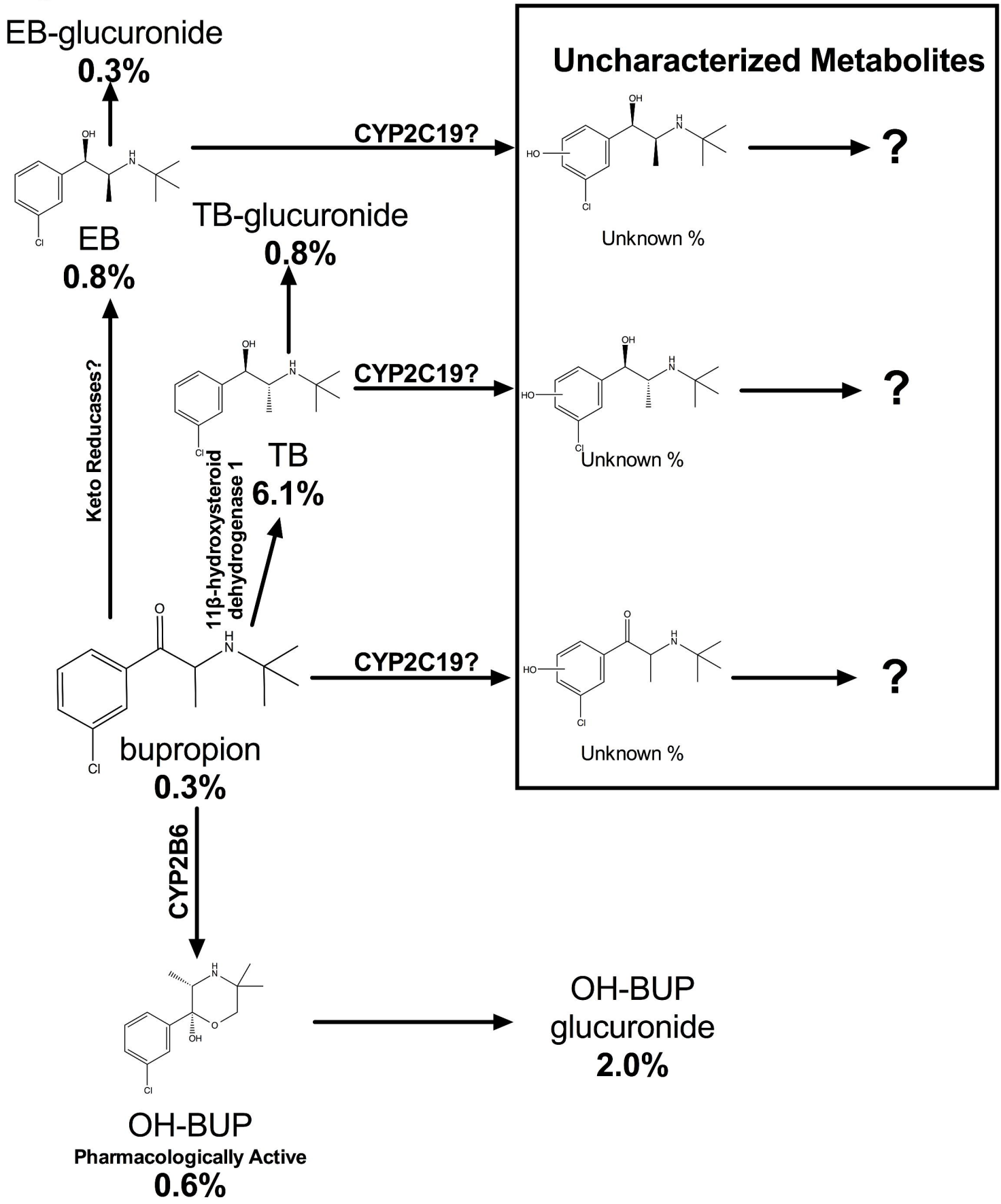

EB-glucuronide
0.3%
EB
0.8%
CYP2C19?
TB-glucuronide
0.8%
TB
6.1%
Keto Reducases?
11β-hydroxysteroid dehydrogenase 1
bupropion
0.3%
CYP2C19?
CYP2B6
OH-BUP
Pharmacologically Active
0.6%
OH-BUP glucuronide
2.0%
Uncharacterized Metabolites
Unknown %
Unknown %
Unknown %

Figure 2

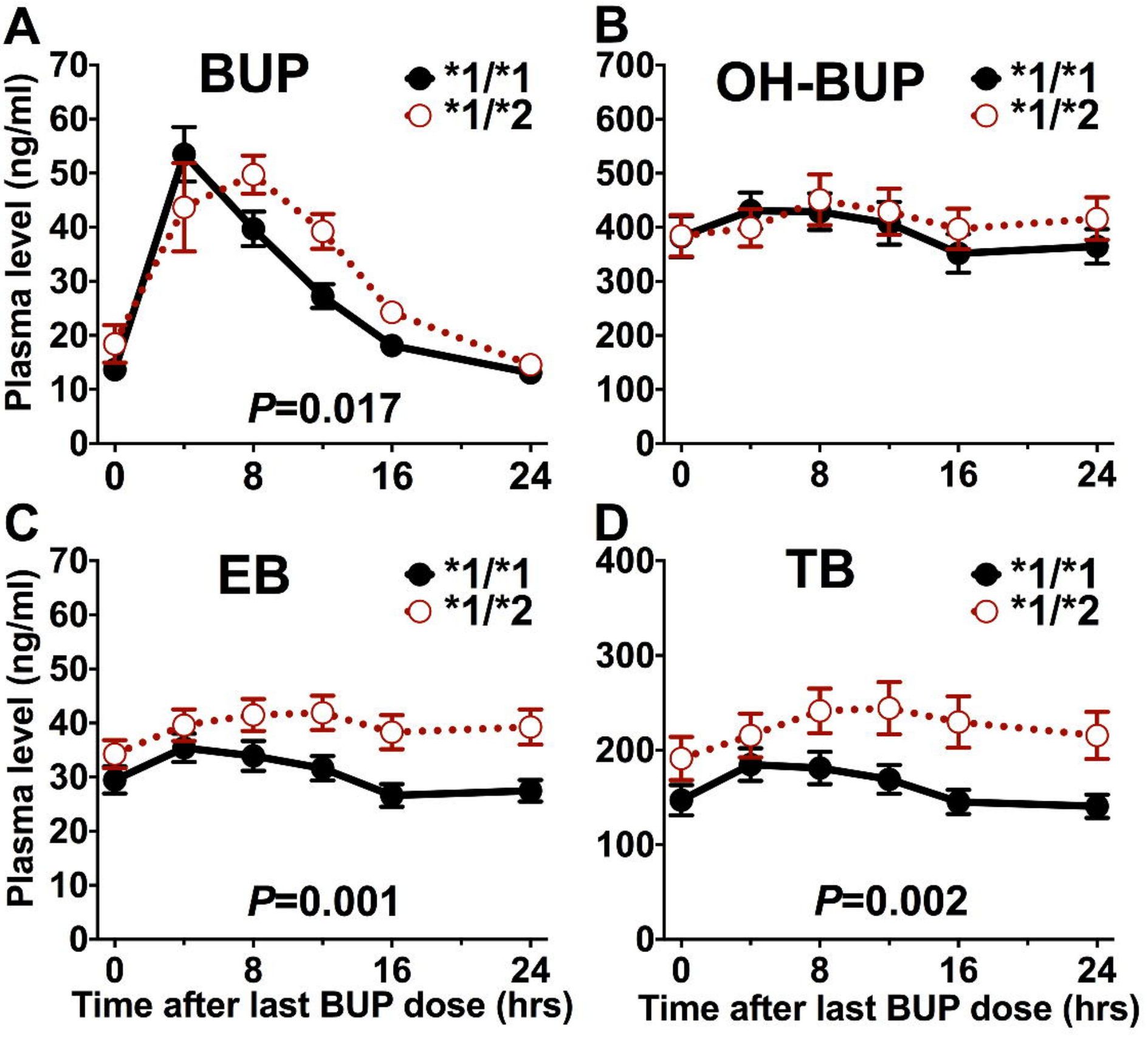

A
BUP
*1/*1
*1/*2
Plasma level (ng/ml)
70
60
50
40
30
20
10
0
P=0.017
0
8
16
24
B
OH-BUP
*1/*1
*1/*2
700
600
500
400
300
200
100
0
0
8
16
24
C
EB
*1/*1
*1/*2
Plasma level (ng/ml)
70
60
50
40
30
20
10
0
P=0.001
0
8
16
24
Time after last BUP dose (hrs)
D
TB
*1/*1
*1/*2
400
300
200
100
0
P=0.002
0
8
16
24
Time after last BUP dose (hrs)

Figure 3

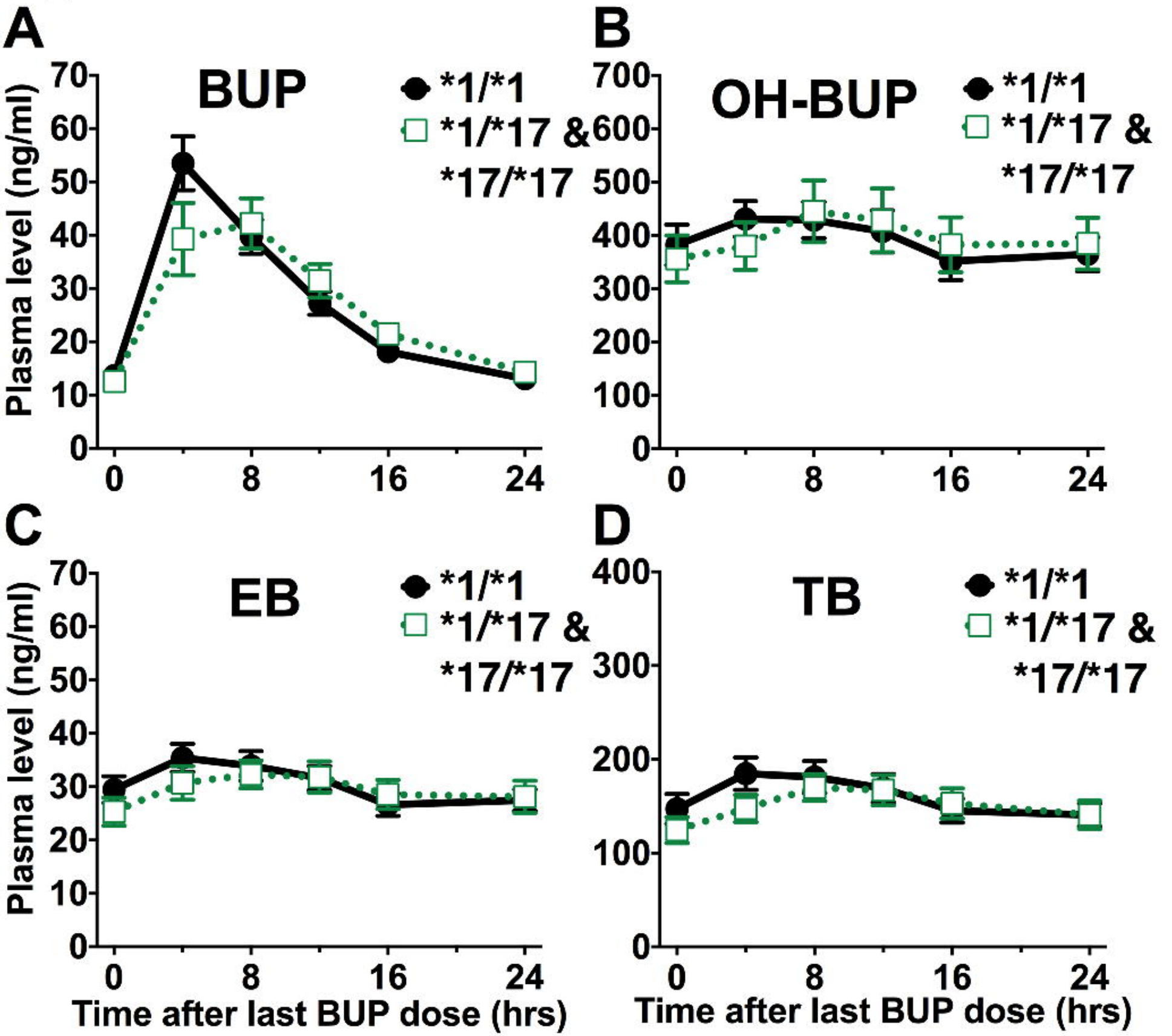
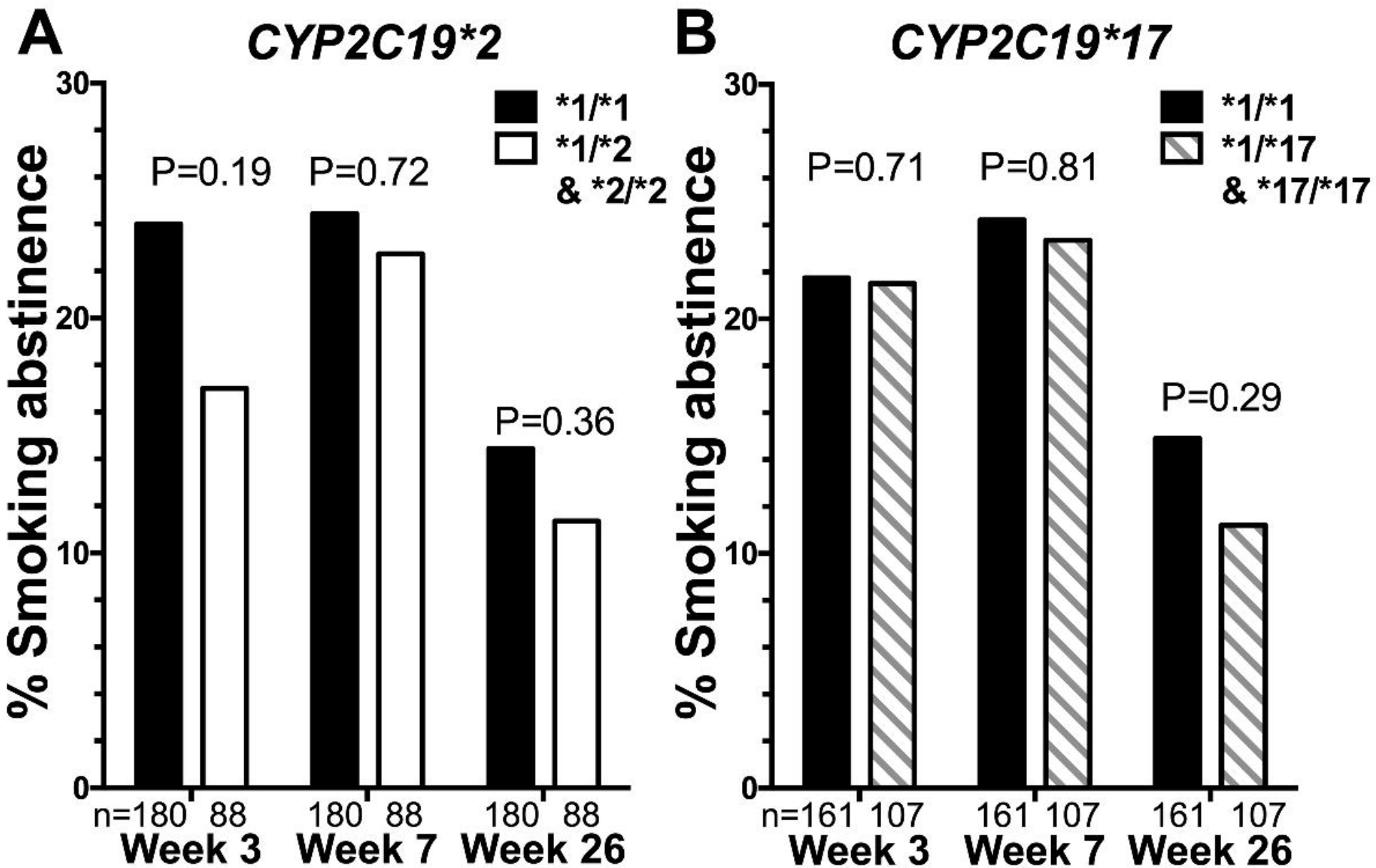

A
BUP
*1/*1
*1/*17 &
*17/*17
Plasma level (ng/ml)
70
60
50
40
30
20
10
0
0
8
16
24
B
OH-BUP
*1/*1
*1/*17 &
*17/*17
700
600
500
400
300
200
100
0
0
8
16
24
C
EB
*1/*1
*1/*17 &
*17/*17
Plasma level (ng/ml)
70
60
50
40
30
20
10
0
0
8
16
24
Time after last BUP dose (hrs)
D
TB
*1/*1
*1/*17 &
*17/*17
400
300
200
100
0
0
8
16
24
Time after last BUP dose (hrs)

Figure 4


A
CYP2C19*2
% Smoking abstinence
30
20
10
0
*1/*1
*1/*2
& *2/*2
P=0.19
P=0.72
P=0.36
n=180 88
180 88
180 88
Week 3
Week 7
Week 26
B
CYP2C19*17
% Smoking abstinence
30
20
10
0
*1/*1
*1/*17
& *17/*17
P=0.71
P=0.81
P=0.29
n=161 107
161 107
161 107
Week 3
Week 7
Week 26

***Drug Metabolism and Disposition***

# Supplementary Information

# Gene variants in *CYP2C19* are associated with altered *in vivo* bupropion pharmacokinetics but not bupropion assisted smoking cessation outcomes

Andy Z.X. Zhu; Qian Zhou; Lisa Sanderson Cox, Jasjit S. Ahluwalia; Neal L. Benowitz and Rachel F. Tyndale

**Supplementary Table S1.** Baseline demographics of Study 1.

| | White (n=21) | African American (n=14) | Asians (n=7) |
|---|---|---|---|
| Sex (% male) | 62 | 50 | 86 |
| Age in years (mean, range) | 29 (19, 51) | 33 (22, 64) | 37(22, 60) |
| BMI (mean, range) | 24 (19, 33) | 26 (20, 34) | 29 (21, 54) |
| *CYP2C19* genotype | | | |
| **1/*1* | n=7 | n=7 | n=3 |
| **1/*2* | n=5 | n=2 | n=3 |
| **1/*17* | n=5 | n=4 | |
| **17/*17* | n=1 | | |
| **2/*17* | n=3 | n=1 | n=1 |

We also genotyped for *CYP2C19*3* in this study, but did not find any participants with the *CYP2C19*3* allele.

**Supplementary Table S2.** Plasma pharmacokinetic parameters and urinary bupropion and metabolite recovery for bupropion and metabolites by *CYP2C19* genotype

| | All (n=42) | No variant (n=17) | with *2 (n=15) | with *17 (n=15) |
|---|---|---|---|---|
| **Plasma** | **Mean (95%CI)** | **Mean (95%CI)** | **Mean (95%CI)** | **Mean (95%CI)** |
| BUP | | | | |
| $AUC_{24}$ (h ng/ml) | 685 (631,739) | 670 (585,754) | **771 (694,848)** | 663 (550,775) |
| Cmax (ng/ml) | 58 (52,63) | 58 (49,67) | 66 (56,77) | 54 (41,67) |
| Css (ng/ml) | 29 (26,31) | 28 (24,31) | 32 (29,35) | 28 (23,32) |
| OH-BUP | | | | |
| $AUC_{24}$ (h ng/ml) | 9524 (8319,10729) | 9401 (7686,11115) | 9929 (7971,11887) | 9558 (6982,12135) |
| Cmax (ng/ml) | 464 (405,524) | 463 (380,546) | 476 (377,576) | 470 (338,601) |
| Css (ng/ml) | 397 (347,447) | 392 (320,463) | 414 (332,495) | 398 (291,506) |
| AUC OH-BUP/BUP | 14.4 (12.6,16.2) | 14.7 (12,17.5) | 13.2 (10.2,16.2) | 14.7 (11.1,18.3) |
| EB | | | | |
| $AUC_{24}$ (h ng/ml) | 772 (689,856) | 732 (618,846) | **947 (802,1093)** | 714 (573,854) |
| Cmax (ng/ml) | 38 (35,42) | 37 (31,42) | **47 (40,53)** | 35 (28,42) |
| Css (ng/ml) | 32 (29,36) | 31 (26,35) | 40 (33,46) | 30 (24,36) |
| AUC EB /BUP | 1.2 (1,1.3) | 1.1 (1,1.3) | 1.2 (1.1,1.4) | 1.1 (0.9,1.3) |
| TB | | | | |
| $AUC_{24}$ (h ng/ml) | 4209 (3612,4807) | 3867 (3134,4601) | **5427 (4168,6686)** | 3669 (2927,4411) |
| Cmax (ng/ml) | 208 (180,236) | 193 (155,231) | **267 (211,323)** | 183 (147,219) |
| Css (ng/ml) | 175 (150,200) | 161 (131,192) | 226 (174,279) | 153 (122,183) |
| AUC TB/BUP | 6.2 (5.5,6.9) | 5.9 (4.9,6.9) | 7.1 (5.6,8.6) | 5.7 (4.8,6.6) |
| **Urinary 24 hours recovery (nmol)** | **Mean (95%CI)** | **Mean (95%CI)** | **Mean (95%CI)** | **Mean (95%CI)** |
| Total | 67824 (51128,84520) | 74825 (48476,101173) | 71393 (33478,109309) | 52890 (38439,67341) |
| BUP | 2087 (1512,2662) | 2465 (1196,3735) | 1762 (1072,2452) | 1896 (1204,2587) |
| BUP/Total Recovery (%) | 0.07 (0.04,0.10) | 0.09 (0.04,0.14) | 0.03 (0.00,0.06) | 0.06 (0,00,0.12) |
| OH-BUP-free | 3672 (2782,4561) | 4420 (2555,6284) | 2941 (1889,3993) | 3234 (2042,4426) |
| OH-BUP-glucuronide | 12283 (10393,14173) | 12249 (10042,14457) | 13574 (9081,18067) | 11041 (8406,13676) |
| OH-BUP/Total Recovery (%) | 28.8 (25,32.6) | 27.5 (21.5,33.5) | 27.8 (22.8,32.8) | 31.3 (23.3,39.4) |
| EB-free | 4872 (3753,5991) | 5574 (3751,7396) | 4564 (2258,6870) | 4111 (2704,5519) |
| EB- glucuronide | 1718 (1438,1998) | 1759 (1432,2086) | 1982 (1284,2679) | 1592 (1141,2042) |
| EB/Total Recovery (%) | 10.3 (9.8,10.9) | 10.5 (9.5,11.4) | 10.1 (8.9,11.3) | 10.9 (9.9,11.9) |
| TB-free | 38376 (27180,49572) | 43345 (25732,60957) | 39718 (14184,65251) | 28779 (18908,38649) |
| TB- glucuronide | 4778 (2539,7017) | 4946 (1476,8417) | 6839 (1722,11957) | 2217 (805,3629) |
| TB/Total Recovery (%) | 57.8 (54.1,61.4) | 58.9 (52.9,65) | 59.4 (54.2,64.7) | 54.3 (47.1,61.5) |
| **% daily dose recovered** | **Mean (95%CI)** | **Mean (95%CI)** | **Mean (95%CI)** | **Mean (95%CI)** |
| BUP | 0.3 (0.2,0.4) | 0.4 (0.2,0.6) | 0.3 (0.2,0.4) | 0.3 (0.2,0.4) |
| OH-BUP-free | 0.6 (0.4,0.7) | 0.7 (0.4,1.0) | 0.5 (0.3,0.6) | 0.5 (0.3,0.7) |
| OH-BUP-glucuronide | 2.0 (1.7,2.3) | 2.0 (1.6,2.3) | 2.2 (1.5,2.9) | 1.8 (1.3,2.2) |
| EB-free | 0.8 (0.6,1.0) | 0.9 (0.6,1.2) | 0.7 (0.4,1.1) | 0.7 (0.4,0.9) |
| EB- glucuronide | 0.3 (0.2,0.3) | 0.3 (0.2,0.3) | 0.3 (0.2,0.4) | 0.3 (0.2,0.3) |
| TB-free | 6.1 (4.3,7.9) | 6.9 (4.1,9.8) | 6.4 (2.3,10.4) | 4.6 (3,6.2.0) |
| TB- glucuronide | 0.8 (0.4,1.1) | 0.8 (0.2,1.3) | 1.1 (0.3,1.9) | 0.4 (0.1,0.6) |

There were five individuals with **2/*17* genotype.

Bolded values indicate statistical significance compared with no variants using linear regression analyses as presented in Table 1.

**Supplementary Table S3**. Baseline demographics of Study 2.

| | Placebo | Bupropion |
|---|---|---|
| % African American | 100% | 100% |
| Sex (% male) | 68% | 64% |
| Age (years) (mean, range) | 46.2 (19, 80) | 46.8 (22, 73) |
| BMI (mean, range) | 31 (15, 68) | 31 (16, 63) |
| *CYP2C19* genotype | | |
| **1/*1* | n=104 | n=93 |
| **1/*2* | n=60 | n=60 |
| **2/*2* | n=6 | n=8 |
| **1/*17* | n=60 | n=77 |
| **17/*17* | n=10 | n=10 |
| **2/*17* | n=27 | n=20 |